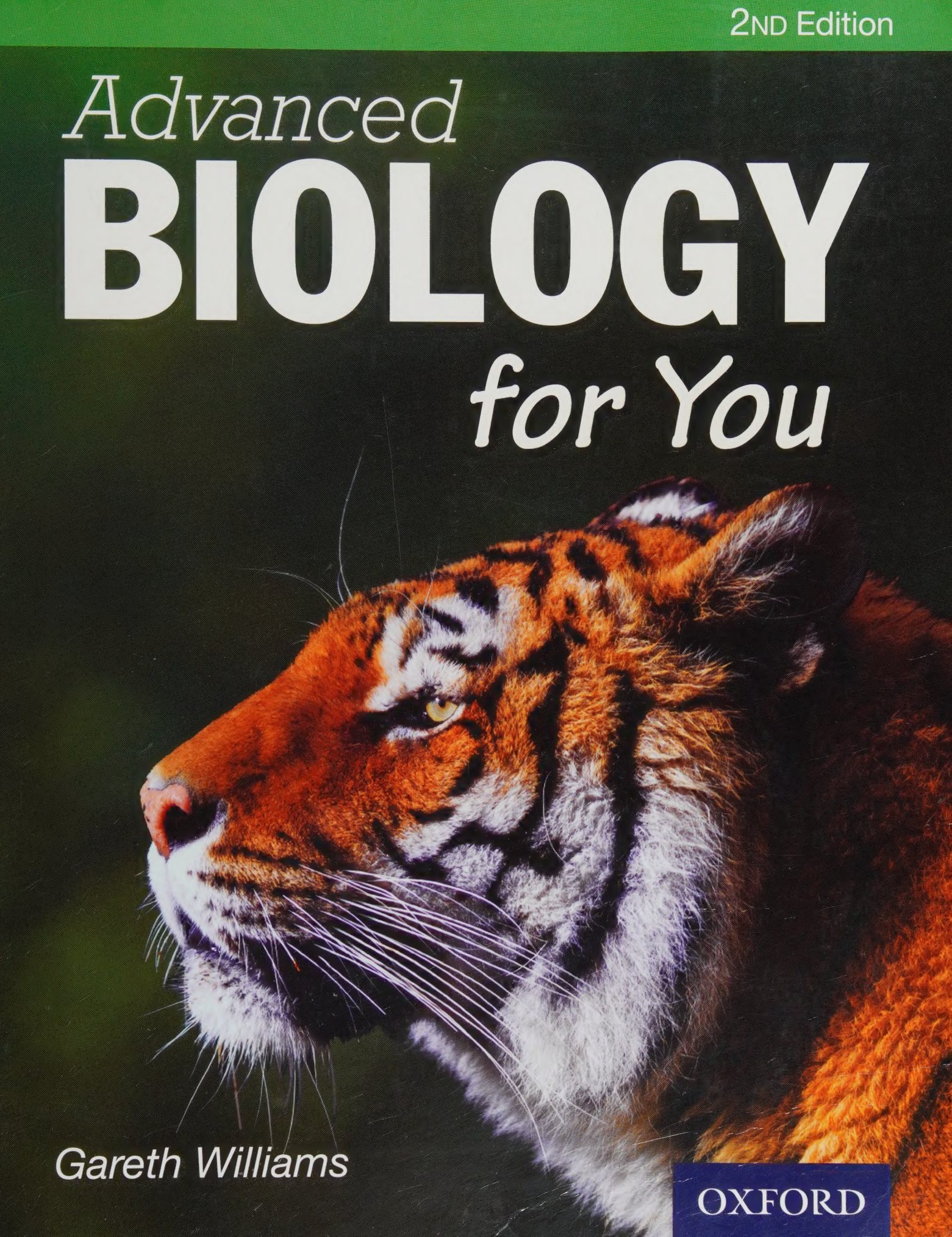
2ND Edition

Advanced BIOLOGY for You



Gareth Williams

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I would like to record my special thanks for the excellent work of Nick Paul and Damian Allen who were responsible for writing the Biology at work sections of this book.

Introduction

Advanced Biology for You is designed to help and support you during your advanced Biology course. No matter which examination you are taking, this book will help you to make the transition to A-level. This revised edition has been fully updated to cover all that you need to know for the new specifications.

The book is carefully laid out so that each new idea is introduced and developed on a single page or on two facing pages. Words have been kept to a minimum and as straightforward as possible, with clear diagrams and useful photographs to support the text. Pages with a red triangle in the top corner are the more difficult pages needed for the full A-level qualification. Key words and facts are clearly printed in **bold** or placed in a highlight box. There is a summary of important facts at the end of each chapter to help you with revision.

At the back of the book there are extra sections giving you valuable advice on study skills, practical work, revision and examination techniques, as well as help with mathematics.

Throughout the book there are 'Biology at Work' pages. These show you how the biological ideas that you learn are used in a wide range of interesting applications.

There is also a useful analysis of how the book covers the different examination specifications, with full details of which pages you need to study on the website at: www.oxfordsecondary.co.uk/advancedforyou

At the end of each chapter there are a number of questions for you to practise your Biology and so gain in confidence. They range from simple fill-in-a-missing-word passage (useful for doing quick revision) to more difficult questions that will need more thought.

At the end of each main topic you will find a section of Further Questions, mostly taken from actual A-level examination papers. The answers to all these Further Questions, as well as answers to the rest of the end-of-chapter summary questions are provided on our website, along with a useful Glossary.

I hope that reading this book will make Biology more interesting for you and easier to understand. Above all, I hope that it will help you to make good progress in your studies, and that you will enjoy using **Advanced Biology for You.**

Gareth Williams

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Chemicals of Life

Cells

Exchange and Transport

Evolution, Classification and Biodiversity

Health and Disease

Energy and Control

Genetics and Gene Technology

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Extra Sections

1 Biological molecules

Explorations in outer space have led to the discovery of large amounts of biological molecules.
These are mainly small molecules made up of carbon, hydrogen, nitrogen, oxygen and sometimes sulfur or silicon.
There is speculation that these molecules could combine to become more complex molecules.

Meteorites landing from outer space have been found to contain organic material.
The Murchison meteorite fell in Murchison, Australia in 1969.
On examination, it was found to contain a variety of different amino acids – the molecules that build proteins.

Could it be that the origin of some of the biological molecules that make up living organisms on Earth came from outer space?

The chemistry in the next four pages is intended to help you understand biological molecules. As such it will not be specifically examined.

Comet Hale-Bopp

Atoms

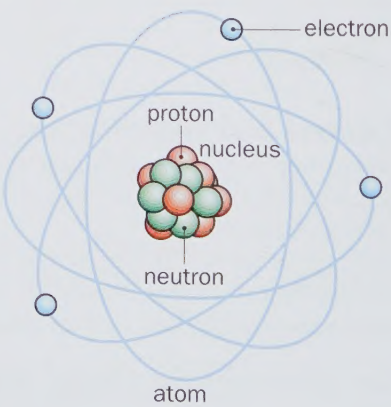
All matter is made up of **atoms**.
But what do you think atoms are made up of?
There are ***three*** different particles that make up an atom:

- **protons**, which have a positive charge,
- **neutrons**, which have no charge,
- **electrons**, which have a negative charge.

The protons and neutrons are found in the nucleus, at the centre of the atom.
The electrons are found circling the nucleus in 'orbitals' or 'shells'.

If the overall charge of an atom is neutral, what do you think this tells us about the number of protons and the number of electrons?

If an atom is neutral, then the number of protons must ***equal*** the number of electrons.



Atoms of a particular element always contain the ***same*** number of protons.
For example, carbon atoms always contain six protons, and oxygen atoms always contain eight protons.

The **atomic number** of an element is the number of protons contained in the nucleus.

The **mass number** of an element is the number of protons ***plus*** the number of neutrons contained in the nucleus.

Molecules

Do you remember what a molecule is?

A **molecule** consists of two or more atoms joined together chemically.

Molecules can be made from atoms of the ***same*** element joined together.
For example, a molecule of oxygen (O_2) contains two atoms of oxygen.
Usually, molecules contain atoms of ***different*** elements joined together.
For example, a molecule of carbon dioxide (CO_2) contains an atom of carbon and two atoms of oxygen.

A molecule that is made up of different atoms is called a **compound**.
So carbon dioxide is a compound.

Ions

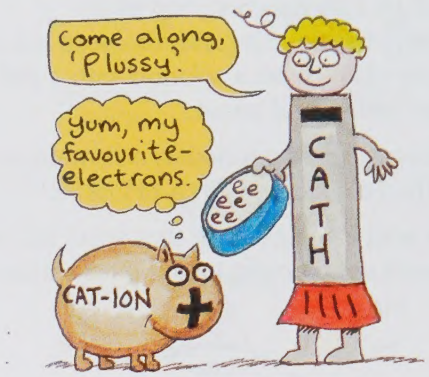
Ions are charged particles. They are formed when atoms or groups of atoms gain or lose electrons.

What charge will atoms have if they ***gain*** electrons?
What charge will atoms have if they ***lose*** electrons?

Since electrons have a negative charge:

- atoms that gain electrons become ions with a negative charge (**anions**),
- atoms that lose electrons become ions with a positive charge (**cations**).

We write ions with the chemical symbol of the elements (or combined elements) and a negative or positive sign to show the charge.
A sodium atom that loses an electron becomes the ion Na^+.
A chlorine atom that gains an electron becomes the ion Cl^-.
Groups of atoms can also form ions.
For example, NO_3^- is a nitrate ion and PO_4^{3-} is a phosphate ion.



Cat-ions are 'pussytive'!
Cations go to the cathode
When they get there they receive electrons

Isotopes

Atoms of the same element always contain the same number of protons.
For example, a carbon atom always contains six protons in its nucleus.

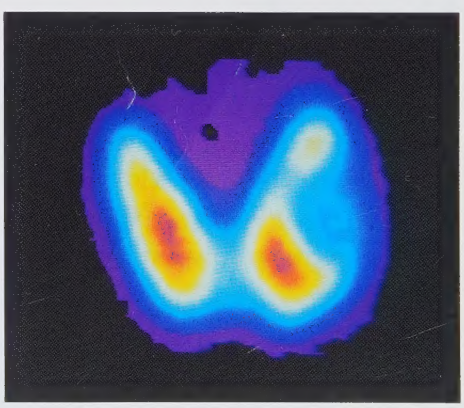
But the number of neutrons in its nucleus can change.
Usually carbon atoms have six neutrons.
So the mass number = 6 protons + 6 neutrons = 12.
But some carbon atoms have eight neutrons.

What do you think their mass number will be?
The mass number of this form of carbon will be 14.

Atoms of the same element with different mass numbers are called isotopes.

Many isotopes are radioactive so biologists have been able to use them as tracers.
For instance, the isotope carbon-14 (written as ^{14}C) is radioactive.
Carbon dioxide containing ^{14}C is said to be labelled, so if it is fed to plants, we can look at the formation of compounds made in photosynthesis by tracing what happens to the ^{14}C.

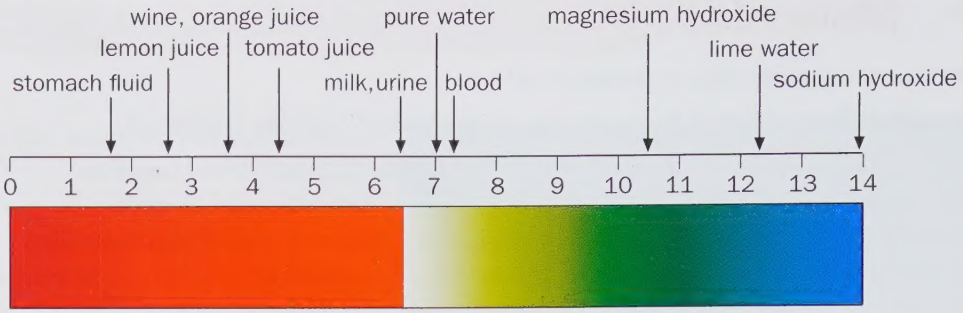
Nitrogen-15 is a 'heavy', non-radioactive isotope (the normal form of nitrogen is ^{14}N).
This isotope has been used to label the nitrogen in DNA to find out how new DNA is made.



Gamma camera scintigram of a human thyroid gland, using radioactive isotope iodine-131 to show cancerous cells

Acids and bases

Hydrogen has an atomic number of 1 and a mass number of 1.
This means that it has one proton and one electron but no neutrons.
A hydrogen ion (H^+), which is an atom that has lost an electron, is simply one proton by itself.



The pH scale

The **pH** of a solution is a measure of the concentration of hydrogen ions present.
The ***higher*** the concentration of hydrogen ions present, the ***more*** acidic the solution.

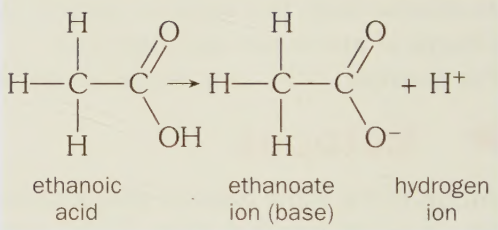
On the pH scale, a value of 1 indicates a strong acid, 7 is neutral and 14 indicates a strong alkali.
The pH scale is logarithmic, so a solution with a pH of 1 is ten times more acidic than a solution with a pH of 2, which is ten times more acidic than pH 3, and so on.

Can you work out how much more acidic a solution with a pH of 1 is than a solution with a pH of 7?

An acid is a substance that splits up, or **dissociates**, into ions when in solution, releasing hydrogen ions. For example:

$$\underset{\text{acid}}{HCl} \longrightarrow \underset{\text{hydrogen ion}}{H^+} + \underset{\text{base}}{Cl^-}$$

Hydrochloric acid is a strong acid since it releases a lot of hydrogen ions into solution.



Carboxylic acids such as ethanoic acid are weak acids.
Relatively few of their molecules dissociate and release hydrogen ions into solution.

Can you see that when an acid dissociates it loses an H^+, leaving a base?
A base is a chemical that can combine with hydrogen ions.

What do you think happens when a base accepts a hydrogen ion?

$$\underset{\text{base}}{NO_3^-} + \underset{\text{hydrogen ion}}{H^+} \longrightarrow \underset{\text{acid}}{HNO_3}$$

It forms an acid.

Buffers

Living organisms have to maintain their cell contents at a fairly stable pH because their enzymes only work effectively over a relatively narrow pH range.
Some chemicals in the cytoplasm are able to:

- act as bases by mopping up hydrogen ions to help neutralise an acidic solution,
- act as acids by donating hydrogen ions to help neutralise an alkali solution.

Some substances are able to carry out both roles.
Chemicals that can act as bases and as acids are called **buffers**.
Plasma proteins act as buffers helping to keep the blood pH constant and preventing it from becoming too acidic or too alkaline.

When acids and bases react together they form salts. For example:

$$\underset{\text{acid}}{HCl} + \underset{\text{base}}{NaOH} \longrightarrow \underset{\text{salt}}{NaCl} + H_2O$$


Using an electronic pH meter

Chemical bonds

Atoms are held together inside molecules by chemical bonds.
The making and breaking of these chemical bonds involves energy.

Ionic bonds

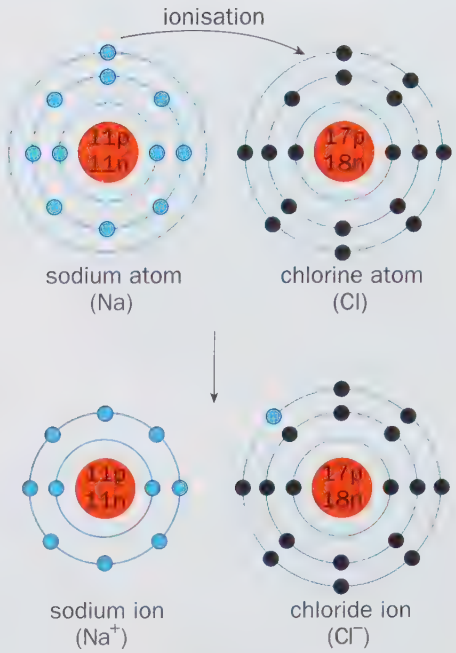
Electrons that circle the nucleus are found in orbitals or shells.
The first shell around the nucleus can hold two electrons,
the second and third shells can each hold eight electrons.
Subsequent shells can hold an increasing number of electrons.

Atoms are stable when their outer electron shell is full.
For example, a sodium atom has one electron in its outer shell.
It becomes more stable if it ***loses*** this electron.
It then forms the positive ion Na^+.

A chloride atom has seven electrons in its outer shell.
It becomes more stable if it ***gains*** an electron to fill the outer shell.
It then forms the negative ion Cl^-.

What do you think will happen to the positive sodium ions and
the negative chloride ions?

The oppositely charged ions will attract each other.
Electrostatic forces draw these ions together forming an **ionic bond**.
Ionic bonds often occur in inorganic compounds such as sodium chloride.



An ionic bond between sodium and chloride ions

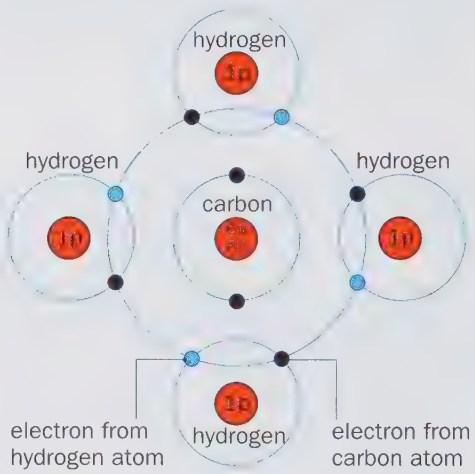
Covalent bonds

Covalent bonds also involve atoms becoming more stable when their
outer electron shells become full.
For instance, a hydrogen atom has a single electron in its outer shell.
It would become more stable if it gained an electron.

Two hydrogen atoms are able to ***share*** their electrons.
This forms a **covalent bond**, which holds the two hydrogen atoms
together in a hydrogen molecule.

A carbon atom has four electrons in its outer shell.
It would become more stable if it gained another four electrons.
Methane (CH_4) consists of a carbon atom and four hydrogen atoms.

It has four covalent bonds, where each of the hydrogen atoms shares its
electron with the carbon atom to make a stable methane molecule.



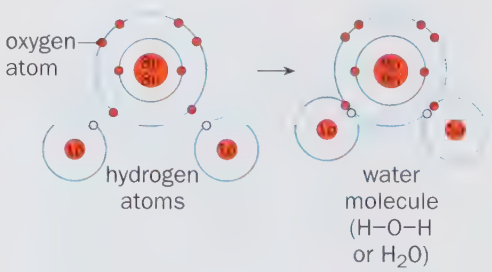
Covalent bonds between a carbon atom and four hydrogen atoms

Hydrogen bonds

A molecule of water consists of two hydrogen atoms and one oxygen atom.
Each oxygen atom has six electrons in its outer shell and
each hydrogen atom has one electron, so each of the two hydrogen atoms
shares an electron with the oxygen atom.
Each hydrogen atom forms a single covalent bond with the oxygen atom,
so forming a stable molecule of water.

But the oxygen atom has more protons in its nucleus than each of the
hydrogen atoms. These positively charged particles tend to pull the shared
electrons in the bond.
So the oxygen end of the molecule has a slight negative charge ($2\delta^-$)
and the hydrogen ends of the molecule have a slight positive charge (δ^+).
The molecule is said to be **polar**.

When two water molecules are in close contact, their opposing charges
attract each other. This forms a **hydrogen bond**. (See top of page 10.)



Covalent bonds between an oxygen atom and two hydrogen atoms

Water

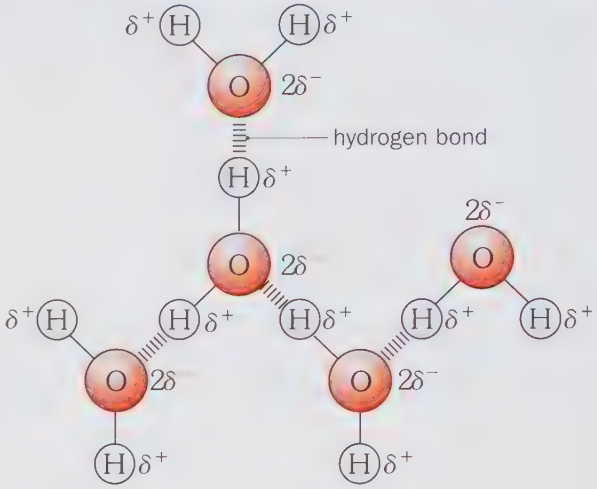
Water is perhaps the most important biological molecule.

- Three-quarters of the Earth's surface is covered by water.
- Water provides the environment for many organisms that live in freshwater or seawater.
- Water makes up 70% of a human cell and up to 95% of the mass of a plant cell.

Let's stick together

The most important property of water molecules is that they can 'stick together' by forming hydrogen bonds with other water molecules.

A water molecule has no overall charge. It is electrically neutral.
Overall there are 10 protons and 10 electrons.
But, as you have seen, the nucleus of the oxygen atom tends to pull electrons away from the nucleus of each hydrogen atom, creating polar molecules.
Polar molecules allow the formation of hydrogen bonds.
Although these hydrogen bonds are weak, there are lots of them, so they stick together in a strong lattice framework.
This sticking together is called **cohesion**.
It gives water many of its special properties.



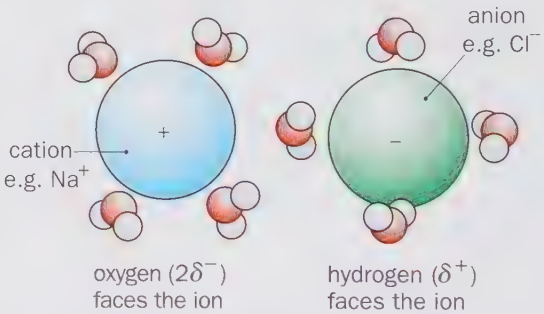
Water molecules are held together by hydrogen bonds

Water as a solvent

Many biological reactions take place in the watery cytoplasm of cells.
When a chemical dissolves in water it is free to move about and to react with other chemicals.
Because water has slightly positive and slightly negative parts, it will attract other charged particles, such as ions, and other polar molecules, such as glucose.
For example, in a solution cations such as sodium ions and potassium ions become surrounded by water molecules.
The slightly negative charge on the oxygen atom is attracted to the positive cation.
The same thing happens with anions such as chloride ions; they attract the slightly positive hydrogen ions.

In contrast, non-polar molecules such as lipids will not dissolve in water.
In this case, the water molecules tend to be attracted ***to each other***, to the exclusion of the non-polar molecules.

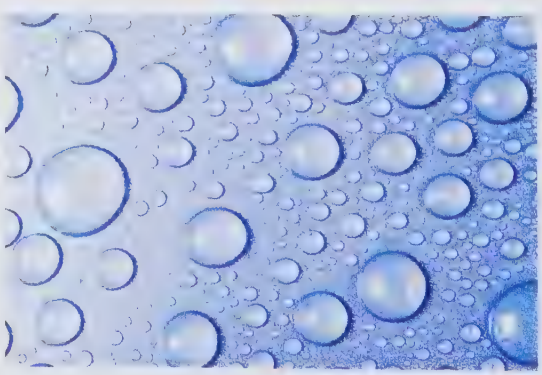
This **hydrophobic** (water-hating) property of lipids is important in giving stability to the cell membrane.



The way in which water molecules arrange themselves around ions in solution

Water as a metabolite and transport medium

In many reactions in cells, water is either used up or produced.
For example, it is used up in photosynthesis and produced in respiration.
Water is a transport medium both inside and outside the cell.
In animals, blood, tissue fluid and lymph transport many dissolved substances.
In plants, water is vital to the ways in which xylem and phloem function.
The properties of water as a solvent are important here.



Thermal properties

Most cells can only tolerate a narrow range of temperature.
This is mainly because the enzymes within the cell can only function effectively over this temperature range.

A large amount of energy is needed to raise the temperature of water.
This is because the movement of water molecules is restricted by the hydrogen bonds that form between them.
We say that water has a high **specific heat capacity**.
This means that it takes a lot of energy to warm water up.
Equally, water has to lose a lot of heat energy to cool down.

As a result, aquatic habitats have relatively stable temperatures.
Aquatic organisms do not have to endure rapid and extreme temperature changes.

It also means that it is easier for mammals to maintain a stable internal body temperature. Sweating involves the conversion of water to a gas.
This requires the transfer of a great deal of energy and is an effective method of cooling the body.

Density changes

Water is unique because its solid form, ice, is less dense than its liquid form. This is why ice floats on the surface of a lake or pond.
It forms an insulating layer, which prevents the water underneath from freezing.
If ice were denser than water, then a pond would freeze from the bottom up, killing all the living organisms within it.

Water is usually most dense at 4 °C. So even when a pond is frozen over, there is a layer of water below the ice at 4 °C where organisms can survive.

Cohesion and surface tension

The hydrogen bonds that hold water molecules together give water its cohesive properties. This cohesion means that long columns of water molecules don't break.
They can be drawn up xylem vessels to the tops of the tallest trees, a bit like sucking water up a straw.

At the air/water interface of a pond, the cohesion between water molecules produces surface tension.
This acts almost like a skin that covers the water.
Insects such as the pond skater exploit this property.
The insect's body is supported by the high surface tension at the water surface.

Incompressibility

Water is virtually incompressible compared with air. It is far easier for organisms to move through air because air is compressed only slightly by their bodies.
Organisms moving through water must completely displace the water.
This results in turbulence and increases the drag. Not surprisingly, many aquatic organisms have developed streamlining.

Freshwater invertebrates

Evaporation of sweat cools the skin of this boxer

Ice floats on the surface of this frozen pond

Surface tension supports this pond skater (Gervis lacustris) on the water surface

Carbohydrates

Carbohydrates are compounds of carbon, hydrogen and oxygen.
In a carbohydrate, there is usually the same number of carbon atoms and oxygen atoms, but twice as many hydrogen atoms.
One molecule of water (H_2O) combines with one atom of carbon to form the sub-unit (CH_2O). These sub-units or **monomers** can be repeated, $(CH_2O)_n$, to form many different molecules.

Carbohydrates are the source of energy in all living organisms.
Carbohydrate **polymers** (chains of monomers) add strength and support to cell-surface membranes, plant cell walls and insect skeletons.

Pasta is rich in carbohydrates

There are ***three*** main types of carbohydrates:

- **monosaccharides** or simple sugars,
- **disaccharides** or double sugars (formed from two monosaccharides),
- **polysaccharides**, polymer chains of many hundreds of monosaccharides.

Monosaccharides

Monosaccharides are the simplest forms of carbohydrate.
They provide the building blocks for larger carbohydrate molecules.
They also act as a respiratory substrate, providing cells with an energy source.

The names of monosaccharides depend upon the number of carbon atoms in the molecule:

- **Trioses** have three carbon atoms ($n = 3$).

For example, glyceraldehyde is an important intermediate in respiration.

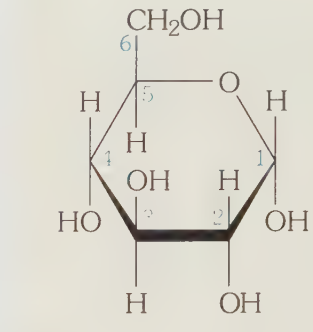
- **Pentoses** have five carbon atoms ($n = 5$).

For example:
Ribose is an important part of the ***RNA*** molecule involved in passing on the genetic code.
Deoxyribose is an important part of the ***DNA*** molecule.
Ribulose helps to fix carbon atoms from carbon dioxide into carbohydrate molecules in photosynthesis.

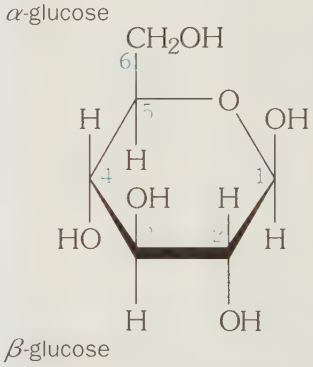
- **Hexoses** have six carbon atoms ($n = 6$) with the formula $C_6H_{12}O_6$.

For example:
Glucose is the main energy source for most living cells.
It is one of the first carbohydrates produced in photosynthesis and forms the building blocks of many other carbohydrates.
Fructose is a very sweet sugar. It combines with glucose to form the disaccharide molecule sucrose, the sugar you put in your tea.
Galactose is found in milk. It combines with glucose to form the disaccharide milk sugar molecule lactose. You put this in your tea too!

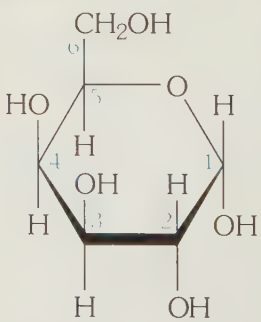
Monosaccharides usually exist as ring structures when they dissolve in water.
Can you see any differences between the α-glucose and β-glucose molecules?
These two molecules have the same chemical formula ($C_6H_{12}O_6$) but they have different structural formulas.
We say that they are **structural isomers**.



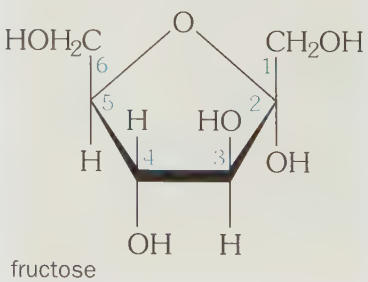
α-glucose



β-glucose



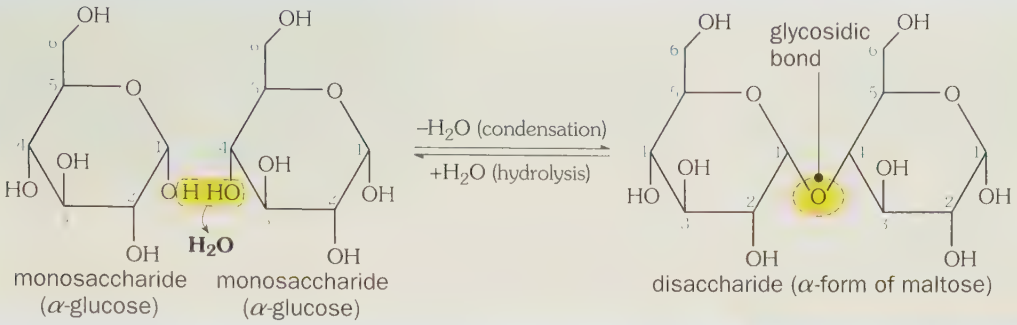
galactose



fructose

Disaccharides

Disaccharides are formed when ***two*** monosaccharides join together. This reaction releases water so it is called a **condensation reaction**. The link between the monosaccharide rings is called a **glycosidic bond**.



Look at the diagram.
Between which carbon atoms does the glycosidic bond form?
It forms between carbon atom number one (C_1) of one α-glucose molecule and carbon atom number four (C_4) of the other α-glucose molecule. This forms a 1,4 linkage.
These two carbon atoms end up sharing an oxygen atom.

Disaccharides can be formed by joining together

- two ***similar*** monosaccharides, as in the case of maltose, which is made from two glucose molecules,
- two ***different*** monosaccharides, as with sucrose and lactose.

Disaccharide	Source	Monosaccharide units
sucrose	stored in plants such as sugar beet and sugar cane	glucose + fructose
lactose	milk sugar – the main carbohydrate found in milk	glucose + galactose
maltose	malt sugar – found in germinating seed such as barley	glucose + glucose

Look at the table to find out which monosaccharide units make up sucrose (cane sugar) and which make up lactose (milk sugar).

Disaccharides can be broken back down into monosaccharides. This type of reaction is called **hydrolysis** (a molecule of water is ***added***).

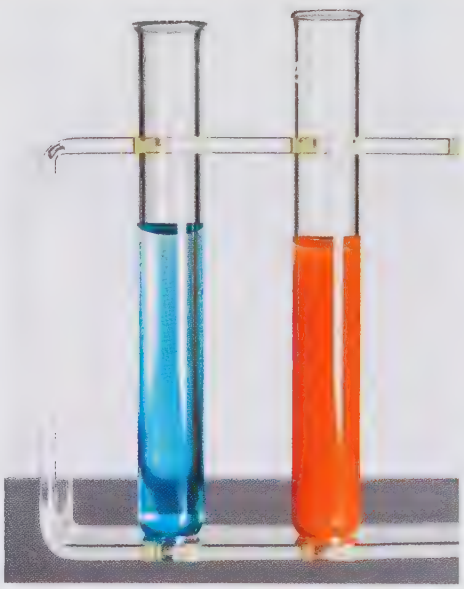
Disaccharides are still relatively small molecules. They are water-soluble and taste sweet. Disaccharides are more suitable for transport and storage than monosaccharides. Sucrose is stored in sugar beet and sugar cane. It is the main form in which carbohydrates are transported in the phloem sieve tubes of plants.

Reducing sugars

Monosaccharides, such as glucose, fructose and galactose, and disaccharides, such as maltose and lactose, are known as reducing sugars. They have **carbonyl groups** (C=O) that can be ***oxidised*** to **carboxylic acids** (—COOH). (Remember, in an oxidation reaction oxygen is added.) Thus they reduce other compounds, such as **Benedict's reagent** when heated, producing a precipitate. The colour of the precipitate depends upon the ***concentration*** of the reducing sugar.

Benedict's test is used to estimate the amount of reducing sugar present in a solution. Sucrose is a non-reducing sugar but it will give a positive result with Benedict's if it is first boiled with dilute acid to hydrolyse (split) it into its monosaccharides.

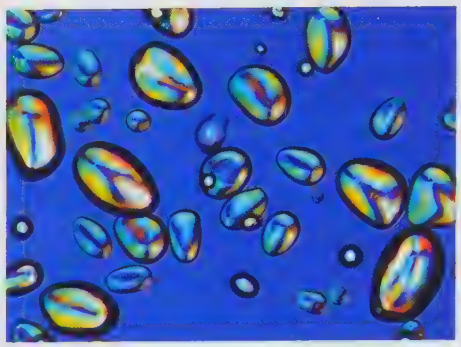
Look at the table to estimate the amount of reducing sugar in the test-tube in the photograph. You could use a **colorimeter** to give a quantitative measurement of the amount of reducing sugar present (see page 491). Reagent test strips can also be used to detect glucose.



A positive Benedict's test

Amount of reducing sugar	Colour of solution and precipitate
no reducing sugar	blue
	green
	yellow
increasing quantity of reducing sugar	brown
	red

Polysaccharides



Starch grains from potato

Polysaccharides are polymers made up of hundreds of monosaccharide units.
A condensation reaction occurs between each monosaccharide unit.
Long chains of monosaccharides are held together by glycosidic bonds.
These long molecules can be branched or unbranched.

Polysaccharides are insoluble and not sweet to taste.
Storage polysaccharides tend to be folded to give a compact molecule like starch.
Structural polysaccharides tend to be coiled or straight-chained (as with cellulose).

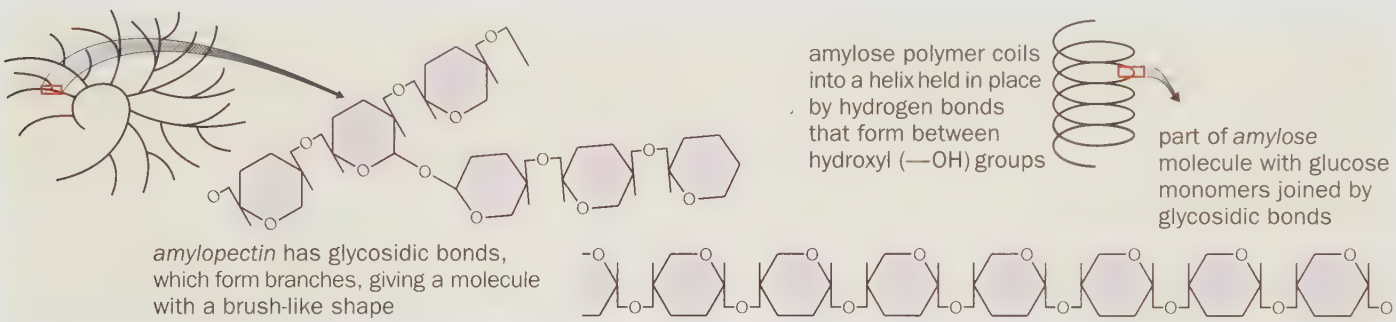
Starch

Starch is the main carbohydrate food reserve in plants.
Many food crops, such as maize, wheat, millet and potatoes, contain a lot of starch.
So it is the major food energy resource for most of the world's population.

Can you name any foods that are rich in starch?

Starch is a polymer made up of many α-glucose molecules.
These α-glucose sub-units are held together by glycosidic bonds.
Starch is actually a mixture of two different compounds:

- **Amylose** is a linear, unbranched polymer, which makes up about 80% of starch.
- **Amylopectin** is a polymer with some 1,6 linkages that give it a branched structure.



What makes starch a good food store?
Try to explain how each of the following properties make starch an ideal storage molecule.
Starch is:

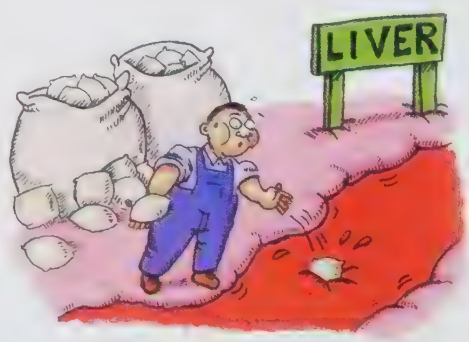
- ***compact*** – think about the space available in cells,
- ***insoluble*** – think about osmosis,
- ***readily converted to sugars*** – when would the store be needed?

Starch gives a blue-black colour with iodine solution

Starch grains are made up of successive layers of amylose and amylopectin, which show up as growth rings in starch grains.

Glycogen is a storage polysaccharide, sometimes called 'animal starch'.
It is the only carbohydrate store in animals.
Chemically it is very similar to amylopectin.
In humans, glycogen is stored in the liver and skeletal muscle.
During prolonged periods of exercise, blood glucose may fall below the threshold level (between 80 and 90 mg of glucose per 100 cm^3 of blood for a normal person).
Liver glycogen is quickly converted to glucose to meet the body's energy needs.

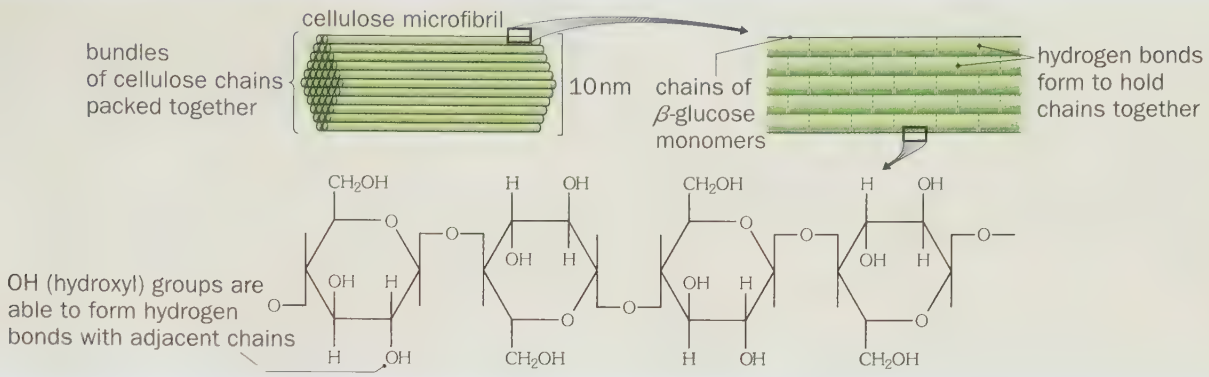
Which hormone promotes the conversion of glucose to liver glycogen when blood glucose is high?



'He must have been out jogging again!'

Cellulose

Cellulose is a structural polysaccharide made up of thousands of glucose units.
In this case, β-glucose units are held together by 1,4 glycosidic bonds, forming long, unbranched chains.



Can you see from the diagram how these chains are linked together to form long **microfibrils**?
The microfibrils are held together by hydrogen bonds to form strong cellulose fibres.
It is these fibres that give plant cell walls their strength and rigidity.

Humans cannot digest cellulose but animals such as cows, sheep and horses can.
These herbivores have bacteria in their guts that make **cellulases**.
These enzymes break down the bonds within the cellulose molecule to release units of glucose.
This is an example of **mutualism**: both the herbivore and the bacterium benefit.
The bacterium gets a food source and a sheltered environment.
The herbivore gets a rich source of energy – glucose.

Some items manufactured from cellulose

We use a lot of cellulose.
It is found in all plant cells and is therefore a readily available raw material.
Probably the best known use is in the manufacture of paper.
The words that you are reading on this page are printed on cellulose.
Cellophane is a clear film used in food packaging.
Celluloid is used in the manufacture of photographic film.
Cellulose films are used in the production of dialysis membranes, Sellotape and sweet wrappers.
Even cotton clothes and tea towels are made from cellulose.
Tough, synthetic rayon, derived from cellulose, is used in tyre cords owing to its high tensile strength.
Viscose is a synthetic fibre derived from cellulose and used in a range of textiles.

Chitin

Chitin has many chemical and structural similarities to cellulose.
It is a polysaccharide, with amino acids added to form a **mucopolysaccharide**.
Chitin forms the exoskeletons of insects and other arthropods.
It is also present in the cell walls of fungi.
Chitin is strong, lightweight and waterproof.
Why do you think chitin is so important to insect body structure?


Chitin makes this insect's exoskeleton strong and light

Lipids

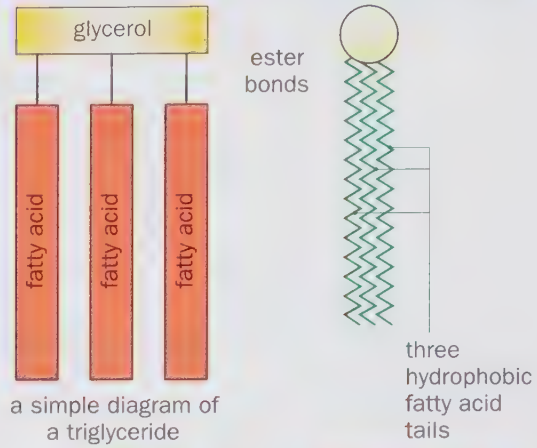
Lipids are a large and varied group of organic compounds. They include **fats** and **oils**. Like carbohydrates, they are made up of carbon, hydrogen and oxygen, though they have a lot less oxygen. They are non-polar compounds and so are insoluble in water. They can be extracted using non-polar solvents, such as alcohol and ether.

Fats and oils are chemically very similar, but at room temperature, fats are solid and oils are liquid.

A field of oilseed rape

Triglycerides are one of the most common types of lipids.
They are formed by joining two ***different*** kinds of organic molecules together: **fatty acids** and **glycerol**.

The glycerol molecule in any lipid is always the same. It is the fatty acids that vary.
Fatty acids are organic acids with a **carboxyl** (—COOH) group at one end.
Joined to the carboxyl group is a long hydrocarbon tail (the R group).
So the general formula of a fatty acid can be expressed as RCOOH.
Different fatty acids have different R groups (hydrocarbon tails).
The properties of any particular lipid depend upon the fatty acids it contains.

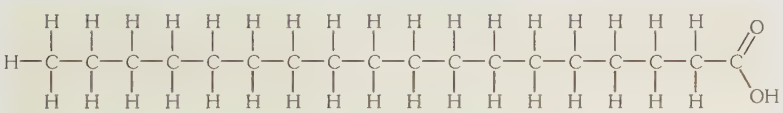


a simple diagram of a triglyceride

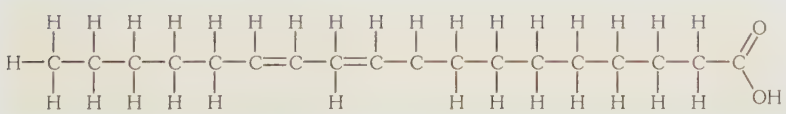
There are hundreds of different fatty acids that might react with glycerol.
They vary in two ways:

- the length of the hydrocarbon tail,
- how saturated the R group is.

Saturated fatty acids only have C—C bonds.
You can see from the diagram below that in stearic acid all the carbon atoms are joined by a ***single*** C—C bond.



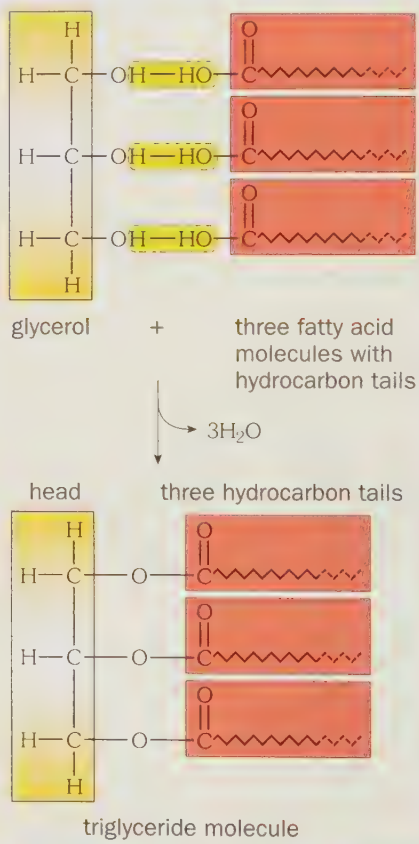
Unsaturated fatty acids have at least one C=C double bond.
Polyunsaturates have more than one double bond, as in linoleic acid below.



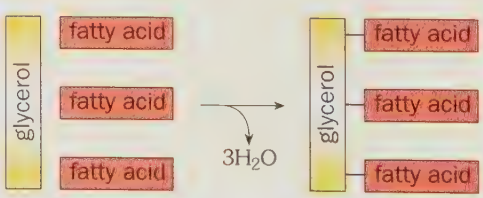
How many double bonds does the whole molecule of linoleic acid have?

Triglycerides

Triglycerides consist of one molecule of glycerol and three fatty acid molecules.
They are formed as a result of three condensation reactions involving the —OH groups of glycerol and the —COOH group of each fatty acid.
For each condensation reaction, an **ester bond** is formed.



When oxidised, triglycerides release more energy for use in respiration than an equal mass of carbohydrate.
Triglycerides also produce a lot of metabolic water when oxidised.
This is important to desert animals – the camel's hump is made of fat.
Triglycerides are stored under the skin where they act as a heat insulator.



Which properties of fats make them ideal as an energy store?

Phospholipids

Phospholipids are similar to triglycerides but one of the fatty acids molecules is replaced by a phosphate group (PO_4^{3-}).

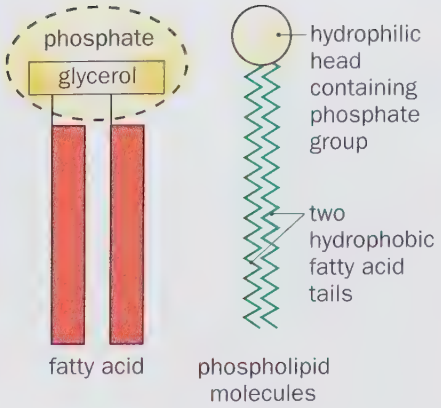
The lipid and the phosphate parts of the molecule have very different properties.
The lipid part is non-polar and insoluble in water – we call it hydrophobic or 'water-hating'.
But the phosphate part is polar and dissolves in water – we say that it is **hydrophilic** or 'water-loving'.

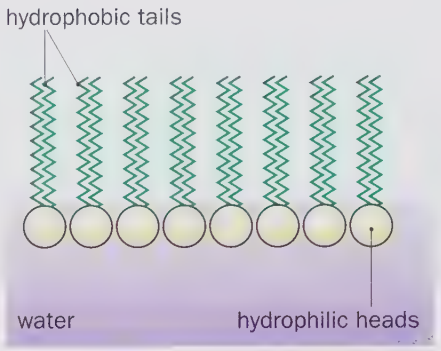
When in contact with water, phospholipids spread out over the surface.
Their hydrophilic heads are attracted to the water and 'dip into' it.
The hydrophobic tails move away from the water.
The molecules can become tightly packed forming a **monolayer**.

What do you think would happen if this arrangement were shaken up?

The phospholipids would form tiny, spherical structures called **micelles**.
The hydrophobic tails turn inwards and become protected from the water by the hydrophilic heads.

As you will see in Chapter 5, these properties are very important in determining the structure and function of the cell-surface membrane.





A monolayer

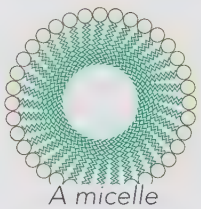
Waxes

Waxes are similar to fats and oils, but their long-chained fatty acids are linked to a long-chained alcohol.

Waxes are very insoluble and form a waterproof layer over some cells.
This stops water getting in, but more importantly for land-living plants and animals it prevents too much water getting out.

Can you think of examples of this property of waxes?

Insects have a waxy cuticle that helps them cut down water loss.
Similarly, many leaves have waxy cuticles to reduce transpiration.



A micelle

Steroids

Structurally, steroids have little in common with other lipids, although they do share some properties.
For instance, they are insoluble in water and soluble in many organic solvents.
Chemically, they have a four-ring structure with various side-chains.
They are of great biological importance, especially as hormones.
Human steroids are synthesised from **cholesterol**.

Many people associate cholesterol with heart disease but in fact it is an important constituent of body cells, especially the cell membrane.
However, ***too much*** cholesterol and saturated fat can produce **atheroma** deposits.
These can reduce the blood flow in arteries and even block them.
High cholesterol diets increase the risk of heart attacks and strokes.
Steroid hormones include **oestrogen**, **progesterone** and **testosterone**.
You can find out more about cholesterol and anabolic steroids on page 24.

A positive test for lipids

The emulsion test for lipids

- add 2 cm^3 of the extract to a clean test-tube
- to this add 5 cm^3 of ethanol
- shake the test-tube to dissolve any lipid in the extract
- add 5 cm^3 of water and shake the mixture
- the mixture turns white as an emulsion, a suspension of fine lipid droplets, forms
- if no lipid is present, the mixture remains clear

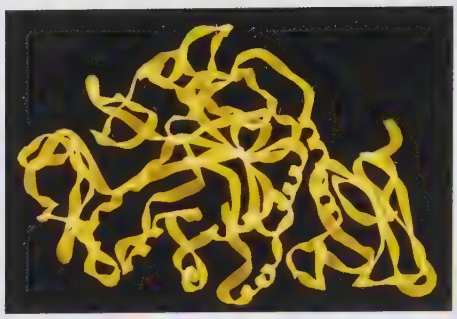
Proteins

Apart from water, proteins make up most of the content of cells.
Proteins perform an enormous range of biological activities.

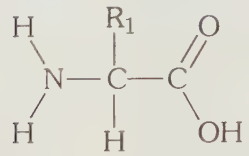
- As enzymes they catalyse chemical reactions.
- Carrier proteins transport materials across cell membranes.
- Antibodies defend the body against disease-causing microbes.
- Structural proteins support cells and tissues.
- Hormones transmit information.
- Transport proteins such as haemoglobin carry oxygen.
- Contractile proteins such as actin and myosin bring about contraction in muscle.

The structure of a protein is closely linked to its function.
For instance, haemoglobin has a different structure to that of an antibody.
Proteins are big compounds with large molecular masses.
The main chemical constituents are carbon, hydrogen and oxygen but, in addition, they ***all*** contain nitrogen and some also have sulfur.

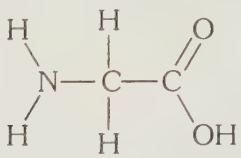
Proteins are polymers composed of sub-units called **amino acids**.
There are only about 20 different types of amino acids but thousands of different proteins, based on how amino acids are joined together in a chain.
The specific function of a protein depends upon its shape.
This is determined by the specific sequence of amino acids in the chain.



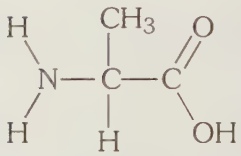
The structure of the enzyme Taka-amylase



basic structure of an amino acid



glycine



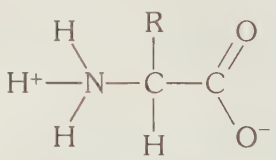
alanine

Amino acids

All amino acids have the same basic structure (see diagram above).
They have an amino ($—NH_2$) group at one end of the molecule and a carboxyl (—COOH) group at the other end.
The R group differs from one amino acid to another.
For example, in glycine the side-chain is a single hydrogen atom, whilst in alanine it is a methyl ($—CH_3$) group.

In solution, the amino end of the molecule can act as a base by accepting hydrogen ions (H^+), so forming a positively charged amino acid ion.
Alternatively, the carboxyl end can act as an acid by giving up hydrogen ions, so producing a negative amino acid ion.

amino group (base) *gains* a hydrogen ion (H^+) and becomes *positively* charged



carboxyl (acid) group*loses* a hydrogen ion (H^+) and becomes *negatively* charged

Molecules such as amino acids, which can act as acids or as bases, are said to be **amphoteric**.
When amino acids form protein chains there is still an amino group at one end and a carboxylic acid group at the other end.
So, like amino acids, proteins can accept or give up hydrogen ions.
This gives a protein the ability to resist pH changes and act as a buffer (see page 8).

The **Biuret test** for proteins involves adding an equal amount of sodium hydroxide solution to the test solution.
A few drops of dilute (0.05%) copper(II) sulfate solution are added and gently mixed.
A purple lilac colouration is a positive result.

The Biuret test gives a lilac colour if positive for proteins

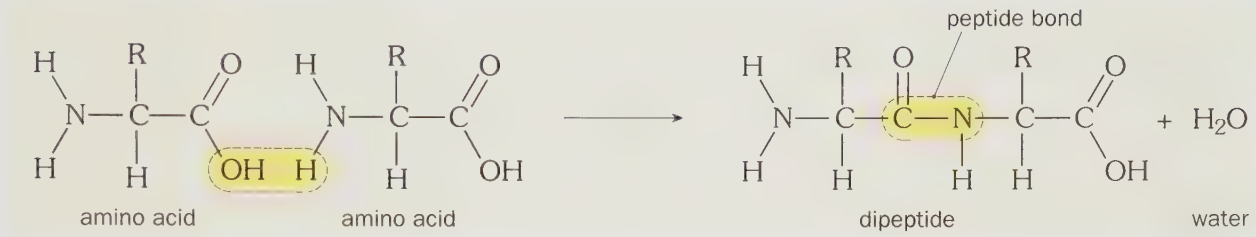
The peptide bond

Amino acids are the sub-units that join together to form polypeptide chains.
Each amino acid is joined to the next one in the chain.
A reaction takes place between the amino group of one amino acid and the carboxyl group of another.
This is a condensation reaction and a molecule of water is lost.
The chemical bond that is formed is called a **peptide bond**.

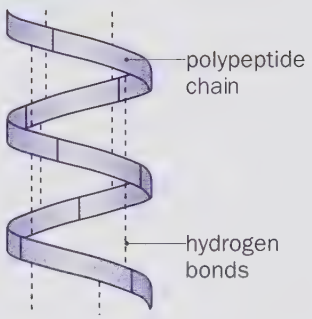
Can you remember other molecules formed by condensation reactions?

Disaccharides, polysaccharides and fats are all condensation products.

Can you see from the diagram how a peptide bond forms between two amino acids to form a **dipeptide**?



A polypeptide contains many hundreds of amino acids.
When a polypeptide bonds with other polypeptides, it forms a protein containing thousands of amino acids.



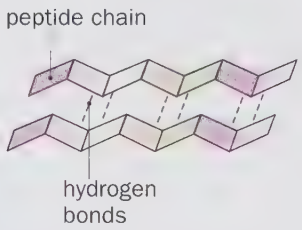
A polypeptide chain wound into an α-helix

Protein structure

The **primary structure** of a protein is the sequence of amino acids in the polypeptide chain.
It determines the eventual shape of the protein and hence its function.
As we have seen, amino acids in the chain are held together by peptide bonds.
For example:

NH_2— leucine—valine—cysteine—arginine—leucine—glutamine—serine—COOH

There are 20 different amino acids, not far off the 26 letters in the alphabet.
Think about all the different words in a dictionary.
This gives you some idea of all the different combinations of amino acids that can build the 100 000 known proteins.



β-sheets of a fibrous protein

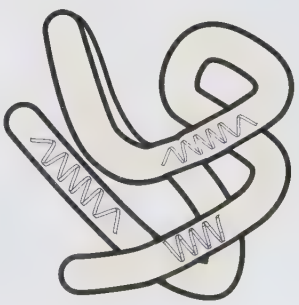
But proteins are three-dimensional molecules.
The amino acid chains do not lie flat like a string of beads on a table.
The chains of amino acids form helices and pleated sheets held together by hydrogen bonds.
This gives the protein its **secondary structure**.

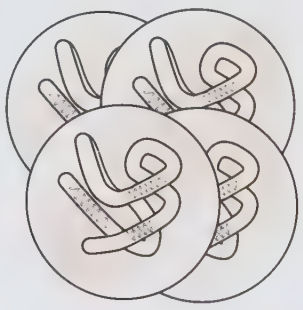
The **tertiary structure** of a protein is the complex three-dimensional shape the molecule takes when the polypeptide helix twists and folds around itself.

How do you think this tertiary structure is held together?

The **quaternary structure** of a protein involves the linking together of a number of polypeptide chains.

Haemoglobin consists of four separate polypeptide chains.

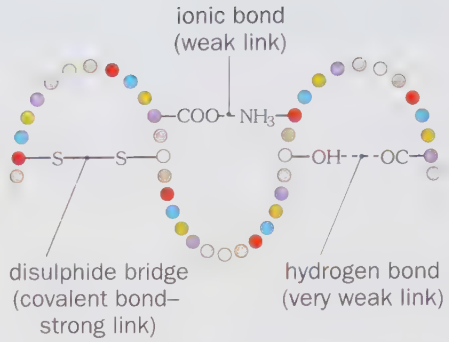

Tertiary structure


Quaternary structure

Protein bonding

The structure of a protein is held together by three types of chemical bond.

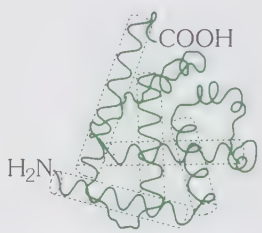
- **Hydrogen bonds** occur between some hydrogen atoms and some oxygen and nitrogen atoms in the polypeptide chain.
  The hydrogen atoms have a small positive charge, and the oxygen and nitrogen atoms have a small negative charge.
  The opposite charges attract to form hydrogen bonds.
  Although these bonds are weak, the large number of them maintains the molecule in a three-dimensional shape.
- **Ionic bonds** occur between any charged groups that are not joined together by a peptide bond.
  These ionic bonds are stronger than hydrogen bonds but they can be broken by changes in pH and temperature.
- Some amino acids such as cysteine and methionine contain sulfur atoms. **Disulfide bonds** can form between the sulfur atoms of amino acids that are close together.
  These disulfide bonds are very strong and contribute to the strength of structural proteins such as keratin and collagen.
- The **hydrophobic effect** helps some proteins to maintain their structure. When globular proteins are in solution, their hydrophobic 'water-hating' groups point inwards, away from the water.



Types of chemical bonds found in a polypeptide

Stability

As you have seen, a protein is held in its three-dimensional shape by hydrogen bonds, ionic bonds and covalent bonds.
Under certain circumstances, such as increasing temperature, the three-dimensional shape can change.



This polypeptide is held together by hydrogen bonds, ionic bonds and disulfide bonds.

An increase in temperature will cause the atoms in the protein molecule to vibrate.
Raising the temperature can cause the atoms to vibrate so violently that the bonds holding the protein together break.
The three-dimensional structure of the protein changes, altering the protein's shape.
These changes affect the tertiary and the quaternary structures.
The primary and secondary structure of the protein is unaffected.

If this loss of shape is permanent, it is called **denaturation**.
Altering the tertiary structure of a protein affects its biological function (as you will see in Chapter 2 on 'Enzymes').
A denatured enzyme is unable to bind with its substrate (the molecule on which it normally acts).

Can you think of anything else that could cause a protein to denature?

Denaturation can also be brought about by extreme changes of pH and by the presence of heavy metal ions and organic solvents.

Heating up an egg denatures the protein albumen so you can't 'un-fry' eggs!

Eddie's feeling the heat, will he ever be the same?

Globular and fibrous proteins

Proteins can be divided into two groups depending on their final three-dimensional structure: **globular** or **fibrous**.

Globular proteins are compact molecules.
The highly twisted polypeptide chains roll up into a ball.

As the protein rolls up, the amino acids with hydrophobic (water-hating) R groups point to the centre of the molecule.
This leaves the amino acids with hydrophilic (water-loving) R groups on the outside.
As a result, globular proteins are water-soluble.

Globular proteins tend to be less stable and are involved in metabolic reactions.
All enzymes are globular proteins, as are antibodies and some hormones.

Haemoglobin is a globular protein involved in metabolism.
It picks up oxygen in the lungs to form oxyhaemoglobin.
Oxyhaemoglobin breaks down (dissociates) in the tissues, giving up its oxygen and reverting to haemoglobin.

Haemoglobin consists of ***four*** polypeptide chains.
Disulfide bridges hold the chains together.
At the centre of each polypeptide chain is an iron-containing group called **haem**.
This is an example of a non-protein **prosthetic group**.
The protein combines with the prosthetic group forming a **conjugated protein**.

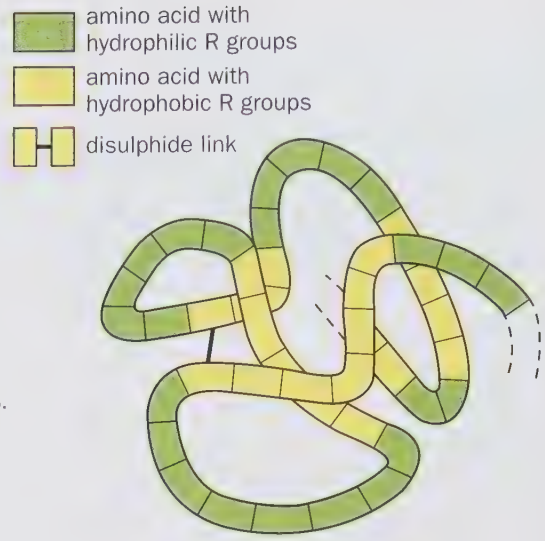
Each haem group contains an iron ion (Fe^{2+}).
Since each iron ion can bind with one molecule of oxygen, each haemoglobin molecule can pick up ***four*** oxygen molecules.

Fibrous proteins consist of polypeptides laid down in parallel chains, linked together to form long fibres or sheets.

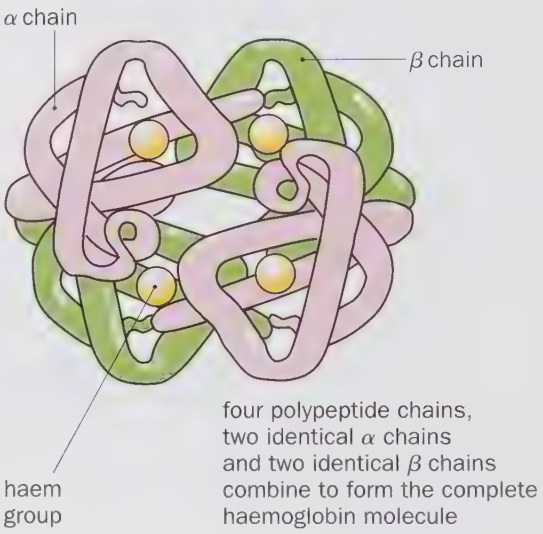
Fibrous proteins are very stable, insoluble and strong.
Collagen is a good example of a fibrous protein.
It provides the tough properties needed in tendons and bone.

A single collagen fibre consists of three polypeptide chains.
Each polypeptide chain is twisted in the form of a helix.
The three polypeptide helices wind around each other like a rope with three strands forming a triple helix.
Hydrogen bonds hold the three strands in place.

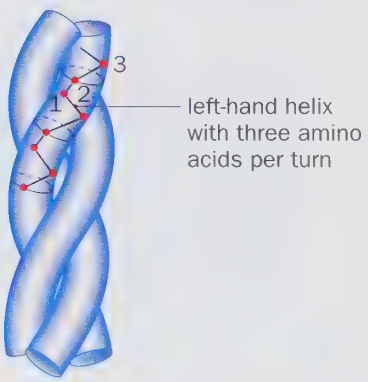
You can find out more about the uses of collagen on page 25.



Part of a globular protein molecule



The haemoglobin molecule



Three polypeptide helices wind together like a rope in this molecule of collagen

Comparison of fibrous proteins and globular proteins	
Fibrous proteins	**Globular proteins**
stable structure	relatively unstable structure
insoluble in water	soluble
strength gives structural functions	metabolic functions
polypeptide chains form long strands	polypeptide chains 'roll up' into spherical shape
e.g. collagen in bone and keratin in hair	e.g. all enzymes, antibodies, some hormones (e.g. insulin), haemoglobin

Inorganic ions

Inorganic ions play many important roles in cell metabolism. They can be divided into two groups:

- **Macronutrients** are only needed in small amounts in living organisms.
- **Micronutrients** are needed in ***minute*** quantities (a few parts per thousand).

The absence of either of these can lead to deficiency diseases.

Milk provides a rich source of calcium

Calcium (Ca^{2+})

- Important in plants because calcium pectate is a major component of the middle lamella. Deficiency can lead to stunted growth.
- The main component in bones and teeth.
- Important role in muscle contraction and blood clotting; deficiency in animals causes rickets and affects blood clotting.

Sodium (Na^{+})

- Important to the cell membrane in maintaining the electrical and osmotic balance.
- Vital in active transport mechanisms.

Potassium (K^{+})

- As with sodium, potassium is important to the functioning of the cell membrane.
- Vital for nerve impulse transmission and other active transport systems.
- Needed for protein synthesis and is an important cofactor in respiration.
- Its absence in plants leads to yellowing of the leaf edges and dead spots.

Chlorosis is a yellowing of the leaves due to magnesium deficiency

Magnesium (Mg^{2+})

- Important constituent of chlorophyll; its absence leads to **chlorosis** (yellow leaves).
- Present in bones and teeth.

Chloride (Cl^{-})

- Important to the electrical and osmotic properties of the cell membrane.
- Needed for carbon dioxide transport in the blood and is a component of stomach acid.

Nitrate (NO_3^{-})

- Important source of nitrogen for many biological molecules including amino acids, proteins, nucleic acids, coenzymes, ATP and some hormones.
- Deficiency in plants causes stunted growth and chlorosis of the leaves.

Spreading pellets of chemical fertiliser

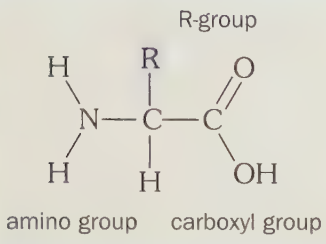
Phosphate (PO_4^{3-})

- Vital in the formation of many important biological molecules such as ATP, nucleic acids and coenzymes.
- Has a role in the phospholipid component of cell membranes.
- Major structural role in bones and teeth.
- Deficiency in plants results in poor root growth and purple younger leaves.

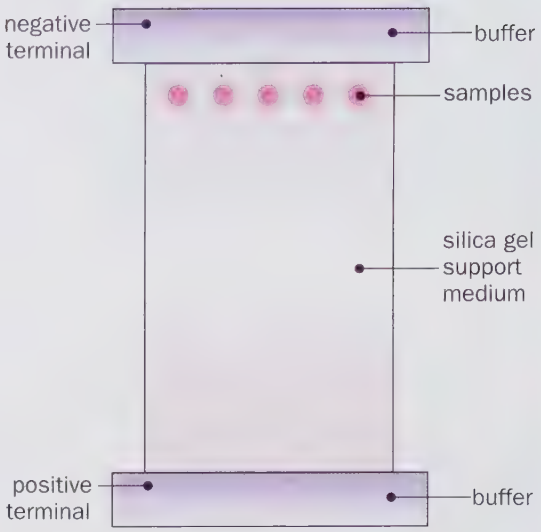
Biology at work: Electrophoresis

Electrophoresis is a technique that allows scientists to separate and identify the proteins present in body fluids such as blood plasma, and also to identify the individual amino acids that make up a protein.

It is similar to chromatography, but whereas chromatography separates chemicals according to their solubility in a given solvent, electrophoresis separates them according to their overall electrical charge. Different proteins have different electrical charges because they are made up from different amino acids.



The basic structure of an amino acid



Gel electrophoresis

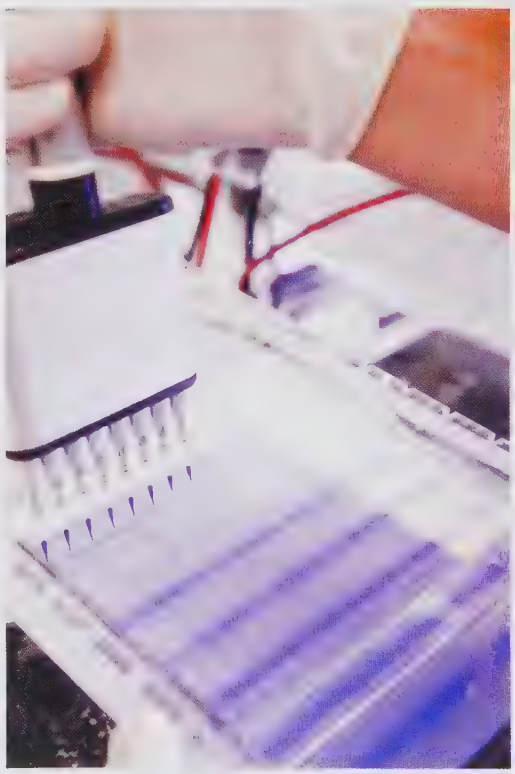
The solution to be analysed is placed on a support medium, for example silica gel, in buffer solution (to keep the pH constant). For most proteins the required pH is typically between 7 and 9.

An electrical current is then passed through the medium, and the proteins move on the medium at different rates according to their overall electrical charge.
As a result of this differential movement, the proteins become separated from each other.

The next step is to reveal the positions of the proteins using a developing agent (essentially a stain).
The distance travelled by each protein can be measured, and this data is compared with standard samples of known proteins in order to identify the particular proteins in the sample.

In order to study the individual amino acids in a protein, the protein must be broken down into its constituent amino acids. This requires protease enzymes, which break the peptide bonds that join one amino acid to another.

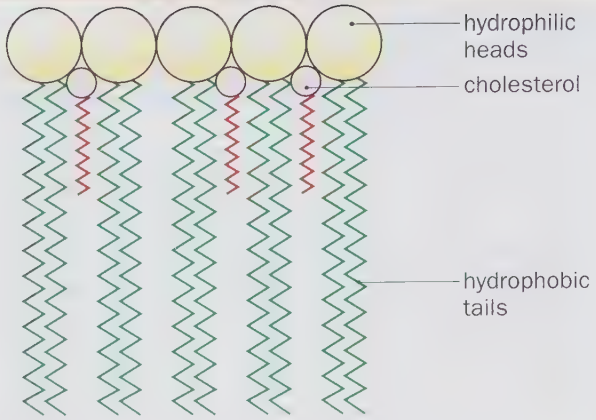
This technique is useful in medicine as it allows doctors to identify abnormalities in blood chemistry, which may lead to the diagnosis of a patient's medical condition.
It is also a significant part of the procedure of genetic fingerprinting, where it is used in the separation of fragments of DNA.



Electrophoresis technique in the laboratory

Biology at work: Cholesterol

Cholesterol is a lipid and although most people associate it with heart disease, it is in fact a normal and important constituent of body cells. It is found in the cell membranes of animal cells, as well as being involved in the formation of hormones and bile salts.

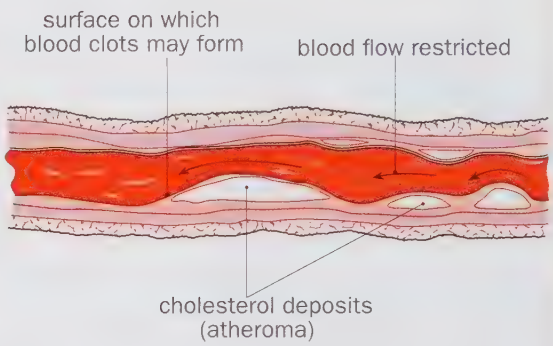


Cholesterol molecules in the cell membrane

Cholesterol is manufactured in the liver, particularly from saturated fats, although a small amount can be absorbed directly from cholesterol-rich foods such as eggs.

Some people have naturally high cholesterol levels irrespective of their diet; this is an inherited condition known as **hypercholesterolaemia**.

Cholesterol, like other lipids, does not dissolve in the blood. It is carried in the circulation attached to a protein. This combination of lipid and protein is called **lipoprotein**. Cholesterol is mainly carried by **low density lipoprotein (LDL)**, and it is in this form that cholesterol can be deposited in arteries. This condition, known as **atherosclerosis**, can reduce the blood flow, and therefore the oxygen supply to vital organs such as the heart.



The development of atherosclerosis due to excessive cholesterol

High density lipoproteins (HDL) carry cholesterol away from the cells and back to the liver. Here it is either broken down or passed out of the body as waste product.

There is strong evidence that high levels of LDL cholesterol are also linked to atherosclerosis, heart attack and stroke. The first step in reducing cholesterol levels is maintaining a healthy balanced diet, together with other lifestyle factors such as not smoking and taking regular exercise. If cholesterol levels still remain high then a GP can prescribe cholesterol-reducing drugs such as statins.

Biology at work: Anabolic steroids

Anabolic steroids are manufactured drugs that mimic the protein building effects of the male sex hormone, testosterone. Research has shown that steroids can pass into the nucleus and directly promote the production of the proteins that make up muscle fibres. Anabolic steroids have very limited medical uses and they should not be confused with corticosteroids which are regularly prescribed to relieve inflammation.

Anabolic steroids are used by athletes to build muscle, and also because they speed up the recovery of muscles after strenuous exercise. This allows the athlete to undertake a more demanding training schedule.

The use of these drugs is banned in international sport mainly because of the unfair advantage one athlete may gain over another. There are also a number of harmful side effects of excessive use of anabolic steroids. These include impotence, sterility, liver damage and heart disease. An additional risk for women is the condition known as **virilisation** – the development of male characteristics such as excessive body hair.


Bodybuilders sometimes take anabolic steroids to increase muscle mass and improve performance

Biology at work: Collagen replacement therapy

Collagen replacement therapy (CRT) is a quick, non-surgical technique, which involves injecting collagen implants to replace the skin's natural collagen. CRT helps to smooth facial lines, scars and deformities on the surface of the skin by supplementing the collagen under the skin. It can also be used to enhance lip shape and definition.

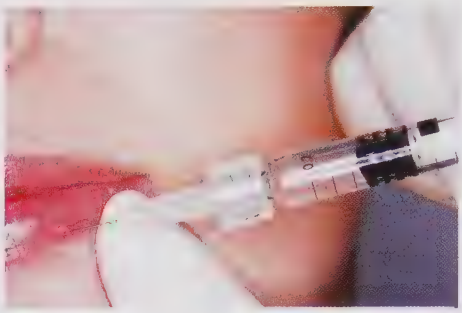
Collagen structure and function

Collagen is a **fibrous** protein found in bone, cartilage, tendons and connective tissue.

Outside the cell, many collagen molecules join to form collagen fibrils. Electron micrographs show that the collagen fibrils are striated, or striped, with alternate light and dark bands due to the displacement of each triple helix.
This adds to the strength of the fibril as well as generating the pattern.

Collagen is also found in connective tissue such as the dermis of the skin. The dermis also contains many elastic fibres (made of the fibrous protein elastin) within its matrix of collagen fibres. One consequence of ageing is that the collagen fibres become compressed whilst the elastin fibres reduce in number. This reduces the skin's elasticity causing wrinkles and lines to develop.

Collagen fibrils



This woman is undergoing CRT on her lips

The development of collagen replacement therapy

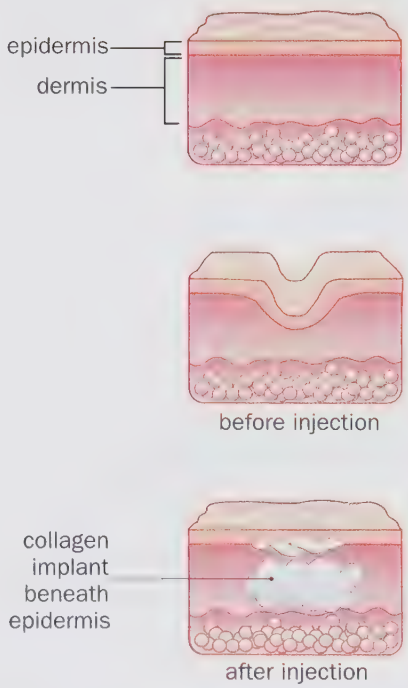
The medical use of collagen dates back to when animal collagen was used in surgical sutures.
Continued research led to the wide use of collagen in a number of applications, including heart valves and as an agent to help stop bleeding during surgery.
A group of American biochemists and medical scientists experimenting with alternative materials for skin grafts pioneered the use of purified bovine collagen to replace lost tissue.
Research by commercial companies has led to the development of various forms of collagen implants. Some can be harvested from a patient's own skin during a previous operation; some from cloned skin from behind a patient's ear; and some can be taken from human donors after their death. These are intended to provide an immediate, visible difference in facial appearance.

How collagen replacement therapy works

The collagen used in CRT is injected to just below the surface of the epidermis, using a fine needle.
Here it is incorporated into the body's own network of collagen fibres.

The natural appearance of the skin may be enhanced as the contour of the support structure is restored.

Like natural collagen, injectable collagen begins to lose its form and will eventually wear down depending on age, skin condition, and amount of sun damage. Full correction is maintained only with ongoing treatment.
There are some risks associated with CRT and all patients undergo a skin allergy test four weeks before treatment.
In 3% of cases patients prove to be allergic to bovine collagen.



Young, ageing and treated skin

Summary

- Carbon atoms bond strongly to each other forming chains and rings.
- Carbohydrates include monosaccharides, disaccharides and polysaccharides. They can act as an energy source or provide structural support.
- Monosaccharides include glucose, fructose and galactose. They have the same chemical formula but different chemical structures – we call them isomers.
- Monosaccharides can become linked together by a glycosidic bond to form disaccharides such as maltose, sucrose and lactose, and polysaccharides, such as starch and cellulose.
- Polysaccharides can perform structural roles, for example cellulose in plant cells, and energy storage roles, for example starch in plants and glycogen in animals.
- Lipids are composed of carbon, hydrogen and a little oxygen. Most are non-polar and insoluble in water.
- Lipids are used as energy stores, for insulation and protection, and in cell-membrane structure. They include fats, oils, phospholipids, waxes and steroids.
- Fats and oils are condensation products (esters) of fatty acids and glycerol.
- Fatty acids vary in the length of their hydrocarbon tails, and whether they are saturated or unsaturated.
- Phospholipids are similar to triglycerides but a phosphate replaces one fatty acid. They form monolayers in water and are vital to the structure of cell membranes.
- Proteins are condensation polymers of up to 20 different amino acids. Proteins have a vast variety of functions as enzymes, hormones, antibodies and as transport and structural proteins.
- All amino acids have an amino group and a carboxyl group but differ in their R groups. Amino acids are linked by peptide bonds as a result of condensation reactions.
- Every protein has a different amino acid sequence, giving it a primary structure. The secondary structure is the arrangement of the polypeptide chain into a helix or pleated sheet. The tertiary structure involves the folding of the secondary structure to give a three-dimensional shape. The quaternary structure involves the association of two or more polypeptide chains to give a protein.
- Fibrous proteins such as collagen, keratin and elastin form tough fibres that give support. Globular proteins such as enzymes, antibodies and some hormones are involved in cellular reactions.
- Water is a fundamental biological molecule. It is an important solvent and is involved in biochemical reactions and in temperature regulation.

Questions

1 Simple carbohydrate molecules can be written as $(CH_2O)_n$.

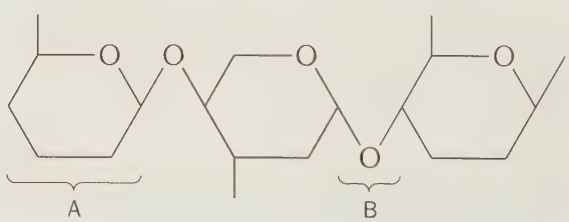
a) What name is given to the carbohydrate in which n is
   i) 6?
   ii) 5?
   iii) 3?

b) State two different functions of carbohydrates in living organisms.

c) Simple carbohydrates can be combined to form disaccharides and polysaccharides. What else is produced in these reactions?

d) i) Name the reagent used to test for reducing sugars.
   ii) Describe the result if this test were to be positive.
   iii) Name a sugar that would ***not*** give a positive result with this test.

2 The diagram shows part of a cellulose molecule.



a) Name the sub-unit labelled A on the diagram.
b) Name the reaction that produced the bond labelled B.
c) What is the name of bond B?
d) Explain how the structure of the cellulose molecule is related to its role as a component of plant cell walls.
e) Name two commercial uses of cellulose.

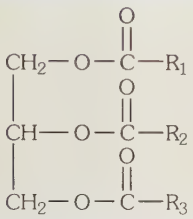
3 a) Copy and complete the table by ticking (✓) which property applies to each biological molecule.

Property	Biochemical compound				
	Monosaccharide	Starch	Cellulose	Lipid	Protein
is a polymer					
contains nitrogen					

b) The diagram shows a small polypeptide with eight amino acids *s* to *z*.

i) Write down the formula of the chemical group that would appear in the box.
ii) Name the type of reaction by which amino acids are joined together.
iii) Name the reagent or reagents used to test for the presence of a protein.

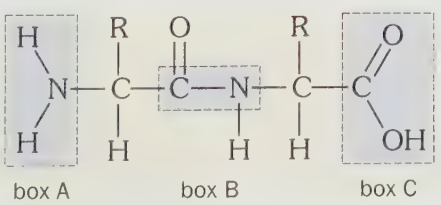
4 Look at the diagram of the triglyceride.



a) Name the two different types of molecule that make up a triglyceride.
b) What is the name of the bonds that form between them?
c) What is the name of the process by which these bonds can be broken?
d) Look at the data in the table. Use the data to suggest possible roles of fats in the metabolism of animals.

	Heat produced in calorimeter (kJ)	Water produced on oxidation (g)
1 g protein	23.4	0.41
1 g carbohydrate	17.6	0.55
1 g fat	38.9	1.07

5 The diagram shows a dipeptide.



a) Draw a diagram to show the two molecules produced if this dipeptide is hydrolysed.
b) What is the name of the chemical group shown in box A?
c) What is the name of the chemical group shown in box C?
d) What is the name of the chemical bond shown in box B?
e) Name two elements, other than carbon, hydrogen and oxygen, that may be present in the groups R.

6 Starch, cellulose, phospholipids and proteins are all macromolecules.
a) Which of these molecules is
i) ***not*** a polymer?
ii) ***not*** found in a chloroplast?
b) Give one chemical element present in all proteins but not present in starch, cellulose and phospholipids.
c) Explain how two features of a starch molecule make it a good storage carbohydrate.

7 The table compares monosaccharides and amino acids. Copy and complete the table by ticking (✓) each statement that you think is correct and putting a cross (✗) where you think the statement is incorrect.

Statement	Monosaccharides	Amino acids
always contain nitrogen		
may be polymerised into macromolecules		
released by complete hydrolysis of nucleic acids		
insoluble in water		
may be linked by glycosidic bonds		
released by complete hydrolysis of cellulose		
always contain carbon, hydrogen and oxygen		

8 Match each level of protein structure with the correct description:
a) primary structure
b) secondary structure
c) tertiary structure
d) quaternary structure
i) the twisting of the amino acid chain into a helix held together with hydrogen bonds,
ii) the association of a number of polypeptide chains,
iii) the sequence of amino acids in the polypeptide chain,
iv) the folding of the polypeptide into a complex three-dimensional shape.

9 a) Name two biological molecules that contain calcium and give the function of each.
b) Name two biological molecules that contain iron and give the function of each.
c) Name one biological molecule that contains magnesium and give its function.

2 Enzymes

Have you ever wondered how they make chocolates with a soft-centre?
How can you pour liquid chocolate over a liquid centre?
Enzymes provide the answer.
The liquid chocolate is poured over a solid centre of polysaccharide.
Then, after the chocolate has set, an enzyme starts to break down the polysaccharide to a runny, sugary centre.

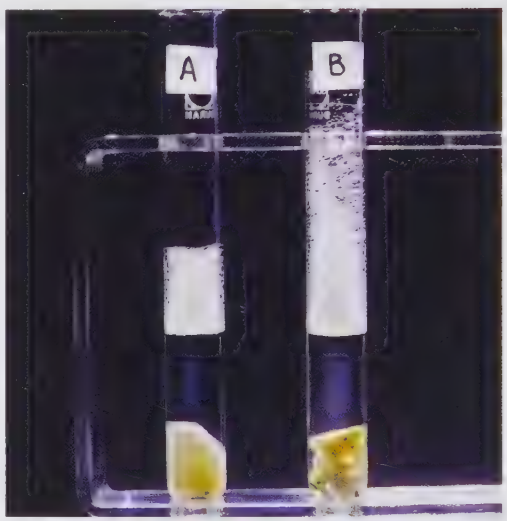
Enzymes are the molecules that control the reactions in your body.
They are the caretakers of your cells and tissues.
They make sure that your body chemistry is kept in good shape.

Each enzyme has a particular job to do. There is:

- an enzyme that clears the fat out of your bloodstream after dinner,
- an enzyme that can detect the level of glucose in your blood,
- an enzyme produced in your liver that stops nerve impulses constantly passing from one nerve cell to the next.

Thousands of chemical reactions take place in your body every second.
Together these reactions make up your **metabolism**.
Enzymes control your metabolism by determining when and how reactions take place.

$$\underset{\substack{\text{substrate}\\(\text{the substance that the enzyme works on})}}{A} \xrightarrow{\text{enzyme}} B + \underset{\substack{\text{products}\\(\text{the substances produced by the reaction})}}{C}$$



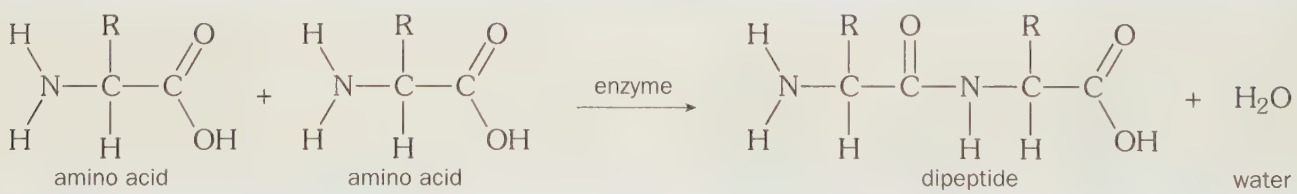
More oxygen bubbles are released in test-tube B than A. The potato is finely chopped in B releasing more catalase

Enzymes are **catalysts** that speed up the rate of metabolic reactions.
These reactions would still take place without enzymes – but in years rather than milliseconds!
There are two types of metabolic reaction:

- Reactions where larger molecules are broken down into smaller ones.
  One example is hydrogen peroxide being broken down to water and oxygen by the enzyme ***catalase***.

$$\underset{\text{hydrogen peroxide}}{2H_2O_2} \xrightarrow{\text{catalase}} \underset{\text{water}}{2H_2O} + \underset{\text{oxygen}}{O_2}$$

- Reactions where small molecules are built up into larger, more complex ones.
  For example, two amino acids can join together by **condensation** to form a dipeptide:



Which of the following reactions do you think break molecules down and which build molecules up?

- a glycogen molecule formed from glucose molecules,
- a protein formed from amino acids,
- the digestion of starch to maltose,
- urea formed from ammonia and carbon dioxide.

The chemical structure of enzymes

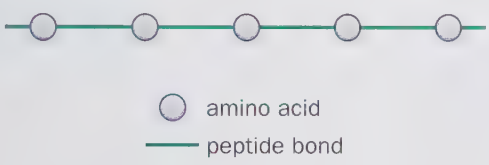
All enzymes are **globular** proteins.
As you already know, proteins consist of long chains of amino acids.
In a globular protein, the amino acid chain is folded and wound into a spherical or globular shape.

Can you see how this protein is held in its globular shape?

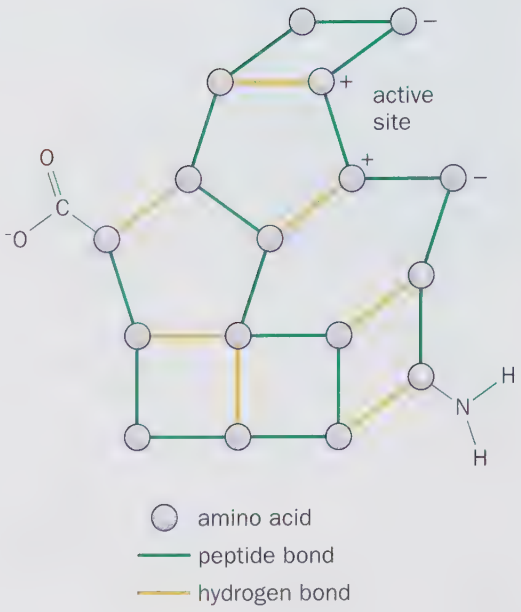
Hydrogen bonds, ionic bonds and disulfide bridges hold the amino acid chain in its distinct three-dimensional shape.
This shape is the tertiary structure of the protein.

Do you remember what the primary, secondary and tertiary structures of a protein are?

The **primary structure** of a protein is the actual sequence of amino acids in the chain.
There are 20 naturally occurring amino acids.
Since there are thousands of amino acids in one protein chain, the number of possible permutations is vast.
Each type of enzyme has its own sequence of amino acids, different from any other type of enzyme.



Primary structure of a protein



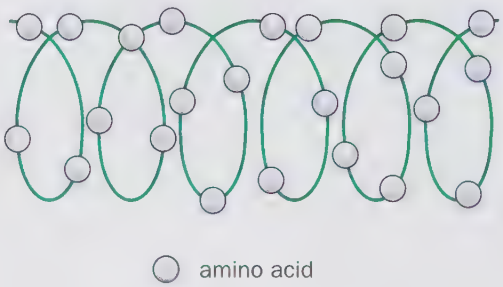
The **secondary structure** of a protein is the way in which the amino acid chain is organised.
In the globular protein shown right, the secondary structure involves twisting the amino acid chain into a spiral or helix.

The **tertiary structure** of a globular protein involves the helix folding back on itself to give the molecule its own complex three-dimensional shape.
The enzyme's tertiary structure is very important, since it gives the enzyme many of its properties.
The exact three-dimensional shape enables an enzyme to form receptor sites for its substrates.
The enzyme molecule is held in its tertiary form by hydrogen bonds, ionic bonds and disulfide bridges.

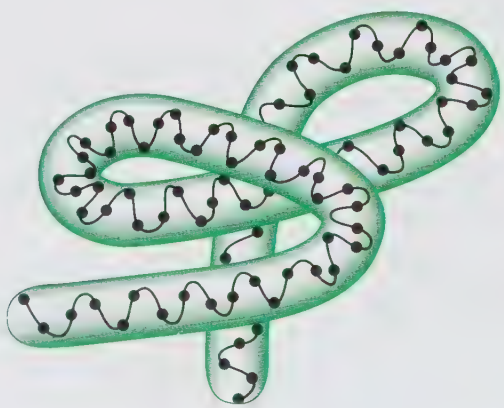
The shape of the protein also depends upon its environment, that is, its immediate surroundings.
So changes in conditions, such as pH and temperature, can cause the three-dimensional shape of the protein to change.

Before going on to see how an enzyme's structure can be linked to its properties, see if you can answer these questions:

What is the primary structure of an enzyme?
What is the secondary structure of an enzyme?
Why is the tertiary structure so important to an enzyme?

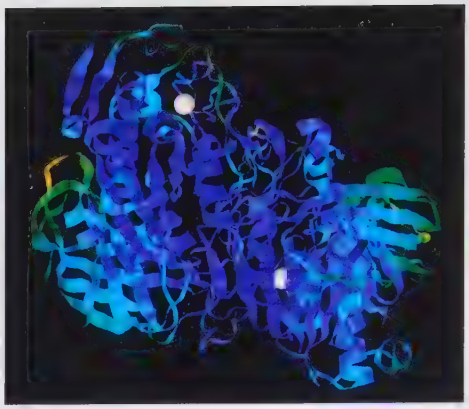


Secondary structure of a protein



Tertiary structure of a protein

Properties of enzymes



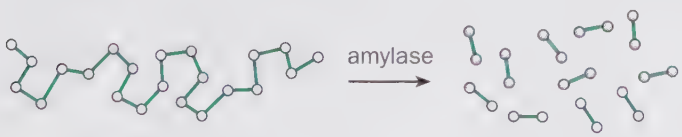
Computer simulation of an enzyme

As you know, enzymes are catalysts. They speed up chemical reactions. But they also have other properties that result from their complex globular shape.

- Enzymes are ***specific***.
  This means that each enzyme will catalyse only ***one*** particular reaction.
- Enzymes are not used up in the reactions that they catalyse, so they can be used again and again.
- When enzymes react, they combine with their substrates to form **enzyme-substrate complexes**.
  When the reaction has taken place, the products are released, leaving the enzyme as it was at the start.
- Only a small amount of enzyme is needed to catalyse a lot of substrate.
- Enzymes are fast acting. We say that they have a high **turnover number**. This means that they can convert many molecules of substrate per unit time.
- Enzymes are affected by changes in temperature and pH.
- Many enzymes are only able to work if another chemical called a **cofactor** is present.
- Enzyme-catalysed reactions can be slowed down or stopped altogether by chemicals called **inhibitors**.
- Some enzymes catalyse intracellular (inside cells) reactions, for example catalase, others catalyse extracellular (outside cells) reactions, for example amylase and trypsin.

Specificity

Each enzyme can catalyse only one particular reaction, because an enzyme can only react with a specific substrate molecule. For instance, amylase can only catalyse the hydrolysis of starch into smaller disaccharide maltose molecules. This is because amylase can only react with starch molecules.



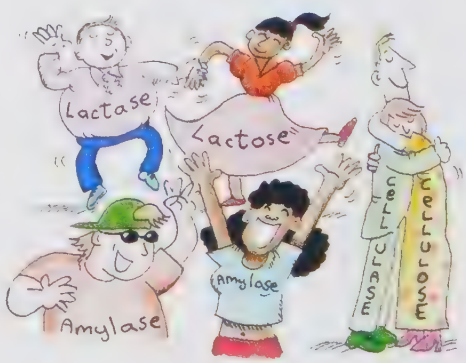
The name of an enzyme comes from the particular substrate on which it acts. In fact, the name of the enzyme gives you a clue about the substrate it reacts with.

- ***Lact***ase acts upon the milk sugar ***lact***ose.
- ***Amyl***ase works on starch, or to give it its proper name ***amyl***ose.
- ***Cellul***ase, made by microbes in the herbivore gut, breaks down ***cellul***ose.

Which substrate do you think each of these enzymes acts upon:

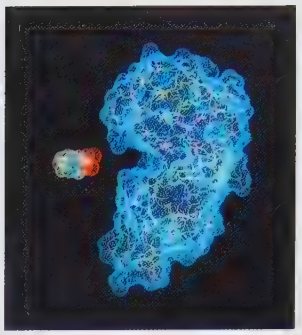
- sucrase,
- lipase,
- protease?

The explanation for the specificity of an enzyme lies in the tertiary structure of the protein molecule, that is, its three-dimensional shape.
As you will see, the enzyme is thought to provide receptor sites where only a certain substrate will fit so that the chemical reaction takes place.



'. . . You're the one that I want'

The lock and key theory



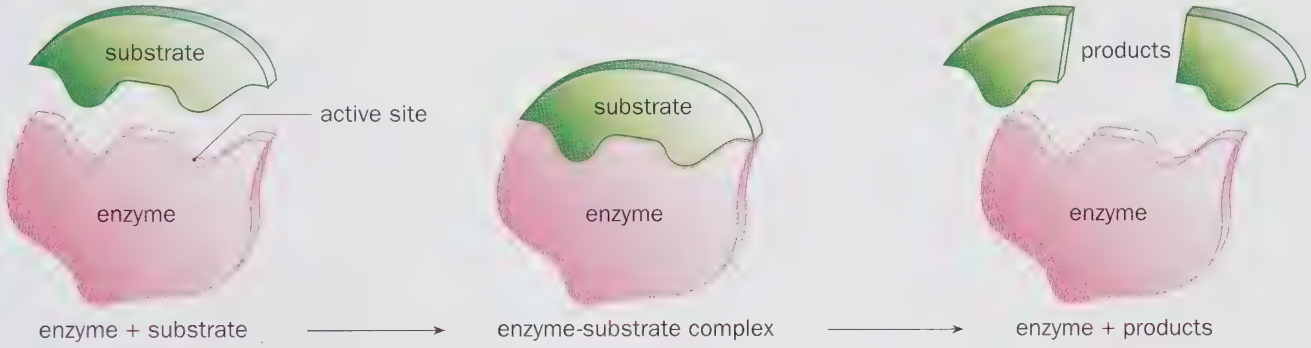
This computer simulation shows the substrate entering the active site

This theory was put forward to try to explain why enzymes are specific and will only work on particular substrates.
You know that enzymes have specific three-dimensional shapes.
They are large molecules, usually much bigger than their substrates.
But only a relatively small part of the enzyme actually comes into contact with the substrate.
This area of the enzyme is called its **active site**.
The active site has a shape into which part of the substrate fits because of its shape.
Only 3–12 amino acids make up the active site, but its shape is an exact fit for the substrate.

The shape of the enzyme's active site and the shape of the substrate are said to be **complementary**, that is they fit each other.
It is a common mistake in examinations to say that the enzyme's active site and the substrate that fits it have the same shape.

The substrate molecule is like the key that fits the enzyme's lock.
The two molecules form a temporary structure called an enzyme-substrate complex.

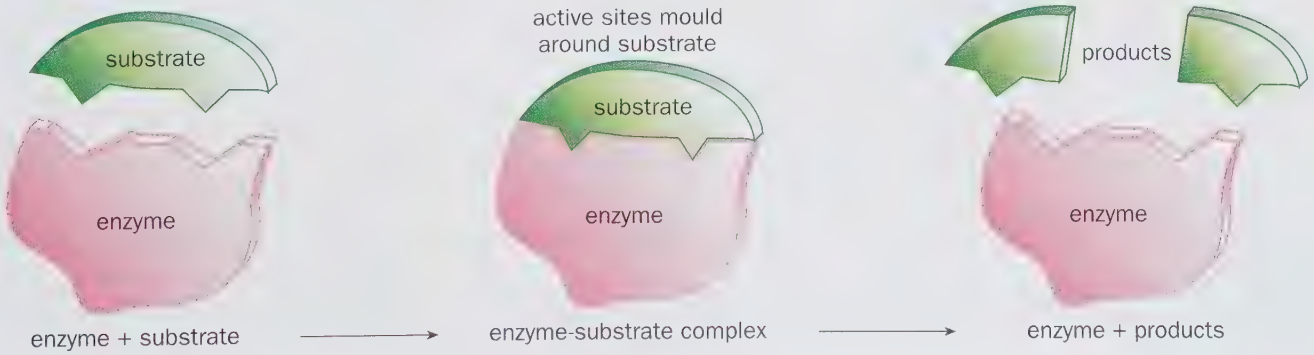
The reaction takes place at the active site and this is where the products are formed.
Since the products have a different shape from the substrate, they no longer fit the active site and are repelled.
The active site is then free to react with more substrate.



The lock and key theory helps to explain many of the properties of enzymes.
For instance, an enzyme is specific because only a particular shape of substrate will fit its active site.

Induced fit theory

This is an updated version of the lock and key theory.
It suggests that the active site of the enzyme may not ***exactly*** correspond to the shape of the substrate.
The active site has a more flexible shape and is able to mould itself around the substrate.
Only when it binds closely to the substrate does the active site catalyse the reaction.
As in the lock and key theory, the products no longer fit the active site and are repelled.
The enzyme reverts to its 'relaxed' state and is able to attach to more substrate.

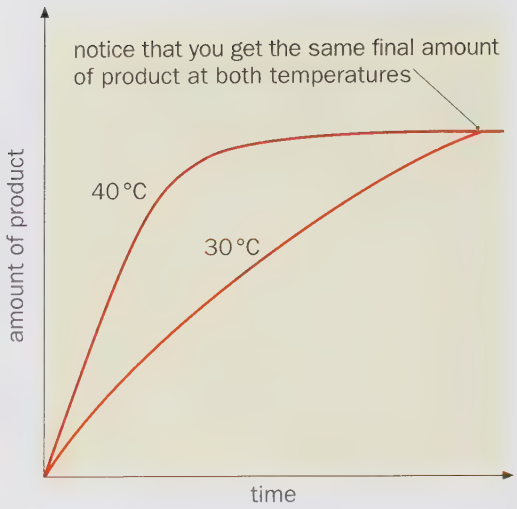


Activation energy

Do you remember about rates of reaction from your earlier studies?
Particles in gases and liquids are continually moving.
If the particles bump into each other, then a chemical reaction may occur.

How would you speed up a chemical reaction?

Heating the particles increases their kinetic energy and so they move about more quickly.
This means that there is a greater chance of collisions and the rate of reaction increases.

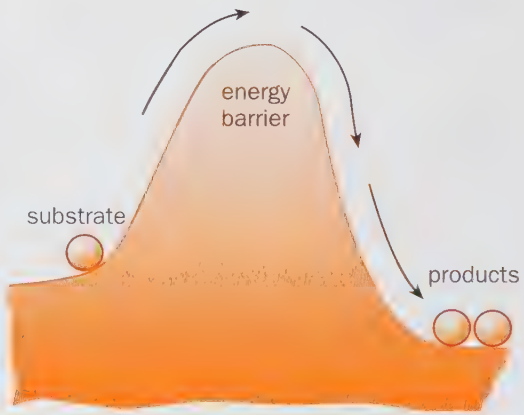


Reactions need energy to start them off.

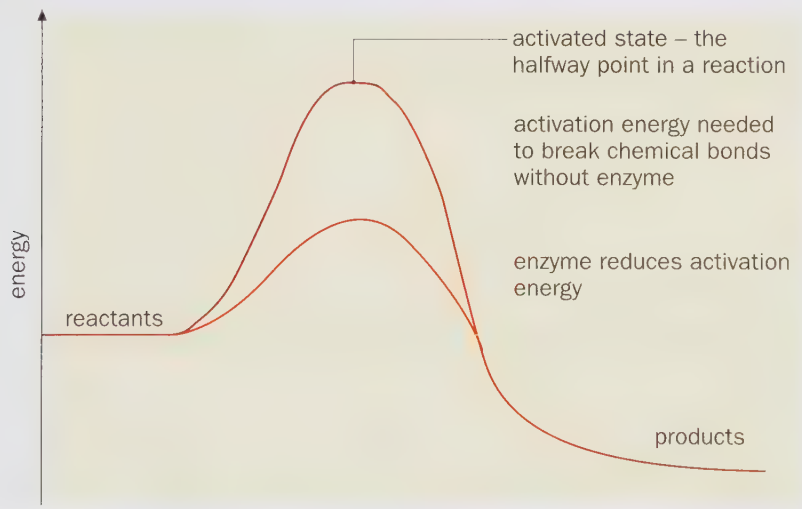
The energy needed to start a chemical reaction is called the activation energy.

Another way of thinking about it is to picture there is an energy barrier to get over before the reaction can get underway.
It's a bit like pushing a boulder over the top of a hill.
First, you have to supply some energy to get it to the top and over before it can roll down the other side.

In the same way chemical reactions need activation energy to start them off.
This energy is needed to break the existing chemical bonds inside molecules.
Activation energy can be supplied in the form of heat.
But why would this be no good for the reactions that take place in your body cells?



Enzymes lower the activation energy needed to make chemical reactions start.
This means that we don't need extra heat for the reactions in our body cells.
The reactions can take place at lower temperatures.



By ***lowering*** the activation energy of a reaction, the enzyme provides a different pathway for the reaction to follow.
By lowering the activation energy, enzymes reduce the input of energy needed, and allow reactions to take place at the lower temperatures found in the cells of organisms.

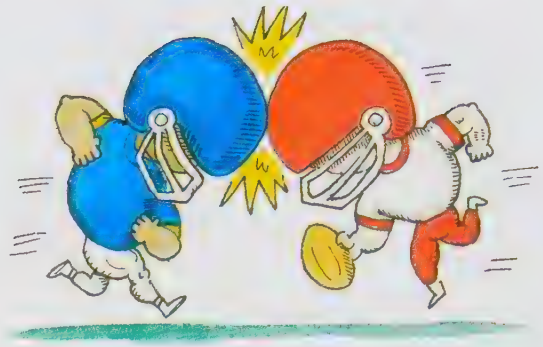
Look back to page 30.
What other factors affect the rate of an enzyme-controlled reaction?
The main factors affecting enzyme activity are shown in the box to the right.

- **temperature**
- **pH**
- **enzyme concentration**
- **substrate concentration**
- **the presence of cofactors**
- **the presence of inhibitors**

Temperature

Heating increases the rate of most chemical reactions.
As you know, heating gives molecules greater kinetic energy and they move around more quickly.

This means there is a greater chance of the molecules colliding and the rate of reaction increases.
Also, since the particles are moving faster, their impact will be greater when they collide, making a reaction more likely.
So raising the temperature makes it more likely that the collisions will result in a reaction taking place.



Particles must collide before they can react!

Increasing the temperature of an enzyme-controlled reaction brings about an increase in rate of reaction, but only up to a point.
For some enzymes, increasing the temperature to about 40 °C brings about a corresponding increase in the rate of reaction.
This is due to the increased kinetic energy of both the substrate and the enzyme molecules.

Maths skills

Over this sort of range, the effect of temperature T on the rate of a reaction can be expressed by the temperature coefficient Q_{10}:

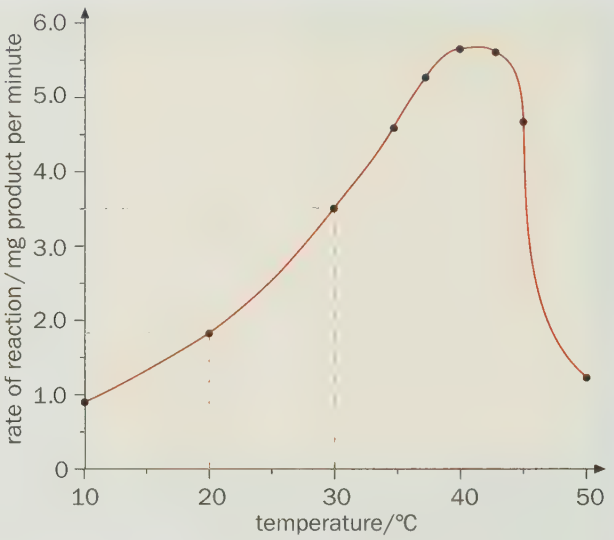
$$Q_{10} = \frac{\text{rate of reaction at T + 10 °C}}{\text{rate of reaction at T}}$$

If we choose T as 20 °C, and taking values from the graph:

$$Q_{10} = \frac{\text{rate of reaction at 30 °C}}{\text{rate of reaction at 20 °C}} = \frac{3.5}{1.75} = 2$$

The rate of reaction ***doubles*** for each 10 °C rise in temperature.

But this does not go on indefinitely.
As you can see from the graph, the rate of enzyme-catalysed reactions reaches a peak at a particular temperature.
This is the **optimum temperature** for the reaction.



What is the optimum temperature for the enzyme in the graph?

Any increase in temperature also causes the atoms making up the enzyme molecule to vibrate more.
Eventually this vibrating causes the breaking of hydrogen bonds and the other bonds that hold the enzyme molecule in its tertiary structure (its specific shape).

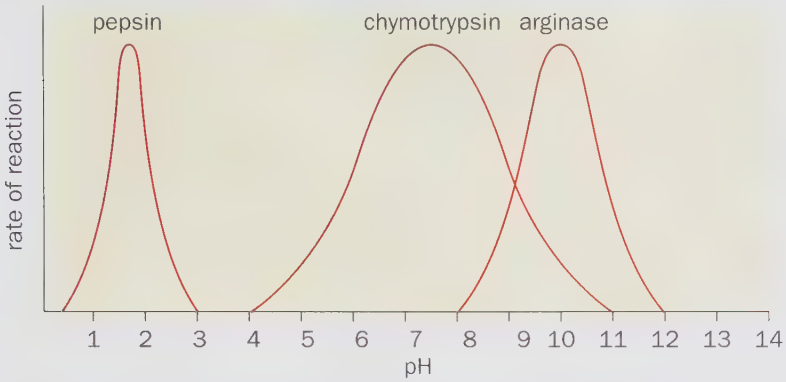
This causes a change in the tertiary structure of the enzyme.
Its three-dimensional shape alters, including the active site, which will no longer fit the substrate molecule.
We say that the enzyme is **denatured**.
This is a ***permanent*** change that cannot be reversed by cooling.
The enzyme has lost its activity since it is no longer able to form enzyme-substrate complexes.

Cooling below the optimum temperature inactivates the enzyme but does not denature it.
The enzyme can work faster again when it is warmed up.

pH (hydrogen ion concentration)

Most enzymes have an optimum pH at which the rate of reaction is fastest.

Look at the graphs.



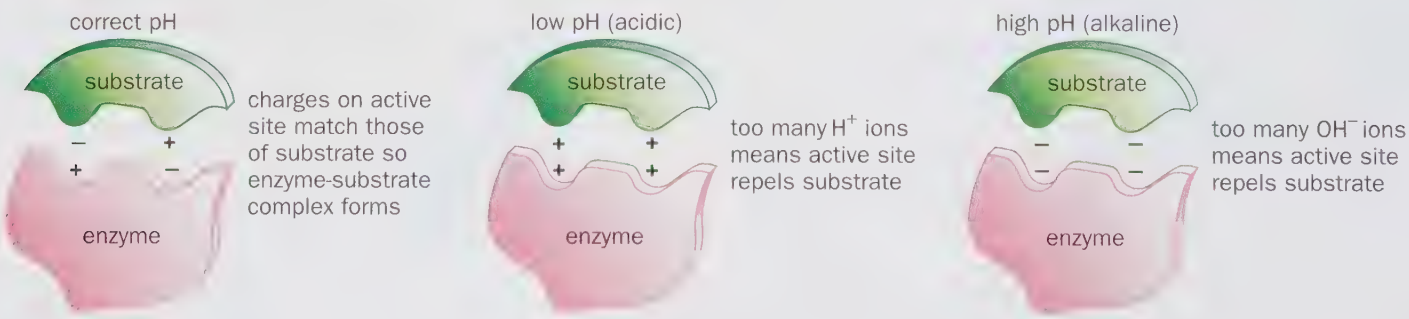
What do you think is the optimum pH for each enzyme?
Why do you think that pepsin and chymotrypsin are active in different regions of the gut?
Which enzyme is active over

a) the narrowest range of pH and,
b) the widest range of pH?

As you have seen, the three-dimensional shape of an enzyme is vital if it is to function properly.
Many of the chemical bonds holding this tertiary structure in place are hydrogen bonds.
Small changes in pH can affect the rate of reaction without denaturing the enzyme.
However, at the ***extremes*** of its pH range an enzyme can become unstable and denature.

Acidity and alkalinity can affect the active site of an enzyme.
Free hydrogen ions (H^+) or hydroxyl ions (OH^-) can affect the charges on the amino acid side-chains of the enzyme's active site.
This will affect the hydrogen bonding and so change the three-dimensional shape of the enzyme and the shape of its active site.
The substrate will no longer fit the active site, the enzyme loses its activity and the rate of reaction falls.

If the active site becomes flooded with hydrogen ions or hydroxyl ions, it can prevent the enzyme and the substrate from fitting together.
Look at the diagrams below to see how this could happen.



If the enzyme and the substrate both have the ***same*** charges, they repel each other and an enzyme-substrate complex is not formed.

Enzyme concentration

As you know, the active site of an enzyme can be used again and again.
So only a small amount of enzyme is needed to catalyse a lot of substrate, because after the reaction takes place at the enzyme's active site the products are released, and the active site is free to accept more substrate.

Enzyme	Turnover number
carbonic anhydrase	36 000 000
catalase	5 600 000
β-galactosidase	12 000
chymotrypsin	6000
lysozyme	60

(Source: Biochemical Society Guidance Notes 3, *Enzymes and their role in biotechnology*.)

The number of substrate molecules that one molecule of enzyme can turn into products in 1 minute is called the turnover number.
You can see from the table that turnover numbers can vary a lot.

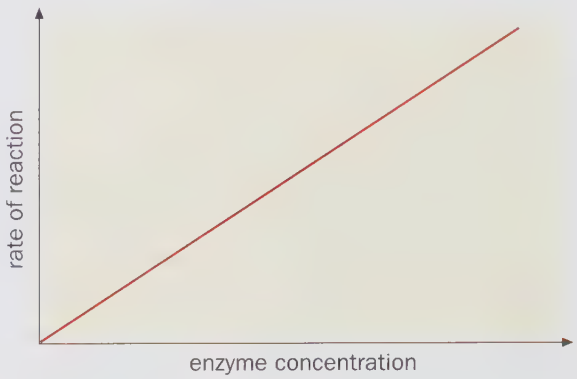
Carbonic anhydrase is found in red blood cells.
It catalyses the reaction in which carbon dioxide dissolves in water.

$$CO_2 + H_2O \xrightarrow{\text{carbonic anhydrase}} H^+ + HCO_3^-$$

This reaction does happen naturally, but it is ten million times faster if the enzyme is present.

Hydrogen peroxide is a common waste product of reactions in cells.
The only problem is that hydrogen peroxide is poisonous.

How many molecules of hydrogen peroxide can one molecule of catalase split in 1 minute?
Why do you think that catalase has such a high turnover number?



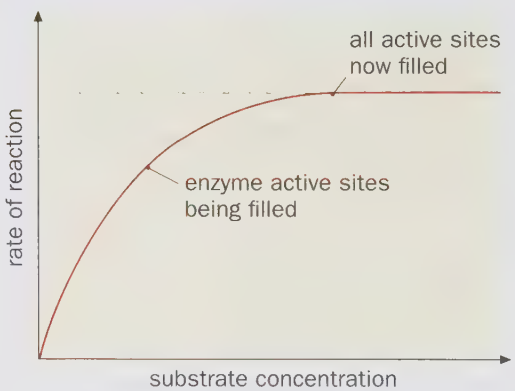
Provided conditions such as temperature and pH are suitable and there is an excess of substrate, then the rate of reaction will be *directly proportional* to the enzyme concentration. So, increasing the enzyme concentration will increase the rate of reaction.

Substrate concentration

If the amount of enzyme stays the same, the rate of reaction will increase with an increase in substrate concentration, ***up to a point***.

Look at the graph.

Are many enzyme active sites being filled at low substrate concentration?
What happens to the active sites as the substrate concentration increases?
What happens to the rate of reaction when ***all*** the active sites are filled?



When the enzyme's active sites are all working as quickly as they can, adding more substrate brings about no further increase in the rate of reaction.
The enzyme is working flat out.

Scientists working in industry often need to know how much substrate to add to an enzyme-catalysed reaction.
Why do you think this is?

Well, if they add too little substrate, then less of the product is produced, and if they add more substrate than is needed, then the costs are higher.

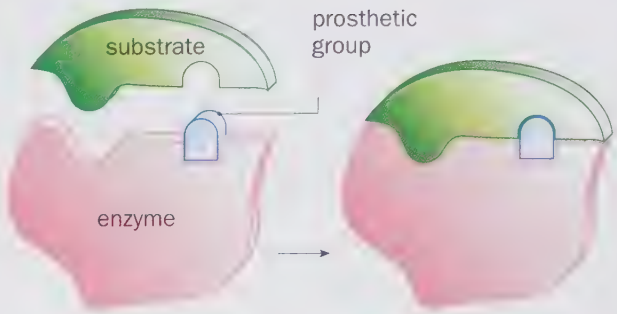
Cofactors

Some enzymes need the presence of another molecule if they are to work.
These molecules are called cofactors.
Cofactors are non-protein molecules.
They modify the chemical structure of the enzyme in some way so that it can function more effectively.
Amylase catalyses the breakdown of starch to maltose.
The enzyme will only function properly if chloride (Cl^-) ions are present.
Without these ions, amylase cannot catalyse the reaction.

There are three types of cofactors:

- prosthetic groups,
- coenzymes,
- activators.

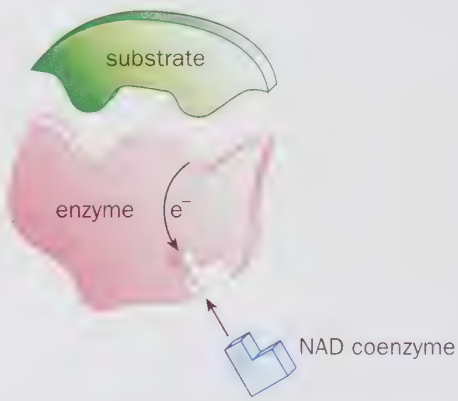
Prosthetic groups are molecules that form a ***permanent*** attachment to the enzyme.
Haemoglobin contains the prosthetic group **haem**, which contains iron and bonds permanently to the protein molecule.
Haem enables the haemoglobin molecule to carry oxygen.
It is also present as the prosthetic group in the enzyme catalase.



enzyme-substrate complex

The enzyme carbonic anhydrase contains a zinc-based (Zn^{2+}) prosthetic group.
This enzyme is a vital component of red blood cells, where it is involved in catalysing the combination of water and carbon dioxide to form carbonic acid.
This enables carbon dioxide to be transported in the blood.

Coenzymes are small, non-protein organic molecules.
Unlike prosthetic groups, they are not permanently attached to the enzyme.
Coenzymes help enzymes and substrates to bond with each other.
The enzyme can only function if the coenzyme is present.



NAD is a coenzyme that works by removing an electron from the active site. The substrate is then able to engage

Many coenzymes are derived from vitamins. An example is the coenzyme ***NAD***, formed from the vitamin ***nicotinic acid***.
NAD is the coenzyme for a number of **dehydrogenase** enzymes.
It acts as a hydrogen acceptor.

Why do you think people develop deficiency diseases if some vitamins are lacking in their diet?

Activators are inorganic metal ions. Examples include magnesium (Mg^{2+}), iron (Fe^{2+}), and calcium (Ca^{2+}).
Activators form a temporary attachment to the enzyme and change its active site so that the reaction is more likely to take place.
For example, the synthesis of any protein in your body cannot take place without magnesium as a cofactor.
The reaction does not take place without it.
Calcium is needed for muscle contraction and nerve impulse transmission.
Most metal activators are obtained from our diet.


Fruit and vegetables are a rich source of vitamins and minerals

Why do you think metal activators are often found in dietary supplements?

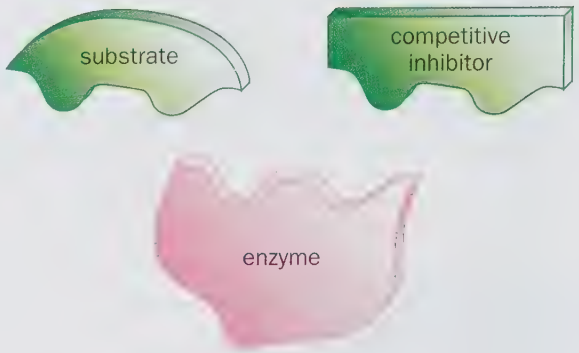
Inhibitors

An inhibitor is a substance that can slow down or stop a reaction. The inhibitor combines with the enzyme and stops it from attaching to the substrate.
Inhibitors are either **reversible** or **non-reversible**.

The effects of reversible inhibitors are temporary, and when the inhibitor is removed the enzyme regains its full activity.

There are two types of reversible inhibitors.

A **competitive inhibitor** has a structure similar to that of the substrate.
The competitive inhibitor competes with the substrate for the active site of an enzyme. This means that it has a shape that allows it to fit into the active site of the enzyme instead of the substrate.
This prevents the formation of enzyme-substrate complexes and the rate of reaction decreases.

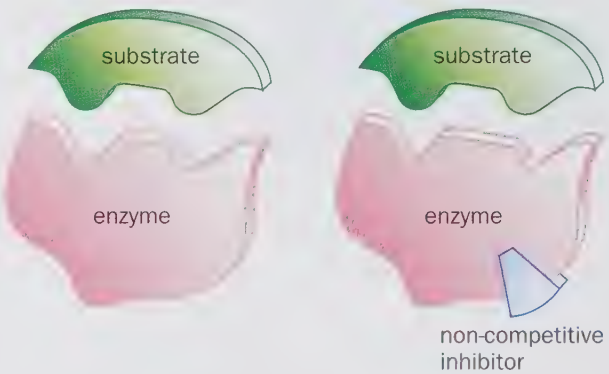


A competitive inhibitor can fit into the active site and exclude the substrate

In respiration, the enzyme ***succinate dehydrogenase*** removes hydrogen from its substrate *succinate*.
But the enzyme's activity can be inhibited if ***malonate*** is present.
Malonate ***competes*** with succinate for the active site on succinate dehydrogenase.

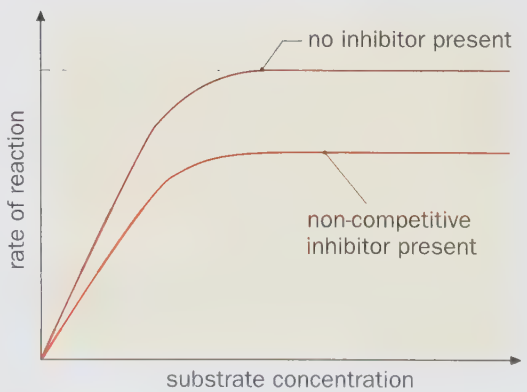
Increasing the concentration of the substrate can reduce the effects of a competitive inhibitor.
Since the inhibitor binds reversibly with the active site, as it leaves there is a chance of the substrate occupying the site.
The more substrate molecules there are present, the greater chance there is of them getting into the active site at the expense of the inhibitor.

A **non-competitive inhibitor** does not bind to the active site of the enzyme. It attaches to some other part of the enzyme molecule.
This alters the overall shape of the enzyme molecule, including the active site, and the substrate can no longer bind to the active site.
Notice that, this time, the inhibitor and the substrate are not competing for the active site.
So increasing the amount of substrate will not reduce the inhibition.



A non-competitive inhibitor does not attach to the active site but indirectly changes its shape

Look at the graph. Can you see that the non-competitive inhibitor affects the action of the enzyme at ***all*** concentrations of substrate? Why do you think this is?



Non-reversible inhibitors alter the enzyme permanently.
Heavy metal ions such as silver (Ag^+) and mercury (Hg^+) cause the disulfide bonds holding the enzyme together to break.
This alters the tertiary structure of the enzyme and it loses its catalytic activity.

Cyanide is a non-reversible inhibitor of ***cytochrome oxidase***, an important enzyme in respiration.

Many pesticides are non-reversible inhibitors (see page 42).
Can you suggest an explanation for the way in which they act?

Controlling metabolic pathways

A metabolic pathway consists of a series of enzyme-controlled reactions.
Look at this pathway:

$$A \xrightarrow{e_1} B \xrightarrow{e_2} C \xrightarrow{e_3} D \xrightarrow{e_4} E$$

Can you see that the product of one reaction becomes the substrate of the next?
For example, B is the product of the first reaction and then becomes the substrate for the second reaction. Each reaction is catalysed by a different enzyme.
This is an example of a **multi-enzyme pathway**.

Enzymes in a pathway like this are often fixed to the inner membrane of cell organelles, such as mitochondria.
This keeps the enzymes close together and so increases the chance of collisions occurring between enzyme and substrate molecules.

A multi-enzyme pathway is often controlled by the end product. This is called **end product inhibition**. This happens because the end product acts as an inhibitor of one of the enzymes at the start of the pathway:

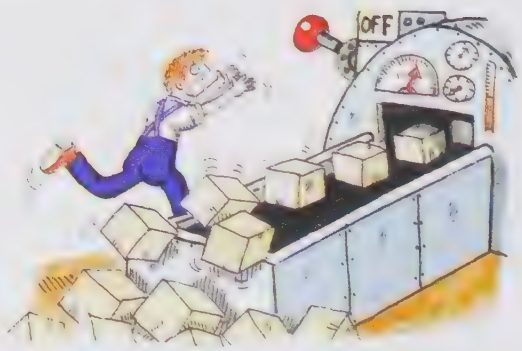
$$A \xrightarrow{e_1} B \xrightarrow{e_2} C \xrightarrow{e_3} D \xrightarrow{e_4} E \text{ (end product)}$$

inhibition

In this example, the end product E inhibits the enzyme e_1.
If too much of E is produced, it inhibits e_1 and the pathway is slowed down. As a result, the level of product falls.

What do you think would happen if there was a shortage of product E?
There would be less of E to inhibit enzyme e_1, so more A would be converted to B. The pathway would no longer be blocked and the level of end product E would rise.

This type of self-regulating mechanism is called **negative feedback**.
This is a concept that you will come across again in this book.
It means that the output from a system affects the input.
In this case, if the amount of product E rises then action is taken to reduce it.

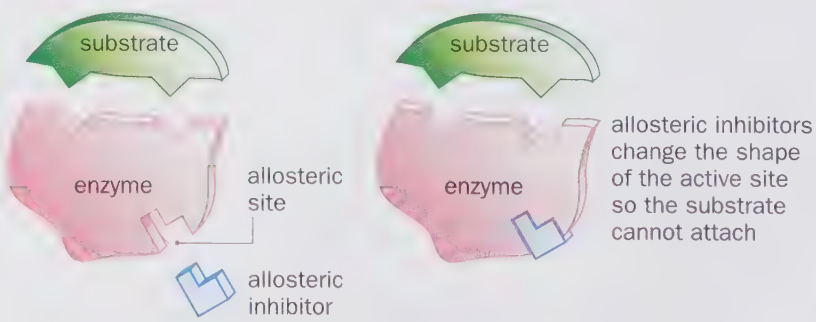


Time for some negative feedback!

This type of control of metabolic pathways must involve reversible inhibitors since the enzymes are not permanently damaged.
The end product acts as a type of non-competitive inhibitor.
It attaches to a specific site on the surface of the enzyme away from the active site.

This affects the overall shape of the active site and slows down the rate of reaction.
Such inhibitors are called **allosteric inhibitors**, because the enzyme has more than one shape.
One shape makes the enzyme ***active***, the other shape makes it ***inactive***.

So the end product acts as a ***molecular switch*** that can turn the enzyme on or off depending upon the concentration of the end product.



Allosteric inhibition

Classifying enzymes

Usually, an enzyme is named by taking its substrate and adding the suffix '-ase'. For example,

- prote***ase*** catalyses the hydrolysis of proteins,
- amyl***ase*** catalyses the hydrolysis of amylose (starch),
- lip***ase*** catalyses the hydrolysis of lipids.

Some enzymes have older names that give no clue to the substrate they catalyse, for example trypsin and pepsin.

Enzymes may be classified according to the ***type*** of reaction they catalyse. For example, alcohol dehydrogenase removes hydrogen from alcohol, and DNA polymerase catalyses the formation (and breakdown) of DNA by polymerisation.

Enzymes are classified into six main groups by the nature of their action.

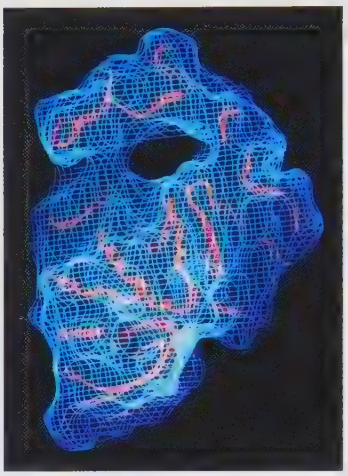
- ***Hydrolases*** catalyse the hydrolysis of a substrate by the addition of a molecule of water:

  $$\underset{\text{substrate}}{AB} + \underset{\text{water}}{H.OH} \xrightarrow{\text{hydrolase}} \underset{\text{products}}{A.H + B.OH}$$

  Hydrolases include the digestive enzymes that work in our gut and the enzyme in a germinating seed:

  $$\text{maltose} \xrightarrow{\text{maltase}} \text{glucose} + \text{glucose}$$

  $$\text{lipid} \xrightarrow{\text{lipase}} \text{fatty acids} + \text{glycerol}$$



Computer graphic of the enzyme Taka-amylase

- ***Oxoreductases*** are involved in oxidation and reduction, or **redox**, reactions. They include:
  dehydrogenases, which oxidise the substrate by catalysing the removal of hydrogen, passing it on to a hydrogen acceptor or coenzyme:

  $$\underset{\text{substrate}}{AH_2} + \underset{\text{coenzyme}}{2NAD^+} \rightleftharpoons \underset{\text{oxidised substrate}}{A} + \underset{\text{reduced coenzyme}}{2NADH}$$

  oxidases, which catalyse the addition of oxygen to hydrogen, forming water:

  $$\underset{\text{substrate}}{AH_2} + \underset{\text{oxygen}}{\tfrac{1}{2}O_2} \rightleftharpoons \underset{\text{oxidised substrate}}{A} + \underset{\text{water}}{H_2O}$$

- ***Transferases*** transfer a group of atoms from one molecule to another. They include:
  transaminases, which transfer amino groups (NH_2) from one molecule to another, so enabling organisms to make certain amino acids.
  phosphotransferases, which control the transfer of phosphate groups in respiration:

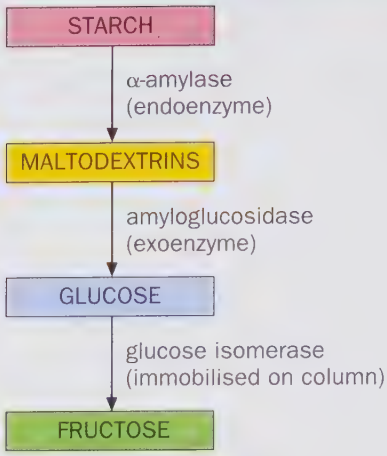
  $$\text{glucose} + \text{ATP} \longrightarrow \text{glucose-6-phosphate} + \text{ADP}$$

- ***Isomerases*** control the conversion of one isomer to another by transferring a group of atoms from one molecule to another.
- ***Lyases*** are able to break chemical bonds ***without*** the addition of water (hydrolysis). This includes ***decarboxylases***, which remove carboxyl groups (COOH) from respiratory substrates to release carbon dioxide.
- ***Ligases*** catalyse reactions in which new chemical bonds are formed, using ATP as a source of energy.
  For example, DNA ligase is involved in the synthesis of DNA.

Biology at work: Commercial use of enzymes

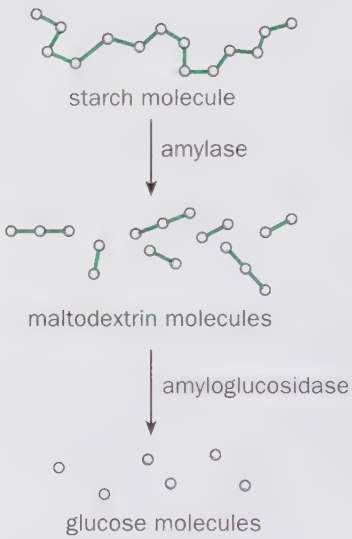
High-fructose corn syrups

Fructose is the sweetest natural sugar.
But naturally occurring sources of fructose, such as honey, are not always available in sufficient amounts to meet commercial demands.
These natural sources also tend to be expensive.
Worldwide, there is a huge demand for sweeteners in soft drinks and confectionery.



Millions of tonnes of ***high-fructose syrup*** are now produced every year from corn starch.
Corn starch is a cheap feedstock, especially in the USA.
Enzymes can break down the starch into glucose.
The glucose is then converted into fructose.

Three enzymes are involved.
Bacterial amylase first hydrolyses the starch.
As the name suggests, the enzyme is extracted from a bacterium.
Amylase is an **endoenzyme**, hydrolysing bonds ***within*** the starch.
So the large polysaccharide molecule is broken down into small chains called **maltodextrins**.

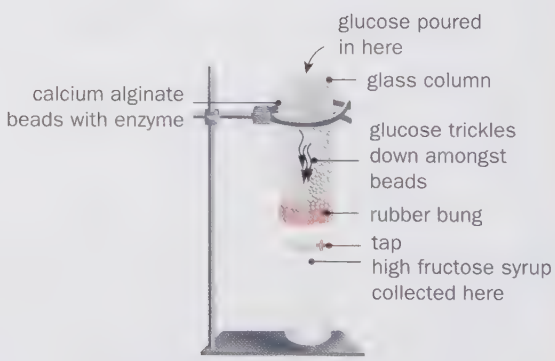


The next step is to hydrolyse the maltodextrins to glucose.
The second enzyme, ***amyloglucosidase***, is an **exoenzyme**, which removes glucose units from the ends of the maltodextrin molecules.
The enzyme is extracted from a fungus.

Why do you think it is useful to hydrolyse a large polymer first with an endoenzyme, and secondly with an exoenzyme?

The endoenzyme chops the large polymer into smaller strips.
Each strip has two ends that the exoenzyme can get to work on.
If the exoenzyme were used first, there would be only two ends of the large molecule that it could attack!

The third enzyme is ***glucose isomerase*** and, like bacterial amylase, it is also made by a bacterium.
Glucose isomerase converts glucose to fructose.
Fructose is much sweeter than glucose.
The enzyme is fixed onto beads of calcium alginate and packed into glass columns.
The enzyme is **immobilised**, because it is fixed to the beads.



An immobilised enzyme column in use

The glucose is run into the top of the column and reacts with the isomerase enzyme as it trickles down the column.
Eventually high-fructose syrup runs out at the bottom of the column.

Can you think of any advantages of using immobilised enzymes in industry?
Once a column has been set up, it can be used many times.
Also, it costs money separating the products of a reaction from the enzyme. If the enzyme is 'fixed' then it does not get mixed up with the products, so the cost of commercial production is lower.

Biology at work: Immobilised enzymes

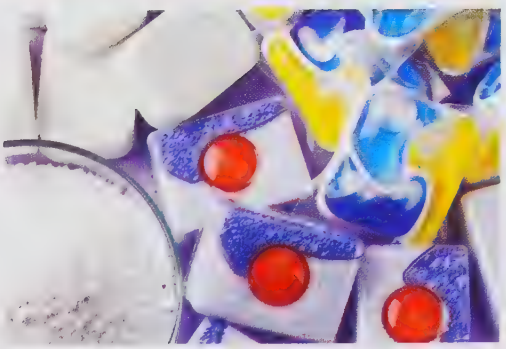
As you have already seen, when enzymes are used commercially it makes economic sense to use them in an immobilised form. However, immobilisation of enzymes did not develop purely for economic reasons.

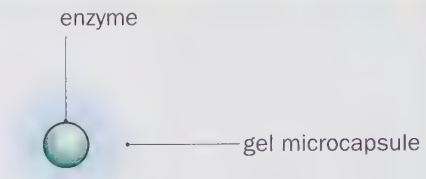
In the mid-1960s, biological washing powders became widely available. These powders contained protease enzymes in a free powdered form. Very quickly there were reports of allergic reactions in both workers and users. This led to bad publicity and a big fall in protease production.
The allergic reactions were thought to be caused by the powdered enzyme coming into contact with the skin.
As a result of these problems, detergents now contain immobilised proteases, which do not react with the skin.

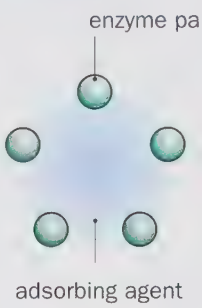
So, just how are enzymes put into an immobilised form?

Entrapment involves the enzyme being mixed with another chemical that forms a gel capsule around it.
The substrate that the enzyme acts upon is able to diffuse through the gel, although this may be quite slow.

Adsorption is a more expensive process that involves the enzyme particles being held by weak forces on an adsorbing agent, for example glass beads or carbon particles.
With this method there is no barrier slowing down the enzyme-substrate contact.

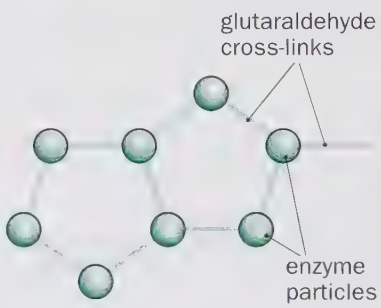






Biosensors

The third method is called **cross-linkage**. This method involves large numbers of enzyme particles being linked together. This is carried out by a so-called cross-linking agent such as glutaraldehyde. The drawback with this method is that it can damage the enzymes.



As you have already seen in this chapter, enzymes are highly specific chemicals and biotechnologists have combined this characteristic together with electronics to develop **biosensors**.
These devices use immobilised enzymes to detect chemicals such as blood sugar, and the reaction between the enzyme and its substrate is measured electronically.

Biosensors have great potential in the areas of medical diagnosis and environmental monitoring, because they can detect minute amounts of chemicals.

Scientists are developing biosensors integrated into microchips that can be implanted in the human body.
These sensors will detect various parameters and send data wirelessly to a smartphone, tablet or computer.

An electronic biosensor

Biology at work: Insecticide resistance in pests

The increased use of pesticides is one of the factors that have led to an increase in the yield of crops.
A particular insecticide does not affect all members of a pest species equally.
Some pests are genetically less susceptible and may survive to pass on their **resistance** to the next generation.
The more frequently an insecticide is used, the more likely resistance in the pest insect species is to evolve.

Mode of action of insecticides

The majority of insecticides in use today belong to the organophosphate group.
They work by blocking the activity of the enzyme *acetylcholinesterase*.
This enzyme is involved in the transmission of nerve impulses across the synapse.

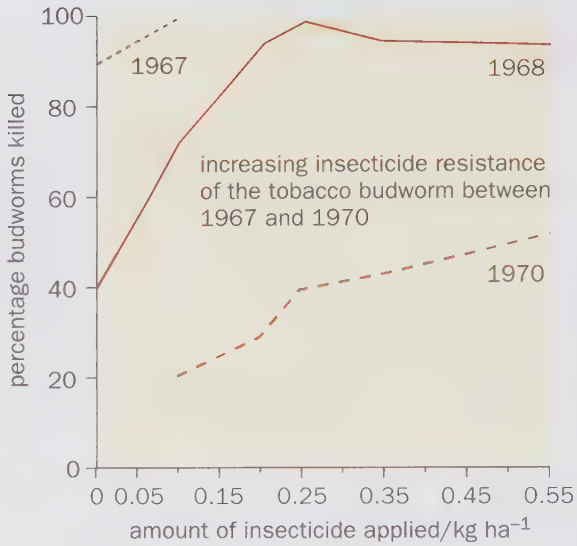
- The chemical transmitter substance ***acetylcholine*** is released from the pre-synaptic membrane.
- It crosses the synaptic gap and binds with the surface receptors on the post-synaptic membrane.
- The enzyme then breaks down the acetylcholine.
- This prevents the continuous transmission of nerve impulses in the post-synaptic membrane.

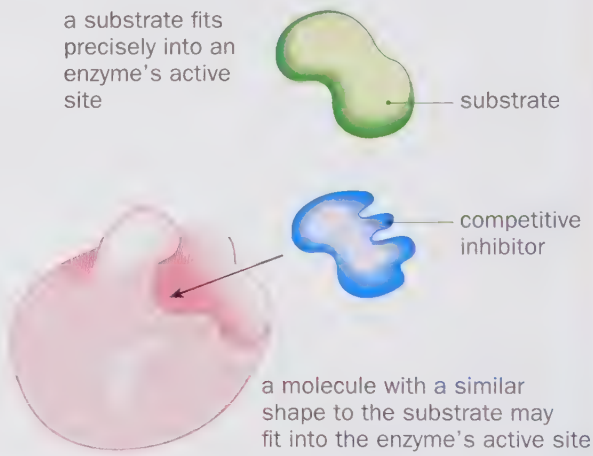
Insecticides mimic the action of acetylcholine by binding with the acetylcholinesterase.
This prevents the enzyme from breaking down acetylcholine and is a form of competitive inhibition.
Nervous dysfunction follows and quickly leads to the death of the insect.

Biochemical basis of resistance

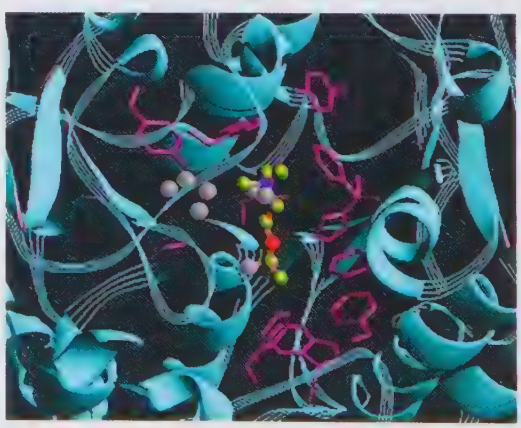
Resistant insects appear to have different forms of acetylcholinesterase to non-resistant insects.
They display a small number of mutations in the base sequence of the DNA in the acetylcholinesterase gene.
Once transcribed, these mutations cause changes in the amino acid sequence of the enzyme.
These amino acid changes occur close to the active site, as revealed by research into the three-dimensional structure of the enzyme.
Changes to the shape of the active site result in it becoming smaller.
Consequently, the larger insecticide molecules are prevented from entering the active site.
This then only allows the smaller acetylcholine substrate molecules to enter the active site.

This knowledge is being used to investigate the identification and synthesis of new insecticides to reduce resistance.





Competitive inhibitors can have a similar shape to that of the enzyme's normal substrate



A three-dimensional computer model of acetylcholinesterase with its substrate acetylcholine (green and red spheres) in the active site

Summary

- Enzymes are globular proteins and their properties are related to their tertiary structure.
- Enzymes speed up the rate of reaction by lowering the activation energy required to start the reaction.
- An enzyme works by combining with a complementary substrate to form an enzyme-substrate complex. The substrate molecule becomes temporarily attached to the enzyme's active site.
- Enzymes are specific because they act only on a particular substrate.
- Enzymes are unchanged after the reaction and can be used again and again.
- Temperature, pH, and the concentration of the reactants affect the rate of an enzyme-catalysed reaction.
- Some enzymes only work in the presence of cofactors. These cofactors include prosthetic groups, coenzymes and activators.
- Enzymes can be prevented from working by reversible and non-reversible inhibitors. Reversible inhibitors can be competitive or non-competitive.
- The name of an enzyme can give a clue to the substrate upon which it works and the type of reaction that it catalyses.

Questions

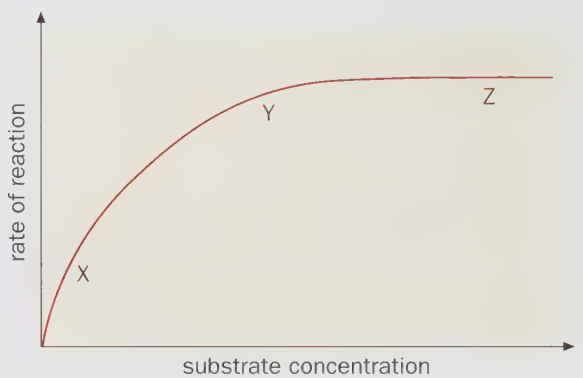
1 Copy and complete the following:
Enzymes are ___ proteins with an exact ___ ___ shape. The ___ structure of an enzyme is held together by ionic and ___ /___ bonds. During a reaction the ___ fits into a region on the surface of the enzyme called the ___ ___. Enzymes are ___ because they will act only on a particular substrate.
Increasing the temperature of an enzyme-catalysed reaction by 10 °C usually ___ the rate. Increasing the temperature beyond 40 °C ___ many human enzymes. Chemicals that slow down or stop the action of an enzyme are called ___.

2 a) Explain what is meant by an enzyme.
b) Explain how each of the following affects the rate of an enzyme-controlled reaction:
i) temperature
ii) enzyme concentration
iii) substrate concentration.

3 a) Define the following terms:
i) enzyme
ii) coenzyme
b) Explain how the following properties of enzymes depend upon the structure of the enzyme molecule:
i) substrate specificity
ii) temperature denaturation
iii) inhibition.

4 a) Explain, with the use of diagrams, the lock and key theory of enzyme action.
b) How does the induced fit theory modify the lock and key theory?

5 The graph shows the effect of increasing substrate concentration on the rate of an enzyme-catalysed reaction.



Explain ***exactly*** what is happening at the points X, Y and Z on the graph.

6 a) Enzyme-catalysed reactions normally have a temperature coefficient (Q_{10}) of 2 between 0 °C and 40 °C. Explain what this means.
b) At higher temperatures the enzyme is rapidly denatured. Explain what happens and the effect that this has upon enzyme action.

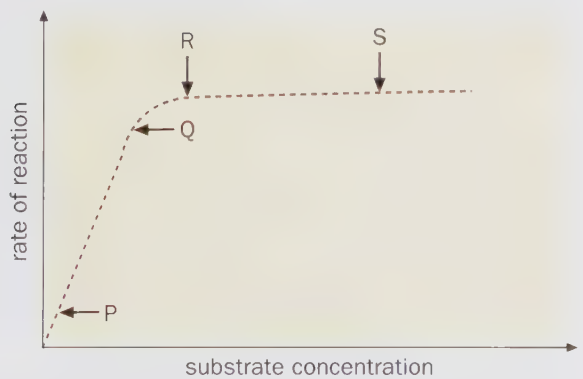
7 a) What is meant by the activation energy of a chemical reaction?
b) Explain how enzymes act to lower the activation energy.
c) In many chemical reactions, heat supplies this activation energy. Why would this be inappropriate for reactions that take place in cells?

8 An experiment was carried out investigating the effects of temperature on the action of the enzyme sucrase on the substrate sucrose. The following data were obtained:

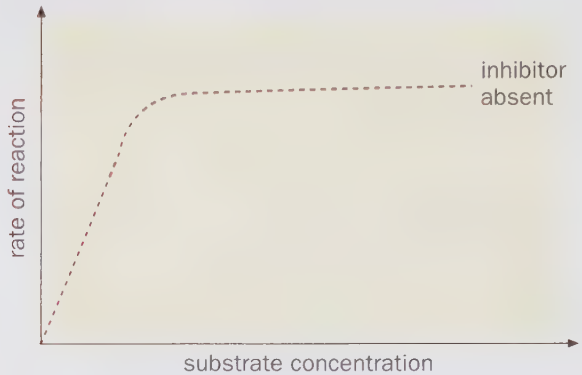
Temperature (°C)	Time taken to complete hydrolysis of sucrose (min)	Reaction rate (1/time)
0	50	
10	15	
20	8	
30	4	
40	6	
50	28	
60	110	

a) Use the data to calculate the reaction rate at each temperature. Using this information, plot a graph to show how the rate of reaction varies with temperature.
b) Describe and explain the results obtained between 10 °C and 30 °C.
c) Explain what is happening to the enzyme molecules between 40 °C and 60 °C.
d) Using information on your graph, calculate the Q_{10} value for this reaction between 15 °C and 25 °C.

9 The results of an investigation into the effect of increasing substrate concentration on the rate of an enzyme-catalysed reaction are shown in the graph.



a) i) Name the factor that determines the rate of the reaction between points P and Q.
ii) What has happened to the rate of reaction between points R and S?
iii) Name two factors that could account for this occurrence.
b) i) State two conditions that should be kept constant in this investigation.
ii) What should be measured in order to determine the rate of an enzyme-catalysed reaction?
c) The investigation was repeated with the addition of a competitive inhibitor. The same amount of inhibitor was added to the substrate at each concentration.
Copy the graph below and add to it the curve you would expect if the inhibitor were present.



10 a) What is a cofactor?
b) What are the three main groups of cofactors?
c) Explain the differences between these three groups.

11 An investigation was carried out into the effects of pH on the action of the enzyme amylase on starch. Eight test tubes were set up at different pHs and incubated in a water bath at 30 °C for 1 hour. The mass of reducing sugar (product) was then estimated. The results are shown in the table.

pH	4.0	5.0	6.0	6.5	7.0	8.0	9.0	10.0
Mass of reducing sugar produced (arbitary units)	1	12	26	32	33	27	13	5

a) Plot a graph to show these results.
b) Explain the effects of pH on the action of amylase in this investigation.

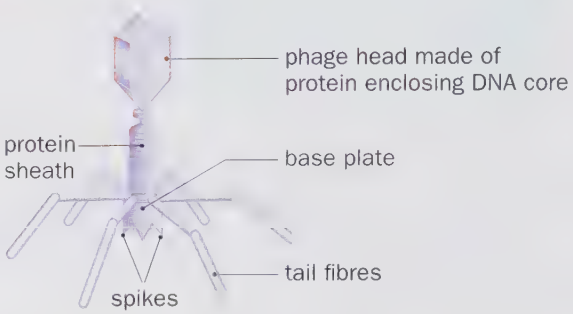
3 Nucleic acids and protein synthesis

The genome is 'all the DNA sequences contained in the chromosomes of an organism'.
The Human Genome Project aims to trace every single human gene and map its particular position on the appropriate chromosome.
It is a truly international project, with contributions from many countries.
It is being coordinated by the Human Genome Organisation.
They intend to unravel the structure of each gene and the protein for which it codes.
The significance of this is that we will be able to understand how people are affected by certain diseases and target early treatments.
The information will also be used to create new drugs to fight cancers, heart diseases, immune disorders and other illnesses.

Dr John Sulston, Director of the Sanger Centre in Cambridge, where the British genome work was based

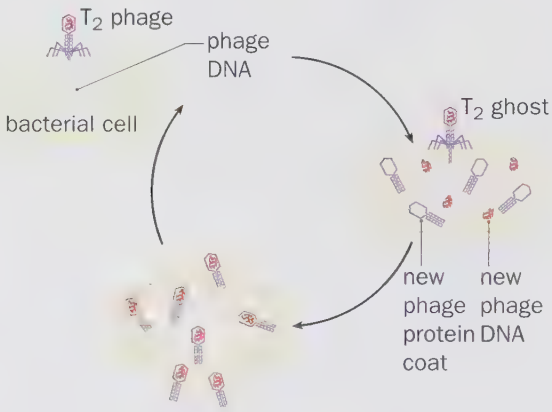
Does DNA carry the genetic information?

For many years scientists were not sure whether it was DNA or protein that carried the genetic information.
In 1952, Frank Hershey and Martha Chase set up an experiment that proved that it is in fact DNA that codes for the production of proteins.



Bacteriophages are viruses that live inside bacterial cells.
The T_2 bacteriophage (phage for short) lives on the gut bacterium *Escherichia coli* (*E. coli*).
The phage consists of a protein coat containing a core of DNA.
The phage is able to inject its DNA into the bacterial cell.
There it takes over the bacterium's biochemical machinery to replicate itself and make lots of new phages.

Hershey and Chase set up two cultures of bacteria and phage to test whether the genetic material was DNA or protein.
DNA contains phosphorus but no sulfur.
Protein compounds contain sulfur but hardly any phosphorus.



The growth medium in culture A contained the radioactive isotope of phosphorus, ^{32}P.
The growth medium in culture B contained the radioactive isotope of sulfur, ^{35}S.
The radioactively labelled phage was then allowed to infect the separate cultures of *E. coli* bacteria.

Which molecules of the phage will be radioactive in
- culture A,
- culture B?

After an appropriate time, the empty phage heads (called ghosts) were separated from the bacterial cells in a blender.
The two fractions were tested for radioactivity, giving the following results:

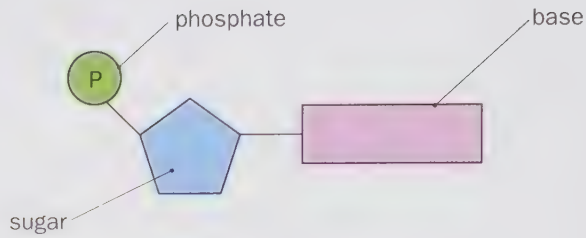
Phage type	*E. coli* bacteria fraction	Phage ghost fraction	Phage offspring
A (^{32}P)	radioactive	non-radioactive	radioactive
B (^{35}S)	non-radioactive	radioactive	non-radioactive

Why do you think that the offspring of phage type A were radioactive?
Why do you think that the offspring of phage type B were not radioactive?
How do the results support the view that DNA carries the genetic information?

The structure of nucleic acids

There are two types of nucleic acid: **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**.
Both are polymers made up of monomers called **nucleotides**.

Each nucleotide is made up of three parts:
- **a phosphate group,**
- **a pentose sugar (either ribose or deoxyribose),**
- **a base that contains nitrogen.**



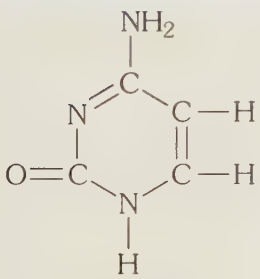
A single nucleotide

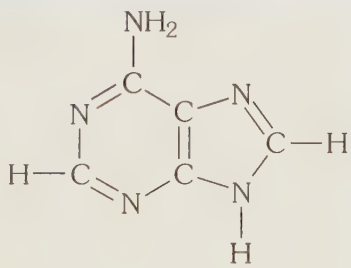
The phosphate, sugar and base join together as shown in the diagram.

Deoxyribonucleic acid (DNA) always contains the sugar **deoxyribose**.
Ribonucleic acid (RNA) always contains the sugar **ribose**.
The phosphate groups are the same in both DNA and RNA.

There are five different types of bases, which we put into two groups.

- **Pyrimidines** are bases with a single ring structure.
  There are three pyrimidine bases: **cytosine**, **thymine** and **uracil**.
- **Purines** are bases with a double ring structure.
  There are two purine bases: **adenine** and **guanine**.


cytosine
(a pyrimidine base)

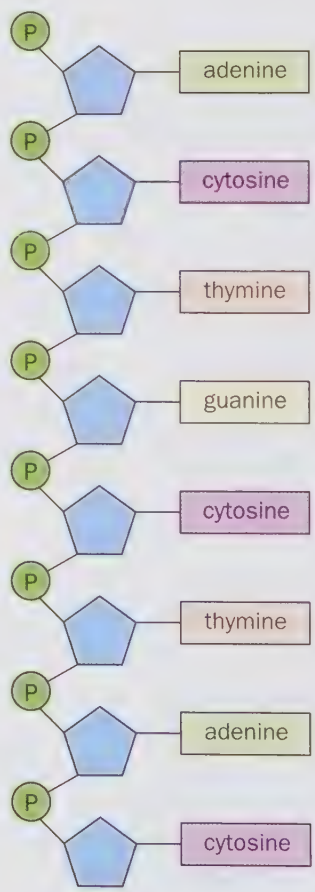

adenine
(a purine base)

One nucleotide can join to another by a condensation reaction.
This takes place between the sugar and phosphate groups with the formation of **phosphodiester bonds**.
Many nucleotides joining up in this way form a polynucleotide chain.

In DNA molecules, the nucleotides contain one of the four bases *adenine, guanine, cytosine* or *thymine*.
In RNA molecules, the nucleotides contain one of the bases *adenine, guanine, cytosine* or *uracil*.
So in RNA uracil replaces thymine.

What are the ***two*** main differences between the structure of DNA and the structure of RNA?

Adenosine triphosphate (ATP) is also a nucleotide derivative. It consists of a molecule of ribose, a molecule of adenine and three phosphate groups. ADP is also a phosphorylated nucleotide (see page 303).



A polynucleotide chain

The structure of DNA

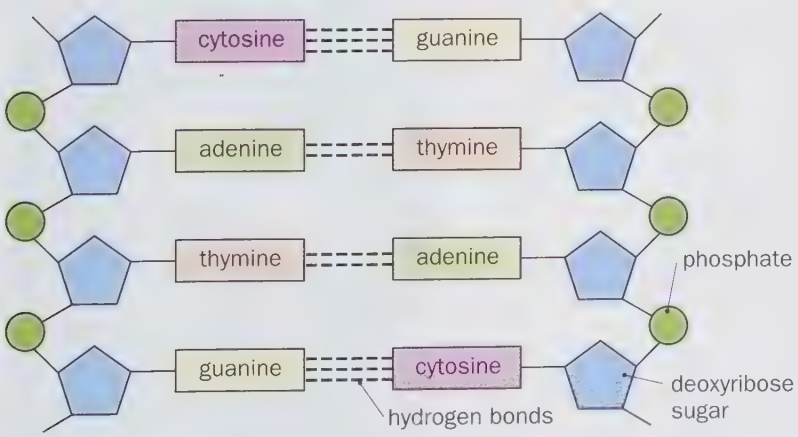
In 1951, the American chemist Erwin Chargaff analysed DNA.
He used chromatography to separate the four bases in DNA from different species.
The amounts of each base were measured quantitatively.
Look at the table showing some of Chargaff's results.
Can you see any pattern in the amounts of the bases estimated?

Organism	Percentage of each base present			
	Adenine	Cytosine	Guanine	Thymine
yeast	32	18	18	32
tuberculosis bacterium	16	34	36	14
locust	29	21	21	29
human	31	19	19	31

Chargaff noted that the amounts of adenine and thymine were similar, and that the amounts of cytosine and guanine were similar.
This was to help later workers, who realised that the bases in a DNA molecule always pair up.
They deduced that adenine must always pair up with thymine because their amounts were always the same.
Similarly they deduced that cytosine must always pair up with guanine.
This is called the '**rule of base pairing**'.

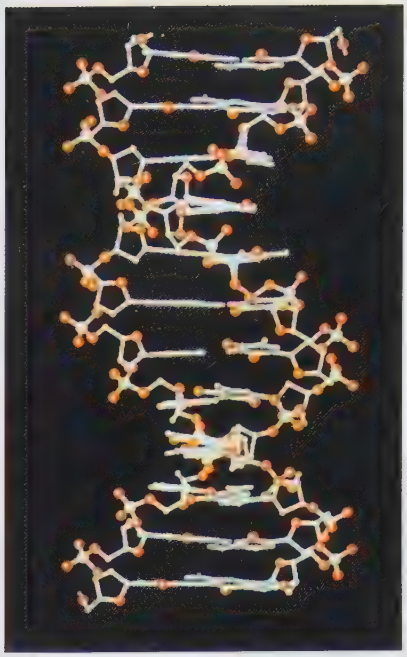
The bases always pair in the same complementary way:
ADENINE with THYMINE
CYTOSINE with GUANINE.

DNA is made up of ***two*** polynucleotide chains.
The structure is a bit like a ladder. The 'uprights' of the ladder are made up of alternating sugar and phosphate groups.
The 'rungs' of the ladder are made up of the bases. The uprights run in the opposite direction to each other and are said to be **antiparallel**.
The bases are held together by weak hydrogen bonds.
Two hydrogen bonds hold adenine to thymine, and three hydrogen bonds hold cytosine to guanine.



The ladder-like structure is twisted into a helix so that it really resembles a spiral staircase with the bases as the steps.
This structure was given the nickname 'the double helix'.

If one half of a strand of DNA has the base sequence TACCTGATGTCAAG, what do you think the sequence of bases on the other strand will be?



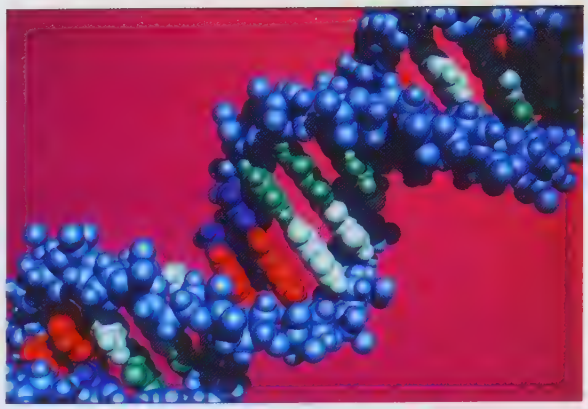
A three-dimensional model of DNA

The double helix

The discovery of the double helix

The working out of the structure of the DNA molecule remains one of the outstanding scientific events of the twentieth century. Scientists recognised the importance of DNA in the cells of living organisms and by the early 1950s the following facts were known about it:

- DNA is a very long, complex molecule made up of nucleotides.
- It contains four bases – adenine, cytosine, guanine and thymine.
- The amount of adenine is the same as the amount of thymine, and the amount of cytosine is the same as the amount of guanine.
- The molecule is likely to be a helix held together by hydrogen bonds.

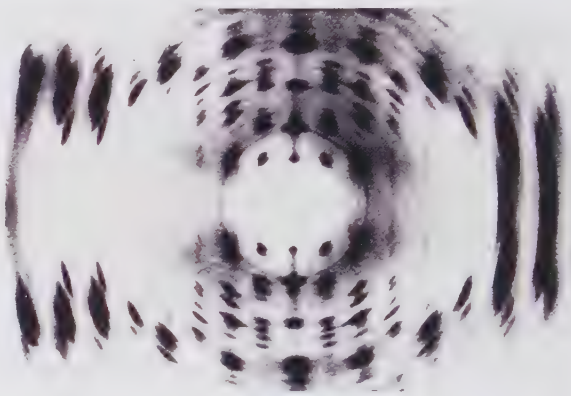


Computer simulation of DNA structure

What scientists could not work out was how the molecular structure was put together.
How could the molecule store all the genetic information of an organism?
How could it copy itself exactly, time after time?

Evidence from X-ray diffraction

A technique called X-ray diffraction has been used to work out the structures of many different molecules, including proteins. The technique involves firing a beam of X-rays at a protein crystal. As the X-rays hit the atoms in the protein, they are deflected and caught on a photographic plate.
The scattered X-rays produce a pattern that can be used by experts to deduce the arrangement of the atoms in the molecule.



X-ray diffraction pattern of DNA

In 1953, Rosalind Franklin and Maurice Wilkins worked at King's College, London, on the X-ray crystallography of DNA. The work was difficult because DNA does not form crystals easily.

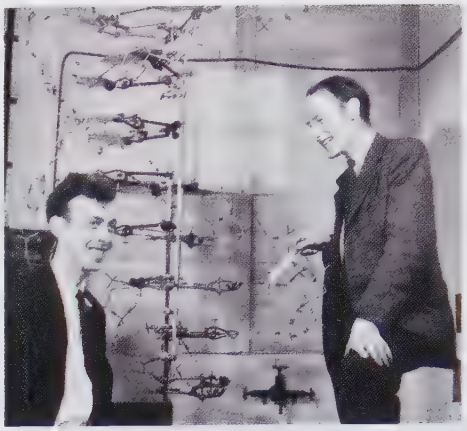
However, they succeeded in producing X-ray diffraction photographs like the one shown above.
From these they were able to deduce that the phosphate groups of DNA must be positioned on the outside of the molecule.

This work proved to be of vital importance to the later discovery of the double helix.
Tragically, Rosalind Franklin died of cancer in 1958 at the age of 37. Four years later, Maurice Wilkins was awarded the Nobel Prize. Unfortunately, Nobel Prizes are not awarded posthumously.

Rosalind Franklin

The DNA detectives

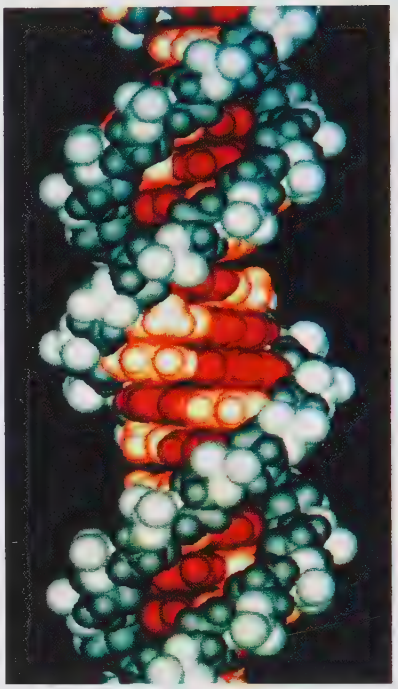
The molecular structure of DNA was finally deduced by James Watson and Francis Crick working at the Cavendish Laboratory in Cambridge in 1953.
Watson was an American biologist visiting Europe and Crick was a British physicist turned biologist.
They collected all the recent information on DNA, including Chargaff's data on base composition, and the X-ray diffraction work of Franklin and Wilkins, and used this to put together a three-dimensional model of DNA.



Watson and Crick with their DNA model

Any model would have to satisfy all the available information and explain how the molecule was able to reproduce itself.
It took hours of discussions and pain-staking manipulation of the models before they hit on the correct solution.
Both Chargaff's results and the X-ray diffraction patterns could be explained if the model consisted of ***two*** polynucleotide chains twisted around each other in the form of a double helix.

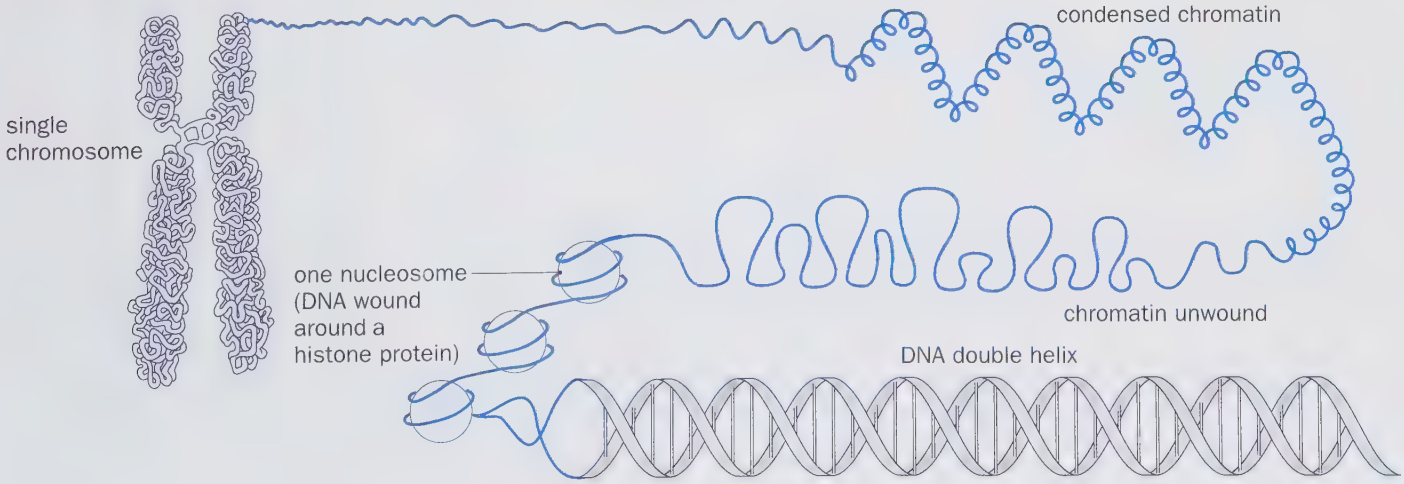
The X-ray diffraction work had shown that every complete turn of the helix measured 3.4 nm.
Watson suggested that if cytosine paired with guanine (a pyrimidine with a purine) and if thymine paired with adenine (again a pyrimidine with a purine) then 10 bases could fit into one complete turn of the helix (3.4 nm).
The bases would be held together by hydrogen bonds.
Watson and Crick made accurate cut-out models of the four nucleotides and were able to fit them together like a jigsaw puzzle.
They went on to make a three-dimensional DNA model, like the one shown in the photograph right.



Three-dimensional model of a DNA molecule

The amount of DNA in a cell is known to double before cell division.
So DNA must be able to make exact copies of itself.
Watson and Crick's model could do this if the hydrogen bonds holding the bases together were to break.
The two strands could separate and each produce a new double helix.
Watson and Crick were awarded the Nobel Prize for their immense contribution to molecular genetics.

Chromosomes are known to consist of DNA and histone protein.
The DNA is thought to be highly coiled so that a huge amount of DNA is condensed into one chromosome containing up to 300 million nucleotides!



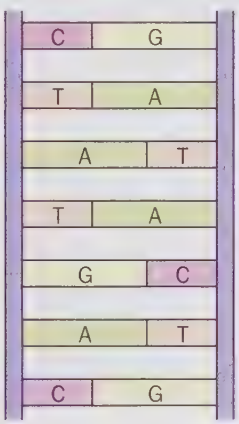
DNA replication

Chromosomes must make copies of themselves so that the new cells formed at cell division have all the correct genetic information. Similarly, chromosomes must make copies of themselves so that the genetic information can be passed on to the offspring via the sperm and the egg. This copying of DNA is known as **replication** and it takes place in every cell before cell division occurs.

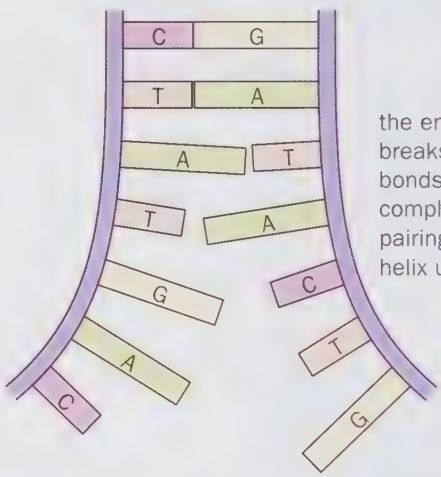
First, the hydrogen bonds holding the base pairs together break. This reaction is catalysed by the enzyme **DNA helicase**. As the bonds break, the two halves of the molecule unwind and separate. It's a bit like a zipper unzipping, and each half of the molecule acts as a mould, or **template**, on which a new strand of DNA can be built.

When the two halves of the molecule separate, the DNA bases are exposed. Free nucleotides enter the nucleus from the cytoplasm and assemble on the template DNA according to the law of base pairing. Nucleotides containing adenine join up with those containing thymine, and nucleotides containing cytosine join up with guanine nucleotides. This happens against each original polynucleotide chain. The enzyme **DNA polymerase** joins the new nucleotides together, forming a new sugar–phosphate backbone.

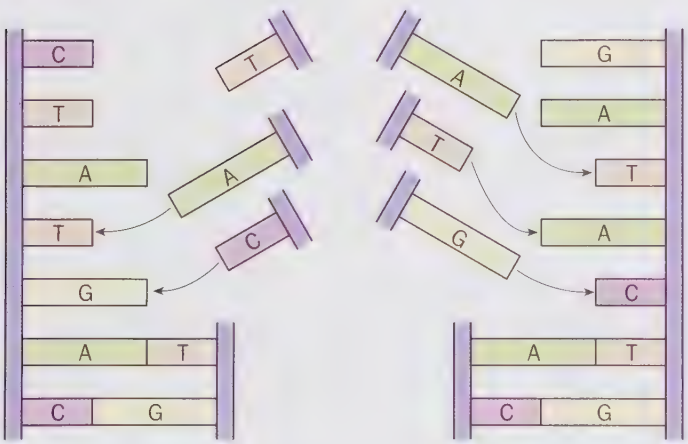
A DNA molecule replicating



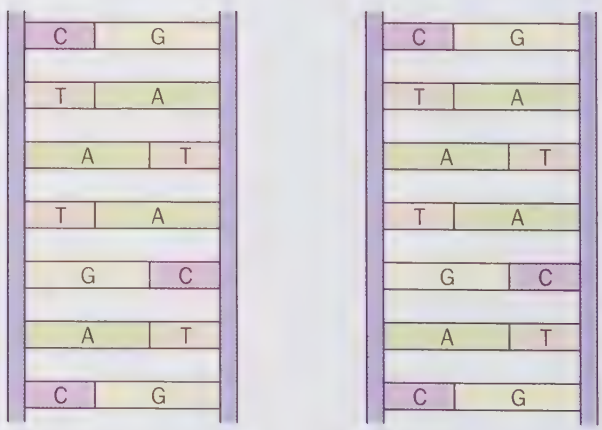
original DNA molecule about to replicate at interphase of cell division



the enzyme DNA helicase breaks the hydrogen bonds between the complementary base pairings: the double helix unwinds



free nucleotides attach to each template DNA where bases are complementary



the nucleotides join up with the formation of phosphodiester bonds between adjacent nucleotides producing two identical DNA molecules – **DNA ligase** catalyses this process

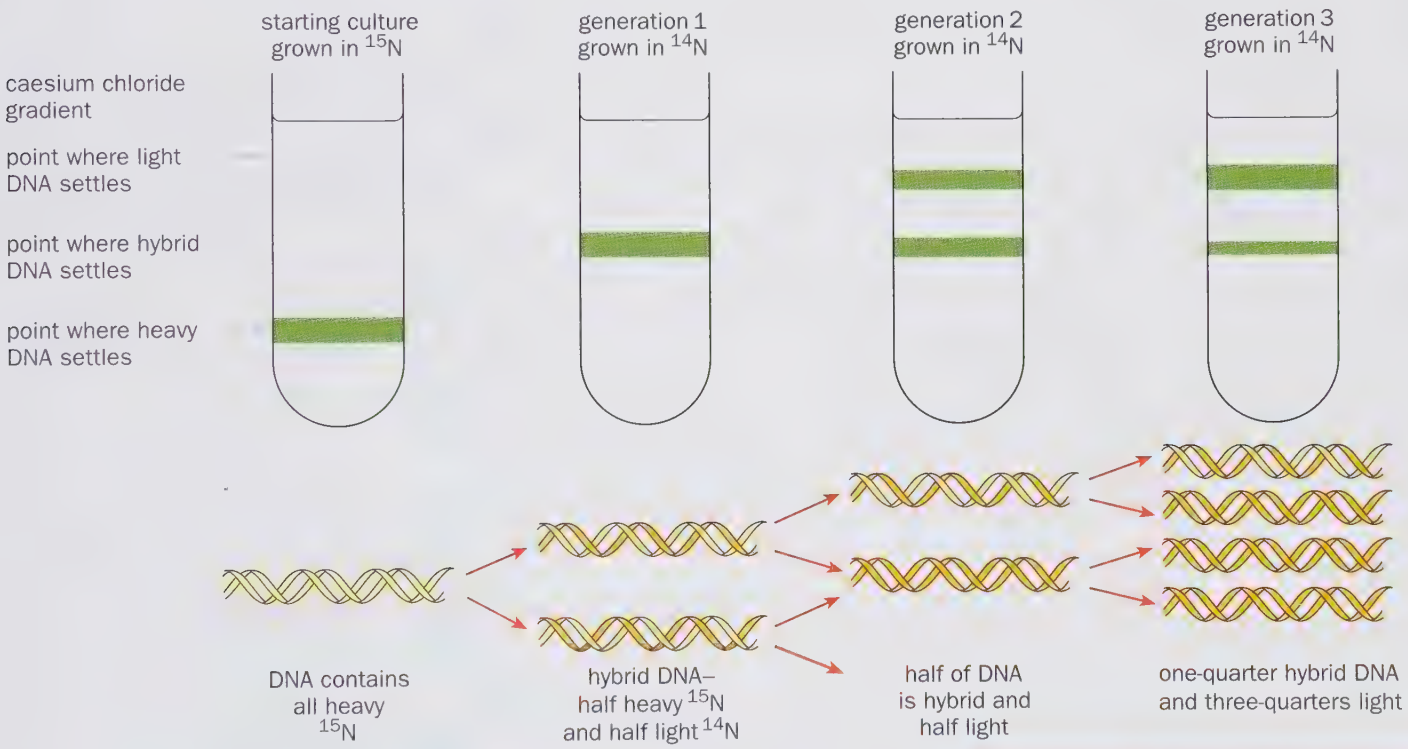
Evidence for DNA replication

This method of replication is known as '**semi-conservative**' because each new DNA double helix contains one complete polynucleotide strand from the original DNA molecule.

A high-speed centrifuge

Shortly after Watson and Crick's discovery, Meselson and Stahl produced evidence to support this method of DNA replication. They grew the bacterium *E. coli* in a medium containing amino acids made with the isotope ^{15}N ('heavy nitrogen'). The bacterium took up the isotope and so all its DNA contained ^{15}N. Meselson and Stahl were able to extract the bacterial DNA and centrifuge it in caesium chloride solution. Depending on the mass of the molecule, the DNA would settle out at a particular point in the tube.

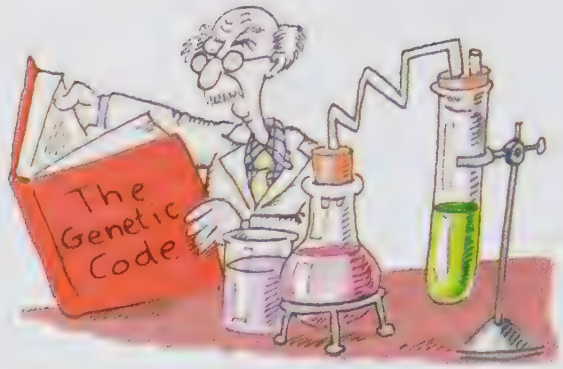
The ^{15}N bacteria were then transferred to a growth medium containing the normal, lighter isotope of nitrogen, ^{14}N, where they reproduced by cell division. Extracts of DNA from the first generation offspring were shown to have a lower density, since half the DNA was made up of the original strand containing ^{15}N and the other half was made up of the new strand containing ^{14}N. At succeeding generation times, the DNA extracts were found to have a lower proportion of ^{15}N as more ^{14}N was incorporated into the bacterial DNA. This was conclusive evidence for the semi-conservative method of DNA replication.



Look at the table showing the proportions of ^{15}N- and ^{14}N-labelled DNA at each generation time.
Predict the percentage of ^{15}N-labelled DNA that would occur in the third and fourth generations.

Generation	% DNA	
	^{15}N	^{14}N
1	50	50
2	25	75
3		
4		

The genetic code



A bit of light reading 'The secret of life'!

You now know how DNA is able to copy itself.
DNA also acts as a store of genetic information.

As you know, each chromosome is made up of a long, super-coiled strand of DNA.
This strand can be divided up into thousands of shorter sections called **genes**.
So a gene is a small part of a DNA strand.

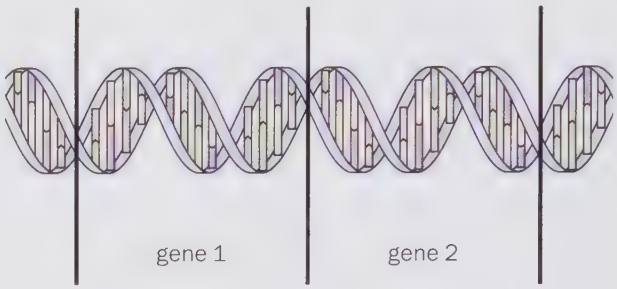
The length of DNA making up a particular gene carries the information needed to make a particular protein.
This information is known as the **genetic code**.

Remember that inside every cell there are thousands of chemical reactions taking place.
Enzymes control all these chemical reactions.
You should remember that ***all*** enzymes are proteins.

So because DNA codes for proteins, it must determine ***which*** enzymes are produced and, therefore, ***which*** chemical reactions can take place in cells.

The information in the genetic code is found in the **sequence of bases** along the length of DNA.
These determine the sequence of amino acids in the protein.

But how many bases do you think code for a single amino acid?



A triplet code?

As you know there are ***four*** different bases found in DNA: adenine, thymine, guanine and cytosine.
These can be represented by the letters A, T, G and C.
There are ***twenty*** different amino acids that can make up any protein.

So how many amino acids could be coded for if ***one*** base coded for ***one*** amino acid?
The answer is only four as there are only four codes: A, T, G and C.

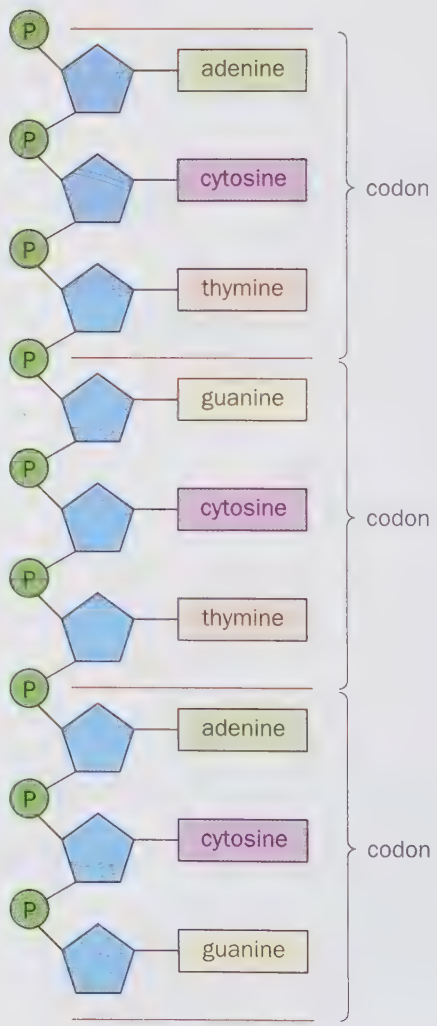
How about ***two*** bases coding for one amino acid?
No, still not enough, although this time we could have 16 different codes for 16 different amino acids:
AA, AT, AC, AG, TT, TA, TC, TG, CC, CA, CT, CG, GG, GC, GA, GT.

Let's try ***three*** bases for each amino acid.
That would give us 64 different codes – that's more than enough for 20 amino acids: ATA, ACG, TAC, etc.

We now know that a triplet code is involved.

In fact, most amino acids are coded by more than one DNA base triplet.
For instance, TTC and TTT both code for the amino acid lysine.
AGC, AGT, AGG and AGA all code for the amino acid serine.
What seems to be important is the first two bases in the sequence.

The code is called a **degenerative code** because most amino acids have more than one triplet code. These base triplets are called **codons**.
The code is **non-overlapping**, which means that each base in the sequence is only read once.
The code is **universal**, that is, it is the same in all living organisms.



Ribonucleic acid (RNA)

Do you remember how RNA differs from DNA?
RNA always has ribose sugar, and the base uracil replaces thymine.

There are other differences between RNA and DNA.
RNA molecules tend to be short-lived.
RNA is found inside the nucleus and in the cytoplasm.
RNA molecules are involved in the synthesis of proteins.
An RNA molecule is a relatively short polynucleotide chain.

There are ***three*** different types of RNA.

Computer simulation model of transfer RNA

Messenger RNA (mRNA)

A mRNA molecule is a single strand (not a double strand) about a thousand nucleotides long.
Messenger RNA is involved in carrying the genetic code from the DNA in the nucleus to the ribosomes in the cytoplasm where protein is made.

Ribosomal RNA (rRNA)

Molecules of rRNA are large and complex, often forming a double helix.
Ribosomes are made up of rRNA and protein.

Transfer RNA (tRNA)

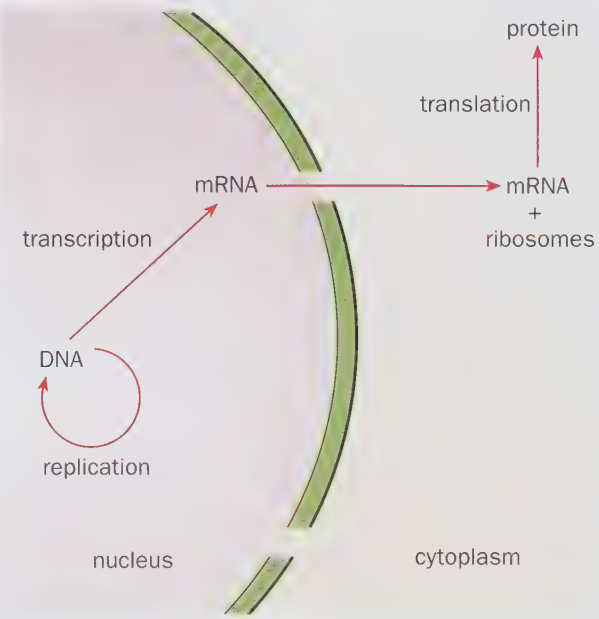
These are small molecules, about 80 nucleotides long.
Transfer RNA molecules bring amino acids to the ribosomes so that the protein can be assembled.

Making a protein

There are ***two*** main steps in converting the DNA code into a new protein.
These are **transcription** and **translation**.

As you know, DNA carries the information to build proteins from amino acids.
But DNA never leaves the nucleus.
So how does the information get from DNA to the site of protein synthesis in the ribosomes?
There must be some kind of 'messenger molecule'.
It must be able to carry the base sequence of DNA to the ribosomes.
Here the message can be ***translated*** into the correct amino acid sequence for the protein.

This 'messenger molecule' was eventually isolated and found to be RNA.
It was given the name **messenger RNA (mRNA)**.
Can you see from the diagram that DNA is now involved in three processes?
Use the diagram to explain what we mean by replication, transcription and translation.



The vital role of mRNA carrying the code to the ribosomes

Transcription

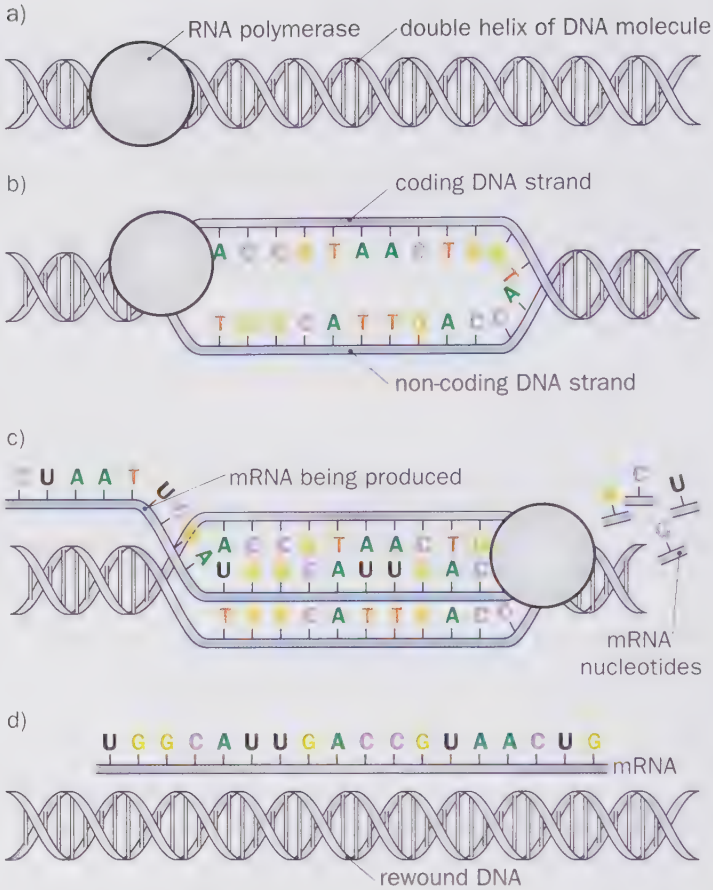
So how do you think mRNA is made in the nucleus?
The mRNA is copied from a specific region of DNA called the **cistron**.
The cistron is a length of DNA that forms a mRNA molecule.
Often a cistron is equivalent to a gene and codes for a specific polypeptide.

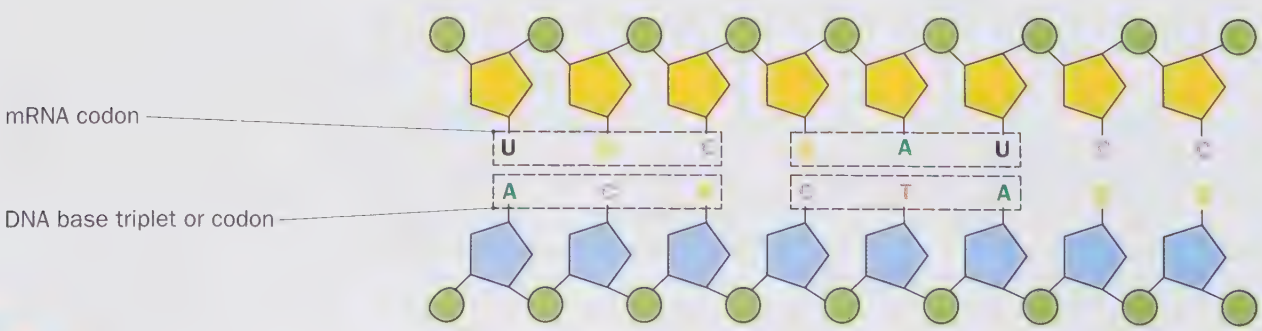
First, the DNA unwinds due to the breaking of the hydrogen bonds between the base pairs by the enzyme DNA helicase.
One of the two DNA strands acts as a **template** against which a matching mRNA strand can be formed.
The DNA strand is called the **sense strand** or the **coding strand**.
The exposed bases on this coding strand of DNA attract RNA nucleotides with a ***complementary*** base, for example cytosine on DNA will attract a guanine nucleotide.
But remember that ***adenine will attract a uracil nucleotide, not one containing thymine***.

The enzyme **RNA polymerase** moves along the DNA, forming bonds that add nucleotides one at a time to the RNA .
Behind the RNA polymerase the DNA strands join up to reform the double helix.
When the RNA polymerase reaches a particular sequence of bases on DNA, it recognises it as a 'stop' codon and detaches.

You should remember that each amino acid was coded for by a DNA codon, or a sequence of three bases.
As you can see from the diagram, the RNA molecules carry mirror images of these base triplets.
These mirror images are called **RNA codons**.
Each mRNA codon codes for a certain amino acid.
In this way, the mRNA carries the DNA code out of the nucleus to the ribosome, where it is used to assemble proteins.

What do you think the base sequence of mRNA will be if it is transcribed from the following DNA sequence?
TAC—CGC—CAT—TTA—ACG—ACT—AAA

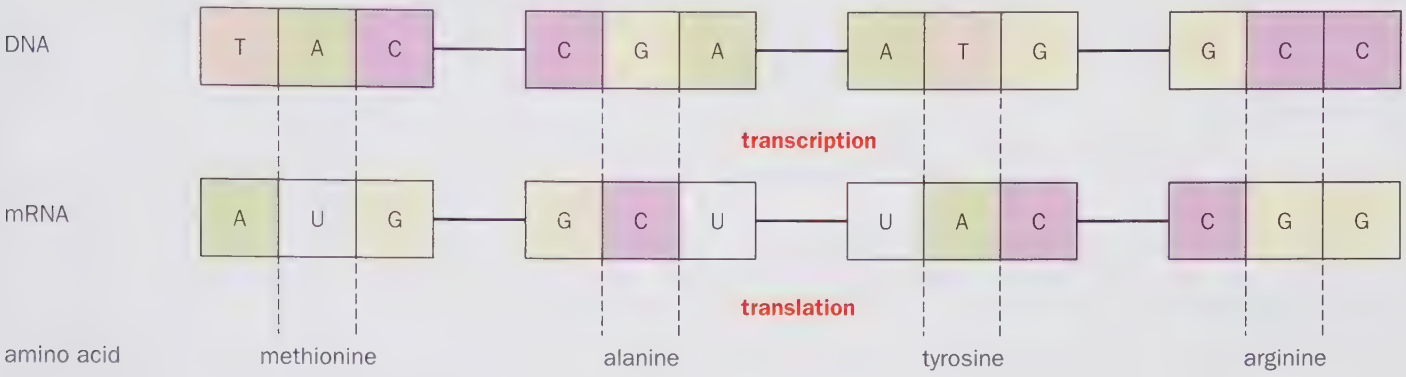




Translation

Translation is the process that converts the coded information of mRNA into the correct sequence of amino acids in a polypeptide.

First of all mRNA arrives from the nucleus with the genetic code.
The mRNA strand contains the codons for each particular amino acid in the protein.
This coded message is then read from the mRNA and ***translated*** into a polypeptide molecule.

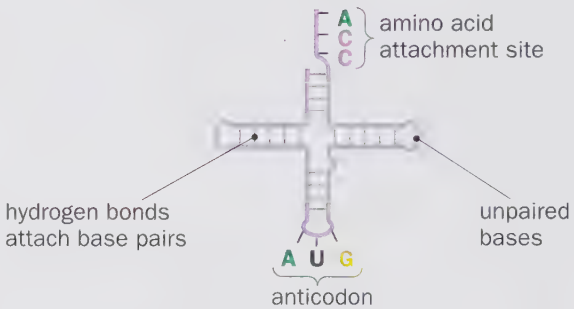


Amino acids are carried to the ribosomes by smaller RNA molecules called **transfer RNA** (**tRNA**).
Molecules of tRNA are single strands folded back on themselves.
They are often referred to as 'clover leaf' structures.
If you look at the diagram, you will see why.

Can you see that the bases are paired to each other?
How are these bases held together?

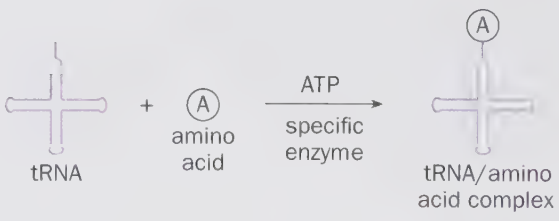
Hydrogen bonds give tRNA a stable structure.

The role of tRNA is to carry amino acids to the ribosomes.
To do this it has an amino acid attachment site at one end.

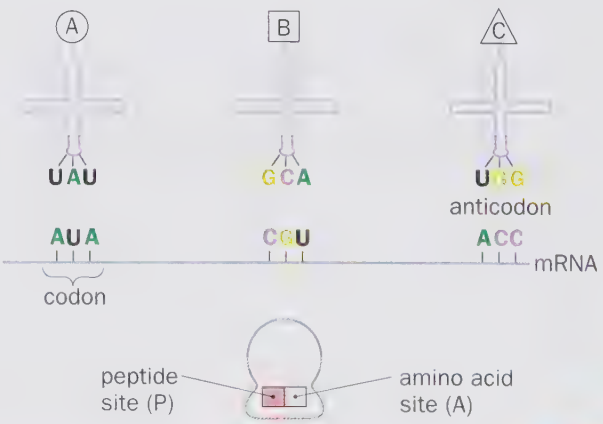


Structure of tRNA

Each amino acid has its own specific tRNA molecule.
Each of the 20 amino acids has a tRNA molecule specific to it.
The tRNA attaches itself to a specific amino acid in the presence of a specific enzyme and ATP.
This is sometimes called **amino acid activation**.



The tRNA/amino acid complex is then ferried to the ribosome where the amino acid becomes attached to the polypeptide chain.
At one end of the tRNA is a base triplet called an **anticodon**.
The anticodon is different for each amino acid: for instance, CGA for alanine, AGU for serine, and UUC for lysine.
The anticodon attaches itself to the compatible mRNA codon.
For instance, anticodon CGA will attach itself to codon GCU.



A ribosome is made up of **ribosomal RNA** (**rRNA**) and protein.
Each ribosome consists of two sub-units.
The smaller sub-unit has two sites for attaching molecules of tRNA: the P site (peptide site) and the A site (amino acid site).
So two tRNA molecules are associated with a ribosome at any one time.

Building a protein

The process of translation begins when the mRNA molecule with the **start codon** AUG attaches itself to the ribosome. AUG codes for methionine, the amino acid that most polypeptide chains start with.
The tRNA molecule carrying the amino acid methionine attaches itself at the P site of the ribosome.

Can you see that the tRNA anticodon UAC attaches to a compatible mRNA codon, AUG?

Now a second tRNA molecule attaches to the ribosome's A site. The two amino acids methionine and alanine are close enough for a **peptide bond** to form between them.
The bond between the first tRNA and methionine now breaks. This provides the energy to form the peptide bond between methionine and alanine.

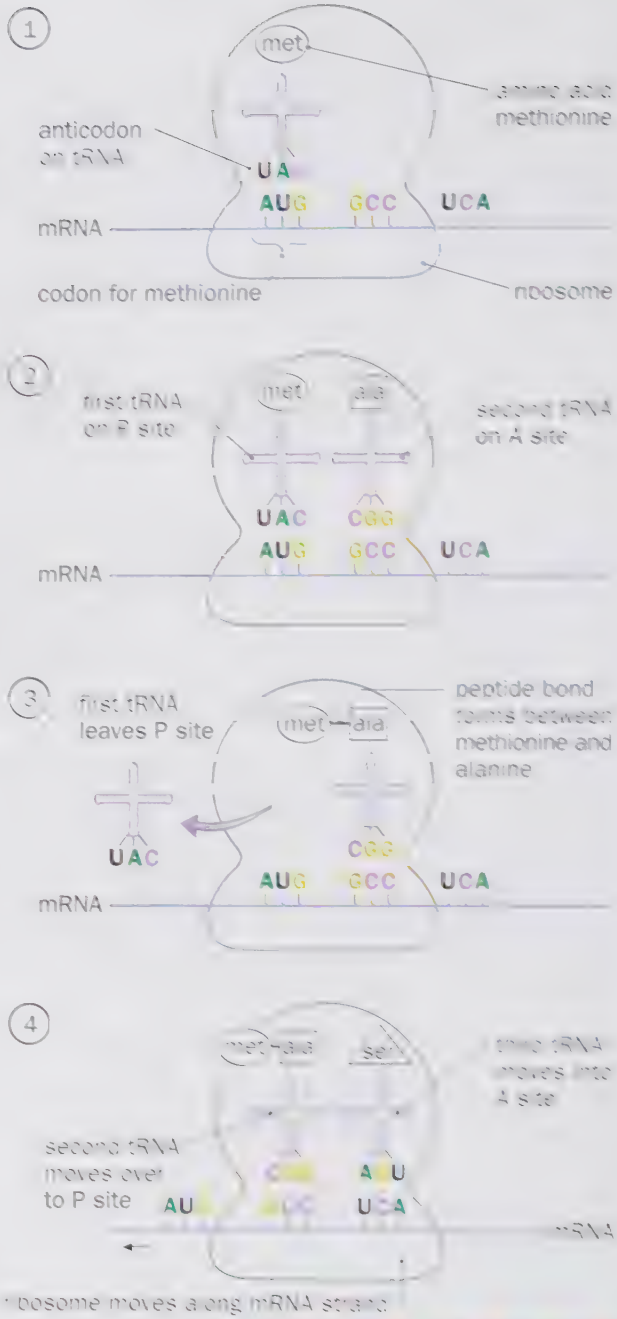
The first tRNA leaves the ribosome and the P site becomes vacant. The whole ribosome now moves one codon along the mRNA strand. So the second tRNA molecule now occupies the P site.
This leaves the A site free. A third tRNA comes in to fill the A site. Can you see its anticodon AGU matches the next codon on mRNA: UCA?
A peptide bond forms between alanine and serine.

The ribosome moves along the mRNA strand reading off the message a codon at a time.
As it does so, more tRNAs slot into the A site and bring with them their amino acids.
And so the polypeptide chain grows.
The process is repeated until a **stop codon** occurs in the mRNA, for example UAG, UGA or UAA.
These do not code for any amino acids.
The mRNA separates from the ribosome and the completed polypeptide chain is released to the cytoplasm.

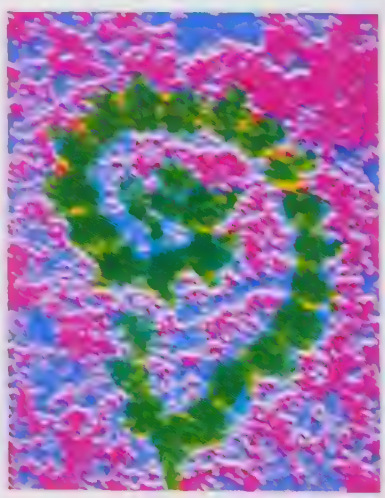
Both the ribosome and the mRNA can be used again.
Each mRNA can code for the production of many molecules of a particular polypeptide before stopping.
Usually a number of ribosomes can be found on a single mRNA, each reading off the coded information at the same time.

Such structures are known as **polyribosomes** or **polysomes**.

Try to complete the table by putting in the correct tRNA anticodon, amino acid and DNA base sequence.
You can find the full table of mRNA codons on page 58.



mRNA codon	tRNA anticodon	Amino acid	DNA base sequence
GGA			
UAC			
CCA			
CUG			
UUU			
AAG			
UGC			



Transmission electron micrograph of a ribosome

Summary

- A chromosome is made up of a long, super-coiled strand of DNA.
- A gene is a length of DNA that codes for the production of a particular protein.
- The DNA molecule consists of a double helix with pairs of bases bonded together.
- The sequence of bases on DNA is called the genetic code. It can be read off in base triplets called codons. Each codon codes for a particular amino acid.
- DNA is able to make new DNA identical to itself by semi-conservative replication.
- DNA can make messenger RNA by a process called transcription. Messenger RNA carries the coded information from DNA in the nucleus to the ribosomes where proteins are synthesised.
- Transfer RNA carries specific amino acids to the ribosomes to be assembled into protein.

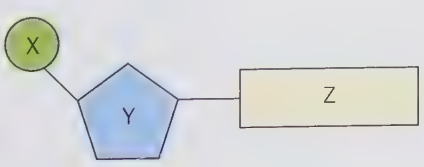
Questions

1 Copy and complete the following:
A molecule of DNA is made up of many sub-units called ___. Each nucleotide is made up of a ___ joined to a ___ sugar with a phosphate group. The DNA molecule consists of two strands running parallel to each other and coiled into a ___ ___. The bases in each strand are held together by ___ bonds. In RNA the base ___ is replaced by ___ and the pentose sugar present is ___. There are ___ different types of RNA and all are involved in the synthesis of ___. One type, called ___ RNA, forms the ribosomes in association with proteins. ___RNA is formed from a single strand of DNA by a process called ___. The third type of RNA is called ___RNA and its role is to pick up specific ___ ___ and carry them to the ribosomes.

2 Copy the table comparing DNA and RNA. If you think the feature is correct put a tick (✓) in the box. If you think the feature is incorrect put a cross (✗) in the box.

Feature	DNA	RNA
contains ribose		
is a single strand		
contains adenine, guanine and cytosine		
contains uracil		
contains equal proportions of purines and pyrimidines		

3 The diagram shows a nucleotide from a DNA molecule.



a) Name the parts labelled X, Y and Z.

b) Write down the base sequence of part of a strand of messenger RNA made from the following portion of a DNA molecule:
ATC GGA CTC TTC ATA GCG ACG GTA

c) The percentage of bases on one strand of DNA is thymine = 28% and cytosine = 22%.
What is the percentage of the bases adenine and guanine in this same strand of DNA?

4 Look at the base sequence of part of a molecule of messenger RNA (mRNA):
A U G A C G C A U G C A G U C C G A

a) How many codons are shown in this section of mRNA?

b) What is the role of each of these codons in protein synthesis?

c) Write down the transfer RNA anticodons specified by this mRNA strand.

5 Copy and complete the table showing three types of nucleic acid involved in protein synthesis.

Type of nucleic acid	Where formed	Function(s)	Where function(s) takes place
DNA			
mRNA			
tRNA			

6 Copy and complete the following table (the table of messenger RNA codons on page 58 will help you).

DNA double helix	non-coding strand			TGT				
	coding strand	AGC						
transcribed mRNA						GUA		
tRNA anticodon				GAG				UUU
amino acid incorporated into polypeptide			cys				met	

Table of messenger RNA codons and the amino acids for which they code

First base	Second base								Third base
	G		A		C		U		
G	GGG	glycine	GAG	glutamic acid	GCG	alanine	GUG	valine	G
	GGA	glycine	GAA	glutamic acid	GCA	alanine	GUA	valine	A
	GGC	glycine	GAC	aspartic acid	GCC	alanine	GUC	valine	C
	GGU	glycine	GAU	aspartic acid	GCU	alanine	GUU	valine	U
A	AGG	arginine	AAG	lysine	ACG	threonine	AUG	methionine	G
	AGA	arginine	AAA	lysine	ACA	threonine	AUA	isoleucine	A
	AGC	serine	AAC	asparagine	ACC	threonine	AUC	isoleucine	C
	AGU	serine	AAU	asparagine	ACU	threonine	AUU	isoleucine	U
C	CGG	arginine	CAG	glutamine	CCG	proline	CUG	leucine	G
	CGA	arginine	CAA	glutamine	CCA	proline	CUA	leucine	A
	CGC	arginine	CAC	histidine	CCC	proline	CUC	leucine	C
	CGU	arginine	CAU	histdine	CCU	proline	CUU	leucine	U
U	UGG	tryptophan	UAG	stop	UCG	serine	UUG	leucine	G
	UGA	stop	UAA	stop	UCA	serine	UUA	leucine	A
	UGC	cysteine	UAC	tyrosine	UCC	serine	UUC	phenylalanine	C
	UGU	cysteine	UAU	tyrosine	UCU	serine	UUU	phenylalanine	U

7 a) What will be the sequence of amino acids in a protein coded by this section of DNA?
GTG ACG GTG CAC ATT

b) The removal or substitution of a base of a DNA strand results in a point mutation. Suppose a point mutation occurs on the DNA strand in part a) producing:
i) GTG ACG GTG CTC ATT
ii) GTG ACG TGC ACA TTC
What effect would each of these mutations have on the protein molecule to be made?

8 The table at the top of the page shows some of the RNA codons (triplets of bases) together with the amino acids for which they code.

a) What sequence of amino acids does the following code in the DNA (deoxyribonucleic acid) give rise to?
— GCAGGACCAGCAACATAC —

b) The following amino acid sequence is from the beginning of the protein molecule of insulin.
Lysine—glutamine—threonine—alanine—alanine—alanine—lysine
What is the DNA code in the chromosome for this fragment of the protein?

c) What are the tRNA anti-codons that will 'plug in' to the code in a)?

9 Indicate whether you think the following statements are true or false.

a) DNA is a protein.
b) DNA is a double helix.
c) The molecular shape of DNA is maintained by hydrogen bonding between complementary base pairs.
d) DNA contains four bases, adenine, guanine, cytosine, and uracil.
e) Transcription, the transfer of information from DNA to RNA, occurs only when DNA is replicating.
f) In messenger RNA (mRNA) there are codons, each consisting of three bases.
g) Transfer RNA (tRNA) carries specific amino acids to ribosomes where complementary anticodons on tRNA match codons on mRNA.

10 The table lists amino acids and the base sequences on a messenger RNA (mRNA) strand which code for them in protein synthesis.

Amino acid	mRNA triplet code
Tyrosine	UAU
Alanine	GCG
Phenylalanine	UUU
Leucine	UUA
Arginine	CGU
Glycine	GGG
Arginine	AGG

A DNA strand has the base sequence:

AATCGCAAATCCCGCATAATTTAG

a) Name the amino acid which would be placed **third** in the growing polypeptide chain.
b) Name the **fifth** amino acid in the chain if a single base deletion occurred after the sequence AAA.
c) Suggest a specific factor which could have caused this change.
d) A transfer RNA (tRNA) molecule carries the anticodon CCC. Name the specific amino acid carried by this tRNA molecule.

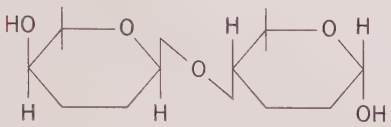
Biological molecules

1 The statements in the table below refer to three polysaccharide molecules. Copy and complete the table. If the statement is correct, place a tick (✓) in the appropriate box and if the statement is incorrect, place a cross (✗) in the appropriate box.

Statement	Starch	Glycogen	Cellulose
Polymer of α-glucose			
Glycosidic bonds present			
Unbranched chains only			
Energy store in animal cells			

Edexcel **[4]**

2 The diagram shows a molecule of lactose.



a) Draw a diagram to show the monosaccharides formed when lactose is hydrolysed. [2]

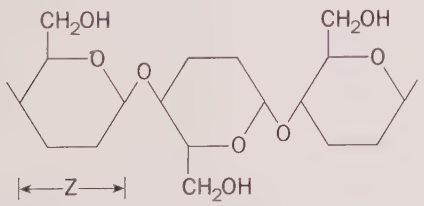
b) Name the enzyme which catalyses the hydrolysis of lactose. [1]

c) Lactose is a reducing sugar. Describe how you would test a liquid to show that it contains reducing sugar. [3]

[6]

3 a) Cellulose is the most abundant naturally occurring carbon compound on Earth. Where is cellulose most likely to be found? [1]

b) The diagram below shows part of a molecule of cellulose.



i) Name the carbohydrate labelled Z. [1]

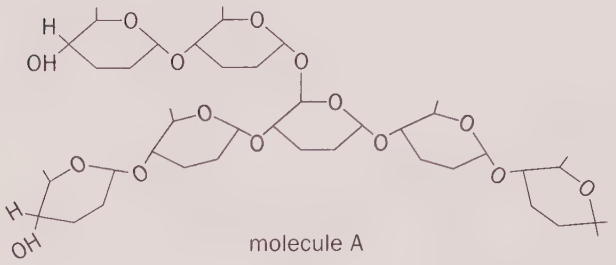
ii) Which of these terms correctly describes the molecule labelled Z?
hexose pentose tetrose triose [1]

iii) 1 Describe how units in this diagram are arranged in a complete molecule of cellulose. [1]

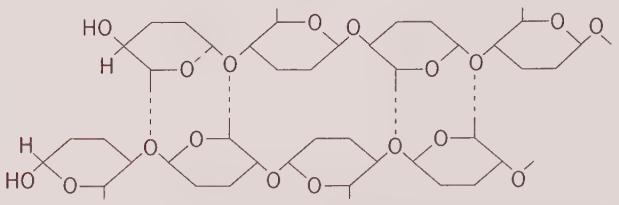
2 Describe *one* property this molecular structure gives to cellulose. [1]

WJEC **[5]**

4 The diagrams of molecule A and molecule B show part of the molecular structure of two polysaccharides. The hexagonal shapes represent hexose sugars.



molecule A



molecule B

Key: ---- Hydrogen bonds

a) Give the name of molecule A. [1]

b) Give *one* difference between the hexose sugars in molecules A and B. [1]

c) Both polysaccharides contain hexose sugars joined by glycosidic bonds.

i) Explain, using an annotated diagram, how these bonds in molecule A are hydrolysed in the process of human digestion. [2]

ii) Using information in the diagram of molecule B, suggest *one* reason why it cannot be digested by humans. [1]

AQA (formerly AEB) **[5]**

5 a) Amino acids form part of the structure of proteins.

i) Draw a diagram to show the general structure of an amino acid molecule. [1]

ii) State the name given to the sequence of amino acids in a protein molecule. [1]

iii) Describe how two amino acids join together. [3]

b) Haemoglobin is an important protein in mammals.

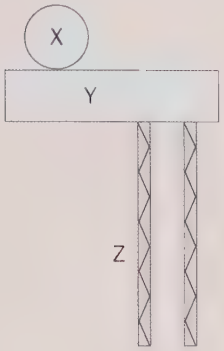
i) State one function of haemoglobin. [1]

ii) Describe the structure of haemoglobin. [3]

[9]

6 a) Describe how you could carry out the emulsion test to show that olives contain lipids. [3]

The diagram shows the structure of a phospholipid molecule.



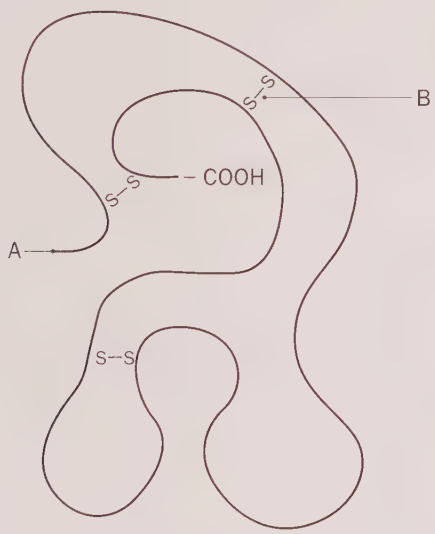
b) i) Give the name of the parts of the molecule labelled X and Y. [2]

ii) Z is a fatty acid. Fatty acids can be saturated or unsaturated.
Describe the difference between a saturated fatty acid and an unsaturated fatty acid. [2]

c) Describe how the structure of a phospholipid molecule differs from that of a triglyceride molecule. [1]

[8]

7 The diagram below shows a molecule of a protein called lactalbumin.



a) Give the name of the chemical group found at A? [1]

b) Lactalbumin has a tertiary structure. Using evidence from the diagram, describe the tertiary structure of a protein. [2]

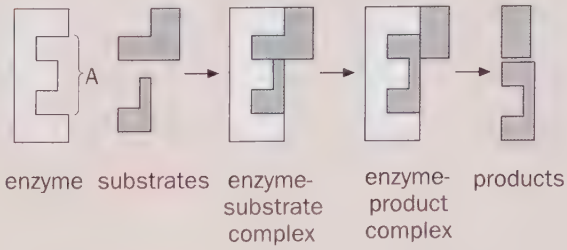
c) Lactalbumin does not have a quaternary structure. Use the diagram to explain why. [1]

d) Lactalbumin is not a conjugated protein. Use the diagram to explain why. [1]

CCEA (formerly NICCEA) **[5]**

Enzymes

8 The diagrams below illustrate one model of enzyme action.



a) Name the part of the enzyme labelled A. [1]

b) Explain how this model can account for enzyme specificity. [2]

c) With reference to this model, explain the effect of a competitive inhibitor on an enzyme-catalysed reaction. [2]

Edexcel **[5]**

9 a) Explain how the following are related to the protein structure of enzymes.

i) The effect of high temperature on an enzyme catalysed reaction.

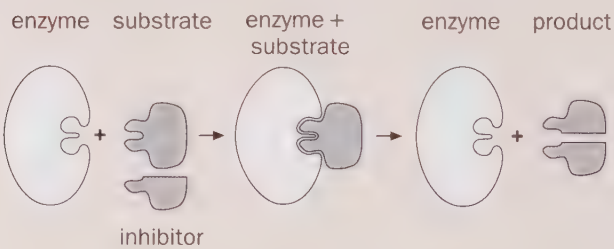
ii) Substrate specificity.

iii) The effect of inhibitors. [10]

b) Suggest a simple method by which you could find out whether an enzyme catalysed reaction is being inhibited by a *competitive* or a *non-competitive* inhibitor. [2]

AQA (formerly NEAB) **[12]**

10 The diagram below illustrates the induced fit model of enzyme action.



a) Use the diagram to explain the following.

i) The induced fit model. [2]

ii) Competitive inhibition. [2]

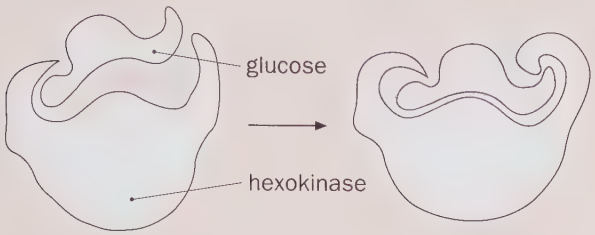
b) How would the diagram be different if it were used to illustrate the lock and key hypothesis? [1]

AQA (formerly AEB) **[5]**

11 Hexokinase is an enzyme. It catalyses the phosphorylation of glucose in cellular respiration.

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

The polypeptide chains of this enzyme are folded into the shape shown in the diagram below. There is a deep groove in the molecule. ATP binds at the surface of this groove. Glucose can also bind at this groove, but as it does this it causes a change in the shape of the enzyme molecule. The product cannot dissociate from the enzyme until the shape reverts to that of the free enzyme.



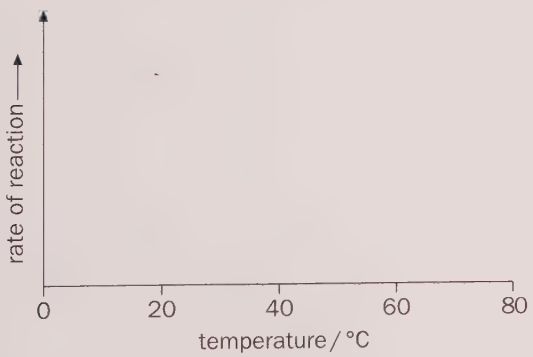
a) i) State another name for the region in the groove where glucose and ATP bind. [1]
ii) Explain how the structure of the enzyme enables the reaction to take place. [4]

b) i) Sketch a curve to show how increasing the substrate concentration affects the rate of an enzyme-catalysed reaction. [3]
ii) Explain the shape of the curve you have drawn. [3]

c) The optimum pH for hexokinase is pH 7. Suggest why only a little glucose-6-phosphate is produced when the enzyme is in a buffered solution of pH 6. [2]

OCR (formerly Camb) **[13]**

12 a) i) Copy the axes below. Sketch a graph to show the expected effects of temperature on the rate of an enzyme-catalysed reaction. [3]



ii) Explain the effects of temperature on this reaction. [4]

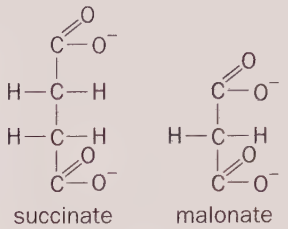
b) The rates of enzyme-catalysed reactions can also be affected by pH. Explain how change in pH affects enzyme structure. [4]

OCR **[11]**

13 Succinate dehydrogenase is an enzyme which catalyses the conversion of succinate to fumarate.

a) Use your knowledge of enzyme structure to explain why succinate dehydrogenase catalyses this reaction only. [2]

b) Malonate is an inhibitor of succinate dehydrogenase. The structural formulae of malonate and succinate are shown in the diagram below.



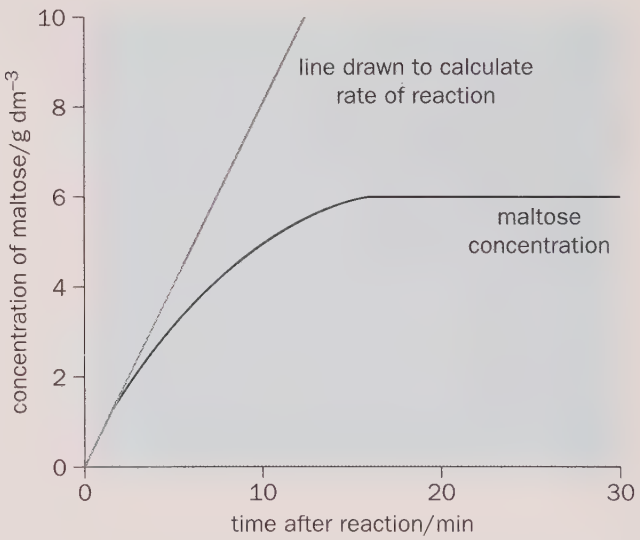
Use the information in the diagram to explain how malonate inhibits the enzyme. [2]

AQA (formerly NEAB) **[4]**

14 Amylase is an enzyme. It catalyses the breakdown of starch to maltose.
Students mixed a starch solution with amylase. They recorded the concentration of maltose at intervals for 30 minutes.
The concentration of amylase and the concentration of starch were controlled.

a) Suggest *two* other factors the students should have controlled. [2]

The graph shows their results.

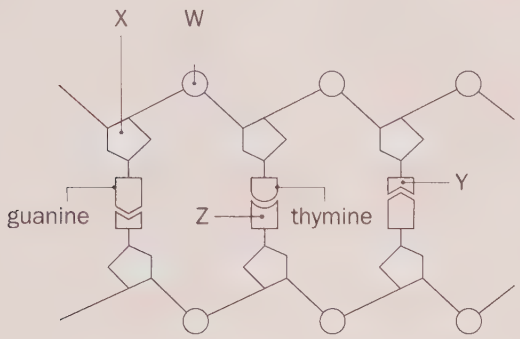


b) Describe how the concentration of maltose changed over the period shown in the graph. [2]

c) i) A tangent has been drawn to the curve in this graph. Explain how you could use this line to calculate the initial rate of reaction. [1]
ii) The rate of reaction was lower after 10 minutes than it was at the start. Explain why. [2]
[7]

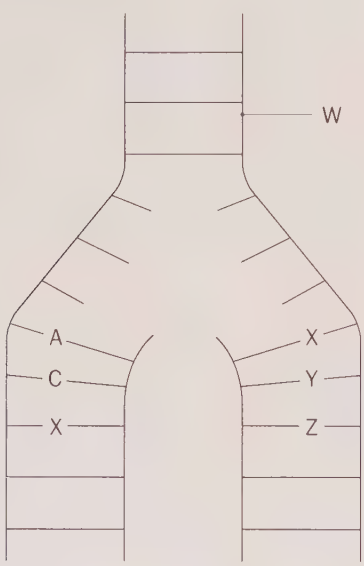
Nucleic acids and protein synthesis

15 The diagram below shows part of a DNA molecule.



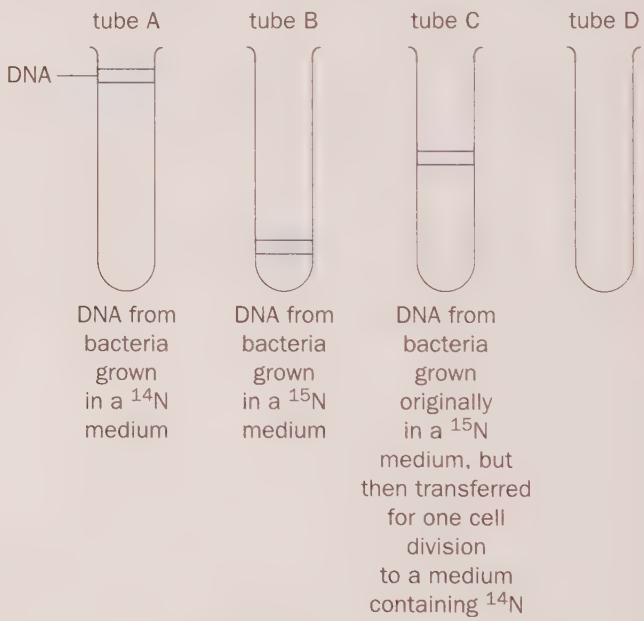
a) Give the name of the parts labelled W, X, Y and Z. [4]
b) A section of a DNA molecule had 500 bases. 44% of these bases were cytosine.
i) Calculate the number of each of the bases present. [4]
ii) Give two reasons why some of the base triplets in a gene do not code for amino acids. [2]
AQA (formerly NEAB) **[10]**

16 The diagram below shows the process of DNA replication. The horizontal lines represent the positions of the bases.



a) In the diagram, A represents adenine and C represents cytosine.
Name the base found at positions X, Y and Z. [3]
b) DNA replication is enzyme controlled.
Describe the role of the following enzymes in DNA replication:
i) DNA helicase, [2]
ii) DNA polymerase. [2]
AQA **[7]**

17 a) The replication of DNA is described as semi-conservative. Explain why. [2]
Bacteria require a source of nitrogen to make the bases needed for DNA replication. In an investigation of DNA replication, some bacteria were grown for many cell divisions in a medium containing ^{14}N, a light form of nitrogen.
Others were grown in a medium containing ^{15}N, a heavy form of nitrogen. Some of the bacteria grown in a ^{15}N medium were then transferred to a ^{14}N medium and left to divide once.
DNA was extracted from the bacteria and centrifuged.
The DNA samples formed bands at different levels in the centrifuge tubes, as shown in the diagram.



b) i) Suggest what tubes A and B show about the density of the DNA formed using the two different forms of nitrogen. [1]
ii) Explain the position of the band in tube C. [2]
c) The bacteria were allowed to divide once more in the ^{14}N medium. DNA was extracted from the bacteria and centrifuged.
Sketch the position of the DNA in the centrifuge tube D. [2]
[7]

18 The diagram below shows the sequence of bases in one strand of the DNA from part of a gene. The base sequence is read from left to right.

DNA base sequence

A C C C C A T T T C A T C C A

The table below shows the anticodons of some tRNA molecules and the specific amino acids each would carry.

tRNA anticodon	Amino acid
CGA	Alanine
CCA	Glycine
UUU	Lysine
GGA	Proline
ACC	Tryptophan
CAU	Valine

a) Using this information, give the amino acid sequence coded for by this part of the gene. [2]

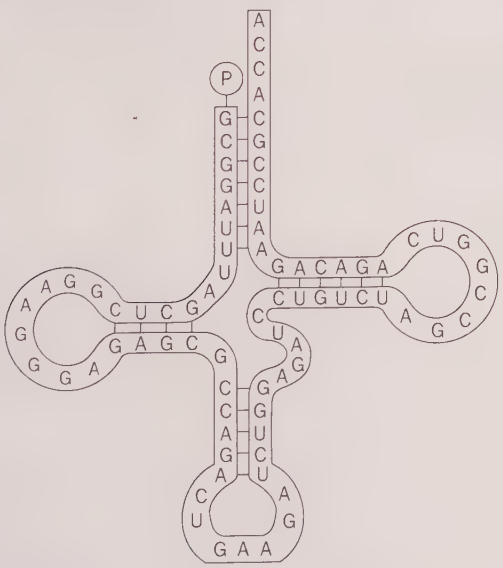
b) The diagram below shows the same length of DNA after it has undergone a mutation.

A C C C G A T T T C A T C C A

This mutation may affect the protein produced. Suggest how. [3]

Edexcel **[5]**

19 The diagram below shows the structure of a tRNA molecule.



a) Give *two* ways in which the structure of a tRNA molecule differs from that of a DNA molecule. [2]

b) Explain how the specific shape of the tRNA molecule shown in the diagram is determined by the pattern of bonding. [2]

c) i) Give the base sequence of the anticodon of this tRNA molecule. [1]

ii) Which mRNA codon would correspond to this anticodon? [1]

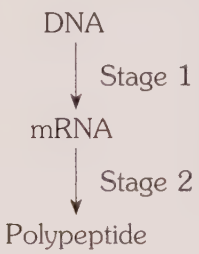
AQA (formerly NEAB) **[6]**

20 Copy and complete the table below to give correct functions of DNA, messenger RNA (mRNA) and transfer RNA (tRNA) during protein synthesis. Place a tick (✓) in the appropriate box if the statement is correct and a cross (✗) in the box if the statement is incorrect. [6]

Statement	DNA	mRNA	tRNA
Attaches to ribosome			
Carries amino acid to ribosome			
Translated			
Transcribed but not translated			
Site of anticodon			
Site of codon			

WJEC **[6]**

21 The diagram below outlines protein synthesis in a cell.



a) Name stages 1 and 2. [2]

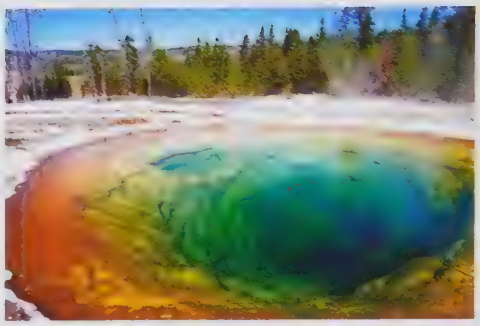
b) Where does stage 2 take place within a cell? [1]

c) Describe the role of tRNA in stage 2. [3]

Edexcel **[6]**

4 Cell structure

Some cells are able to tolerate extreme conditions.
Microbes are single-celled organisms. Some can be found in water heated to boiling point by volcanoes, and at temperatures below zero.
They can be found in pools saturated with salt or sodium carbonate.
These microbes are known as **extremophiles** and some are thought to be closely related to the earliest forms of life on Earth.



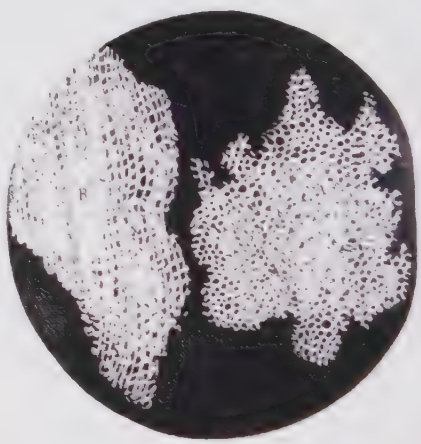
Thermophilic bacteria can live in these hot springs in Yellowstone National Park, USA

Extremophiles include **thermophiles**, bacteria that are able to thrive at 70 °C in hot volcanic springs.
Halophiles are able to tolerate the extreme salinity in salt lakes where evaporation concentrates and crystallises the seawater.
Alkalinophiles can survive at a pH of 10 and above.
They are found in soda lakes rich in sodium carbonate and sodium chloride.
Acidophiles, such as the bacterium *Thiobacillus ferro-oxidans*, are able to tolerate pHs below 1!

These conditions would be enough to kill most cells because their enzymes would denature, or water would be drawn out of the cells by osmosis.
These organisms may provide clues about how and where life originated.

Beginnings

Robert Hooke was the first person to observe cells in 1665.
He looked at a thin slice of cork under a primitive microscope.
It had a honeycomb appearance made up of lots of 'little boxes' that he called cells.



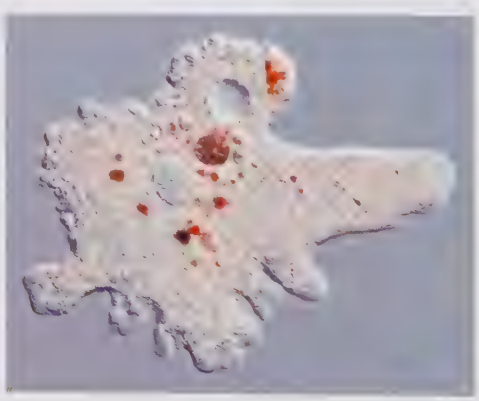
The cork cells observed by Robert Hooke

Cork is in fact dead bark taken from the cork tree.
Hooke didn't realise that the small, dark holes that he was seeing were once filled with a living material – **protoplasm**.
Following his discovery, Hooke and other early microscopists found that different types of plant material were all composed of cells.

In 1883, botanist Matthias Schleiden and zoologist Theodor Schwann proposed that all plants and animals were composed of cells, and that cells were the basic building blocks of life.
In 1855, Rudolf Virchow stated that new cells could only arise from the division of pre-existing cells, and that the chemical reactions of life took place inside cells.

These observations led to the formulation of the modern **cell theory**.

All living things are made up of cells.
New cells are formed by the division of pre-existing cells.
Cells contain genetic material, which is passed on from parent to daughter cells.
All metabolic reactions take place inside cells.

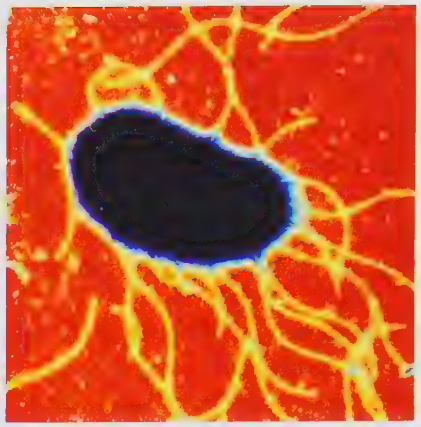


Amoeba is unicellular

Some organisms are made up of just ***one*** cell.
These are described as **unicellular**.
Other organisms are **multicellular** and are composed of millions of cells.
Each of us is made up of over 50 million million cells.

What are the four main principles of the cell theory?

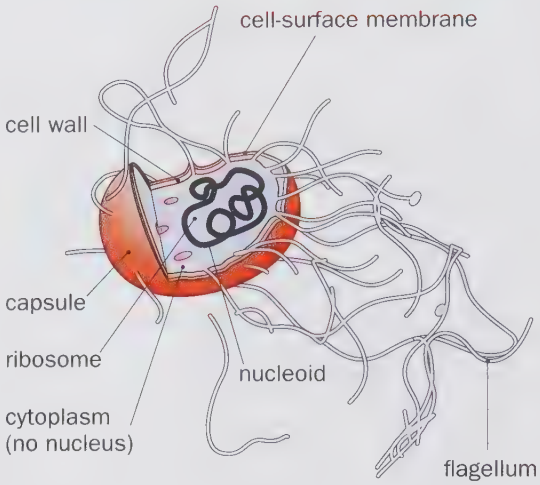
Prokaryotic cells



There are two distinct types of cell: **prokaryotic cells** and **eukaryotic cells**. Prokaryotic cells are more primitive than eukaryotic cells and have a simpler structure.
They have no nucleus nor do they have distinct membrane-bound cell organelles.

Organisms made of prokaryotic cells are called **prokaryotes**.
Organisms made up of eukaryotic cells are called **eukaryotes**.

Prokaryotes are relatively simple organisms, such as bacteria and blue-green bacteria. Cells like these were probably the first living things to evolve on Earth – a few billion years ago.
The word 'prokaryote' means 'before the nucleus' and if you look at the diagram right of the bacterium you can see why.
The single chromosome containing the bacterial DNA is not surrounded by a nuclear membrane and is not associated with proteins.
It is referred to as a **nucleoid**.



In addition to the main chromosome, bacteria may have other small rings of DNA called **plasmids**.
Plasmids can replicate themselves independently of the main chromosome.

Bacteria have a rigid **cell wall** outside the cell-surface membrane containing a strengthening material called **murein** to protect the cell.

The **cell-surface membrane** sometimes develops inner extensions called **mesosomes**.
This is where respiration takes place.

Bacteria feed by **extracellular digestion**.
They release enzymes onto their potential food and absorb the digested products. These enzymes are made inside **ribosomes**.

Some bacteria have **flagella**, which beat to propel the cell along.

	Prokaryotes	Eukaryotes
organisms	bacteria and blue-green bacteria	plants, animals, fungi and protoctists
size	small, ranging from 1 to 10 μm	large, usually between 10 and 100 μm
nucleus	a nucleoid no distinct nucleus as the main chromosome is not surrounded by a nuclear membrane	distinct nucleus with linear chromosomes surrounded by a nuclear membrane
DNA	DNA is circular and not associated with proteins plasmids present	DNA is linear and associated with histone proteins plasmids absent
cell walls	rigid cell wall containing the glycoprotein murein	present in plants, fungi and some protoctists but not containing murein
organelles	few organelles except for small ribosomes (70S type) and none are membrane-bound	many membrane-bound organelles such as mitochondria and chloroplasts ribosomes are larger (80S type)
flagella	flagella, if present, lack microtubules	flagella have microtubules

1 m = 1000 mm
1 mm = 1000 μm
1 μm = 1000 nm

Eukaryotic cells

Eukaryotic cells probably evolved from prokaryotic cells some 1 billion years ago.
'Eukaryote' means 'true nucleus'.
The DNA in these cells is held within a nuclear membrane.
Eukaryotic cells have **organelles** such as **mitochondria** and **chloroplasts**.
These are surrounded by membranes, which allow metabolic processes such as respiration and photosynthesis to take place within the organelle.
The enzymes controlling these processes are often located ***within*** the membranes.
This greater degree of organisation has resulted in an increase in cell size.

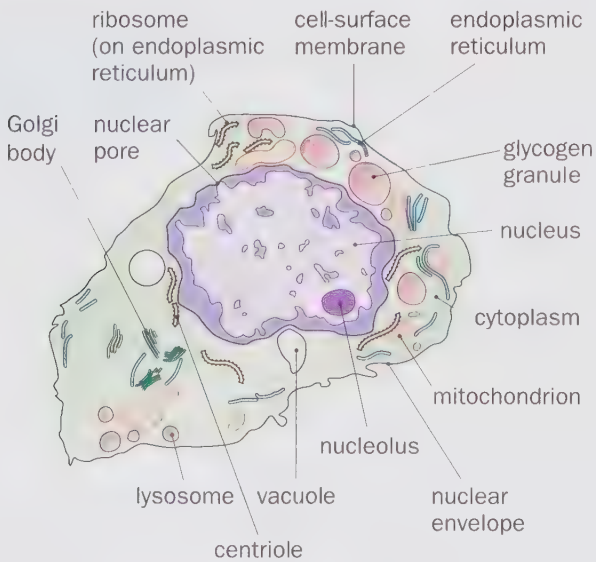
Eukaryotes have a proper nucleus: their DNA is enclosed by a nuclear membrane.
Prokaryotes do not have a nucleus: their DNA is in direct contact with the cytoplasm.

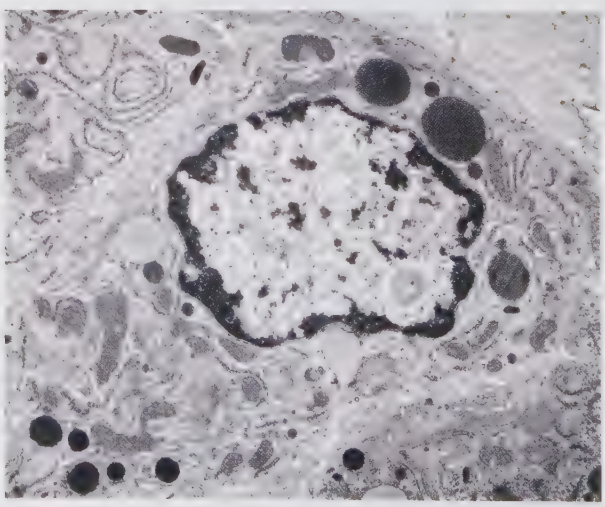
Animal cells

What does a typical animal cell look like?

That's not an easy question, because there are many different types.
The structure of a cell is adapted to carry out a particular function.
For instance, a sperm cell has a tail to swim; a nerve cell has a long cell process called an axon to transmit impulses.
However, animal cells have many things in common.

Animal cells are surrounded by a cell-surface membrane or **plasma membrane**.
Inside the membrane is the jelly-like **cytoplasm**, which contains the **nucleus** and other organelles.
These other cell components include the **endoplasmic reticulum**, mitochondria, **Golgi bodies**, **centrioles**, **lysosomes**, ribosomes and **cytoskeleton**.
This detailed organisation is known as the **ultrastructure** of the cell.



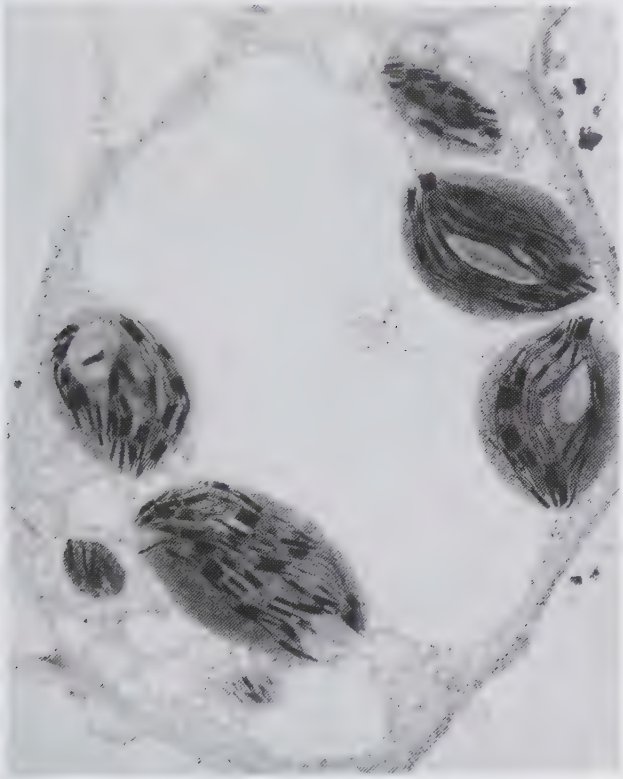


A liver cell as seen under the electron microscope

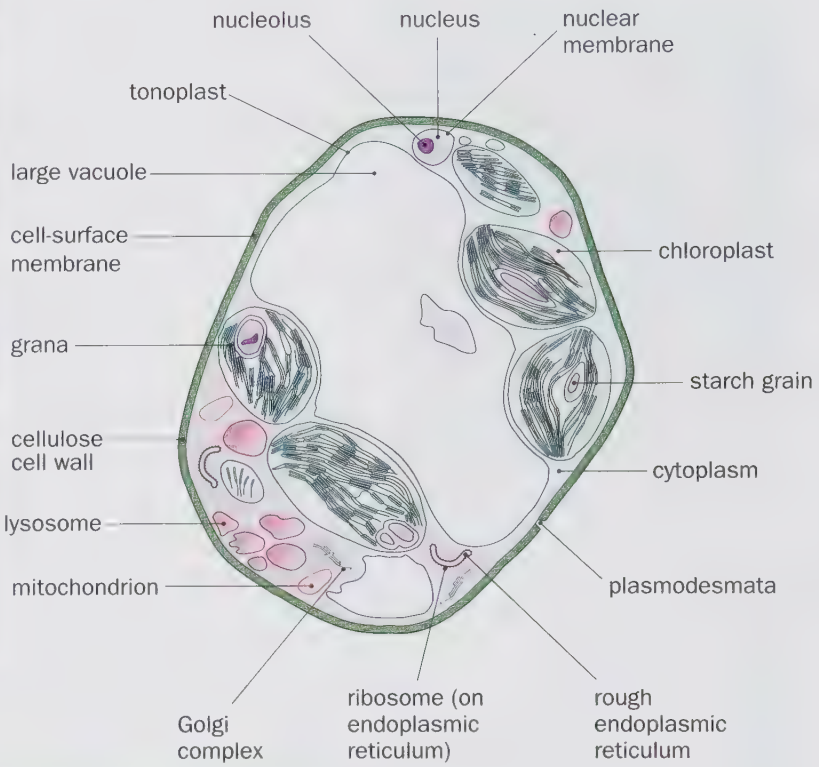
Plant cells

Plant cells have all the structures found in animal cells.
Look at the diagram. Can you see any ***additional*** features?

All plant cells are enclosed by a **cellulose cell wall**, outside the cell-surface membrane.
This supports the cell and gives it its regular geometric shape.
The cell wall may contain a number of small holes called **pits**.
The pits allow strands of cytoplasm, called **plasmodesmata**,
to link different cells together.
Most of the inside of the cell is taken up by a fluid-filled **vacuole**.
A large vacuole is a permanent feature of plant cells.
It is surrounded by a membrane called the **tonoplast**.
The presence of chloroplasts is one of the most important features of plant cells
as this is where **photosynthesis** takes place.



Note the typical plant cell features in this palisade cell: chloroplasts, starch grains, cellulose cell wall and a large vacuole

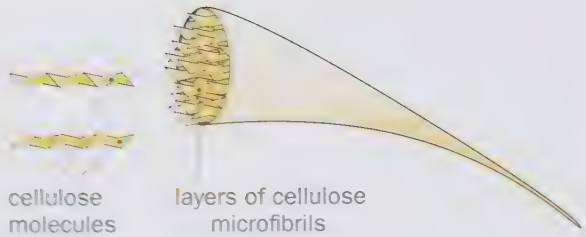


Differences between plant and animal cells

Plant cells	Animal cells
cellulose cell wall	no cell wall
plasmodesmata	no plasmodesmata
chloroplasts	no chloroplasts
large, permanent vacuole filled with cell sap	small, temporary vacuoles
tonoplast around vacuole	no tonoplast
no centrioles	centrioles present
starch grains for storage	glycogen granules for storage

Cellulose cell wall

The plant cell wall consists of tiny cellulose fibres called **microfibrils**, glued together by a mixture of polysaccharides. Each microfibril is made up of thousands of cellulose molecules bound together by pectins and hemicelluloses.



Cell walls have the following functions:

- They provide support for the cell by allowing it to become **turgid**. As water enters the vacuole by osmosis, the plant cell expands. The cell wall has to be strong enough to resist this expansion and so enable the cell to become turgid.
- They provide mechanical strength to support the cell. The cellulose microfibrils are very strong. The strength may be increased by the addition of **lignin** in tissues such as xylem. In cells such as **collenchyma**, extra cellulose is added to the cell wall to increase mechanical support.
- They are freely permeable to water and substances in solution.
- They have narrow pores (pits) through which fine strands of cytoplasm (plasmodesmata) are able to pass. These connections allow exchange of materials between the living cell contents.
- The cell walls of adjacent cells are glued together by the **middle lamella**. This is a jelly-like substance made up of calcium pectate and magnesium pectate.

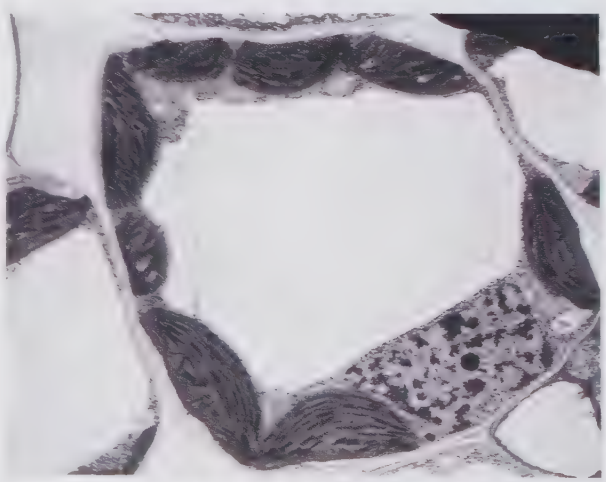
Microfibrils make up this cellulose cell wall

Vacuole

Plant cells have a large, permanent vacuole bounded by a membrane called the tonoplast. The vacuole contains cell sap, which is a solution of sugars, amino acids, mineral salts and waste chemicals dissolved in water. In some cells the sap contains pigments; for instance, beetroot cells contain betacyanin, which give them their characteristic purple colour.

Plant cell vacuoles have a number of functions:

- Water tends to enter the vacuole by **osmosis**. The cell contents expand and push against the cell wall, which resists this stretching with an equal and opposite force. The cell can take in no more water and is said to be turgid (see page 91).
- Vacuoles act as stores for foods like sugars and amino acids.
- Vacuoles accumulate waste products such as tannins. If these wastes accumulate in the vacuoles of leaf cells, they are excreted when the leaves fall.
- The vacuoles of some cells contain pigments, which give colour to parts of the plant like petals.



In this mature plant cell, the vacuole, bounded by the tonoplast takes up most of the cell volume

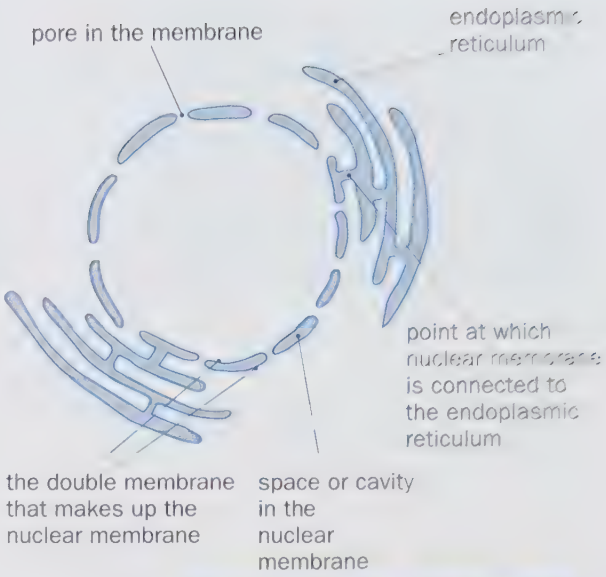
Nucleus

The nucleus is the largest organelle in the cell and can be seen with a light microscope.
Nuclei are spherical and each is about 10 μm in diameter.

The nucleus is surrounded by a nuclear membrane.
The nuclear membrane is a double membrane with a space in between.

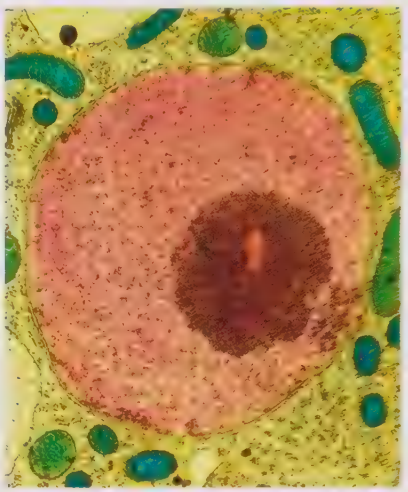
There are many large pores in the membrane, which allow materials to pass in and out of the nucleus.

As you can see in the diagram, the nuclear membrane is connected to a system of membranes called the endoplasmic reticulum.



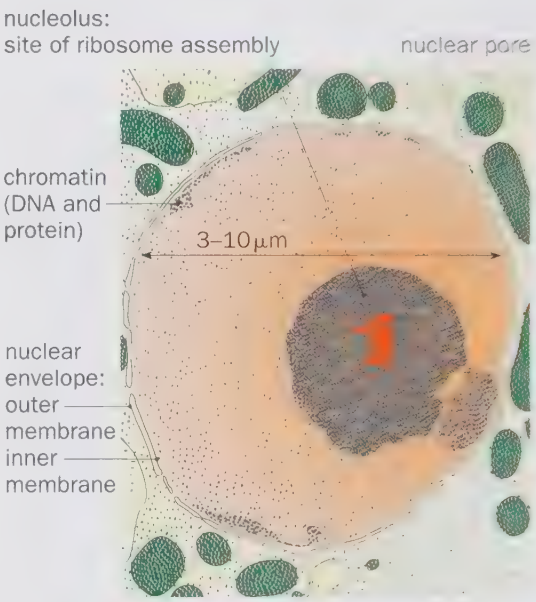
Nucleoplasm is the name given to the material inside the nucleus. It contains **chromatin**, which is made up of DNA attached to proteins called **histones**.
In a non-dividing cell the chromatin is spread throughout the nucleus in the form of tiny granules.
At times of cell division, the chromatin condenses, pulling the DNA into thread-like **chromosomes**.
This is why chromosomes only become visible at times of cell division.

The nucleus also contains a spherical structure called a **nucleolus**.
The nucleolus is important as it makes ribonucleic acid (RNA), which is needed to make ribosomes.
This is looked at in Chapter 3.



A false colour electron micrograph of a cell nucleus

- The nucleus contains the genetic material DNA, together with histone proteins in the chromosomes (see page 49).
- The DNA carries genetic information for the synthesis of proteins, including enzymes.
- The DNA in the nucleus codes for the production of enzymes. Enzymes control the chemical reactions taking place in the cell. So the genetic material of the nucleus controls the metabolism of the cell.
- The nucleus produces new chromosome material at cell division so that each daughter cell has the same amount of DNA and is genetically identical.

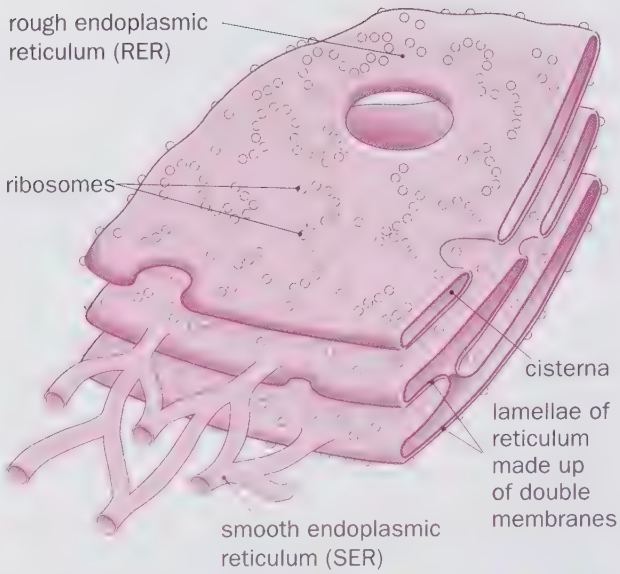


Can you think of any cells that ***do not*** have a nucleus?

Red blood cells lose their nuclei and this enables them to carry more **haemoglobin** and so pick up more oxygen.
Phloem sieve tubes provide the transport system for sucrose in plants. They have lost most of the cell organelles including their nuclei, which makes it easier for materials to flow through the cell.

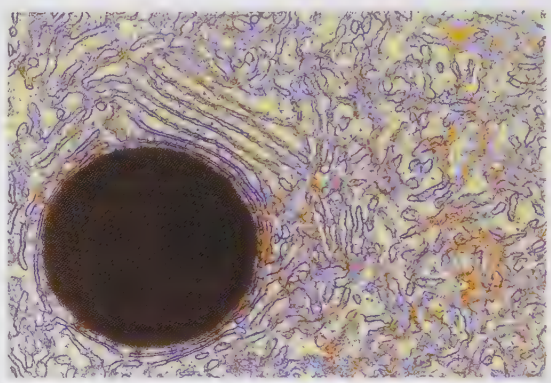
Endoplasmic reticulum

The endoplasmic reticulum (ER) is a complex system of double membranes.
The fluid-filled spaces between the membranes are called **cisternae**.
As the ER is connected with the nuclear membrane and the cell-surface membrane, the cisternae form a system of little passages, which allow materials to be transported throughout the cell.



Where ribosomes are present on their outer surface, the membranes are called **rough endoplasmic reticulum** (**RER**).
The main function of rough ER is to package and transport proteins made by the ribosomes.
Cells that manufacture a lot of protein, for instance those making digestive enzymes in the gut, have large amounts of rough ER.

Not all ER is covered in ribosomes.
ER with no ribosomes is called **smooth endoplasmic reticulum** (**SER**).
Smooth ER is the site of lipid synthesis.
So large amounts of smooth ER are located in cells that make lipids and steroids, for example cells of the liver and testis.



A transmission electron micrograph (TEM) of SER no attached ribosomes

ER has the following functions:

- to form an extensive transport system throughout the cell,
- production and packaging of proteins (rough ER),
- synthesis of lipids and steroids (smooth ER),
- collection, storage and distribution of these materials.

Ribosomes

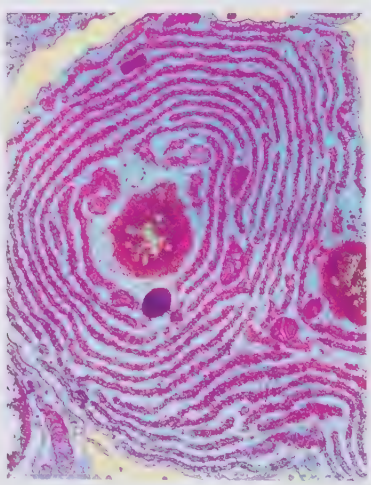
Ribosomes are small, dense organelles that are found in huge numbers in all cells.

There are two types of ribosomes depending on the cells that they are found in:

- **80S type** is about 20 nm in diameter and found in eukaryotic cells,
- **70S type** is slightly smaller and found in prokaryotic cells.

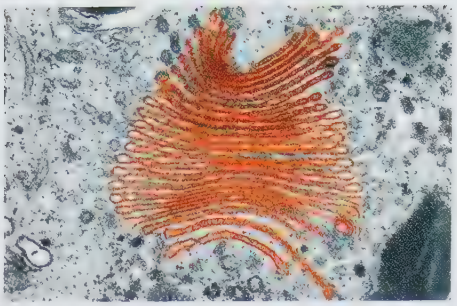
Ribosomes can occur free in the cytoplasm where they synthesise enzymes that are used in the cytoplasm, such as those enzymes involved in glycolysis (the first stage of respiration).
Many of the cell's ribosomes are attached to ER (rough ER).

Ribosomes are manufactured in the nucleolus from ribosomal RNA and protein.
Each ribosome is made up of one small sub-unit and one large sub-unit.
Ribosomes are the sites of protein synthesis in the cell.



Can you see ribosomes on this TEM of RER?

Golgi body



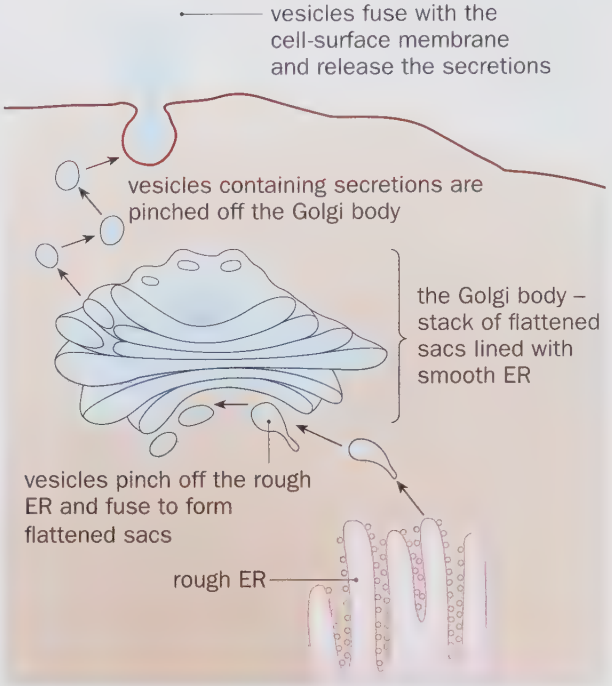
TEM of a Golgi body in section

These organelles were first observed by the Italian scientist Camillo Golgi at the end of the nineteenth century. They appear as stacks of flattened sacs.

Electron micrographs show how Golgi bodies are formed.
Small pieces of rough ER are pinched off at the ends to form small vesicles.
A number of these vesicles then join up and fuse together to make a Golgi body (also known as Golgi apparatus).

Proteins that were made and stored in the rough ER are transported in the small vesicles and collect in the Golgi bodies.
Here the proteins are modified and combined with carbohydrates or fats to make new molecules such as glycoproteins.

Small vesicles can then become pinched off from the Golgi body, carrying the new chemical products away. These chemicals can be secreted when the vesicle moves to the cell-surface membrane.
Sometimes the vesicles that are pinched off from the Golgi body become lysosomes.



The main functions of the Golgi body are:

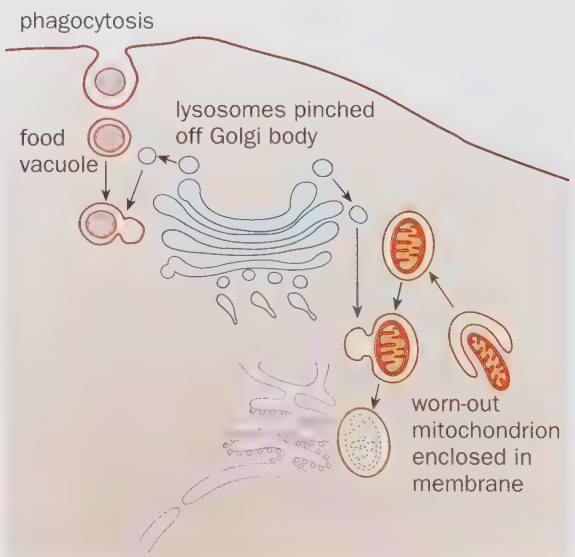
- assembling glycoproteins, such as **mucin**, by combining carbohydrate and protein,
- transporting and storing lipids,
- formation of lysosomes,
- production of digestive enzymes,
- secretion of carbohydrates for the formation of plant cell walls and insect cuticles.

Lysosomes

Lysosomes are small vesicles formed when small pieces of the Golgi body are pinched off at the end.
They contain a variety of hydrolytic enzymes, referred to as lysozyme, which can digest material within the cell.
The membrane around the lysosome prevents these enzymes from digesting the cell itself.

The main functions of lysosomes are linked to the actions of these enzymes.

- Lysosomes release enzymes, called lysozymes, that destroy worn-out organelles in the cell.
- They digest material that has been taken into the cell.
  For instance, some white blood cells are able to engulf bacteria.
  The bacterium is taken into the cytoplasm inside a vesicle.
  Lysosomes discharge their contents into the vesicle and digest the bacterium.
  This process is known as **phagocytosis** (see page 94).
- Lysosomes release their enzymes to the outside of the cell to digest other cells. This process is called **exocytosis** (see page 94).
- They can cause the cell to self-destruct.
  The lysosome's membrane breaks down, releasing its enzymes and digesting the entire cell (**autolysis**).



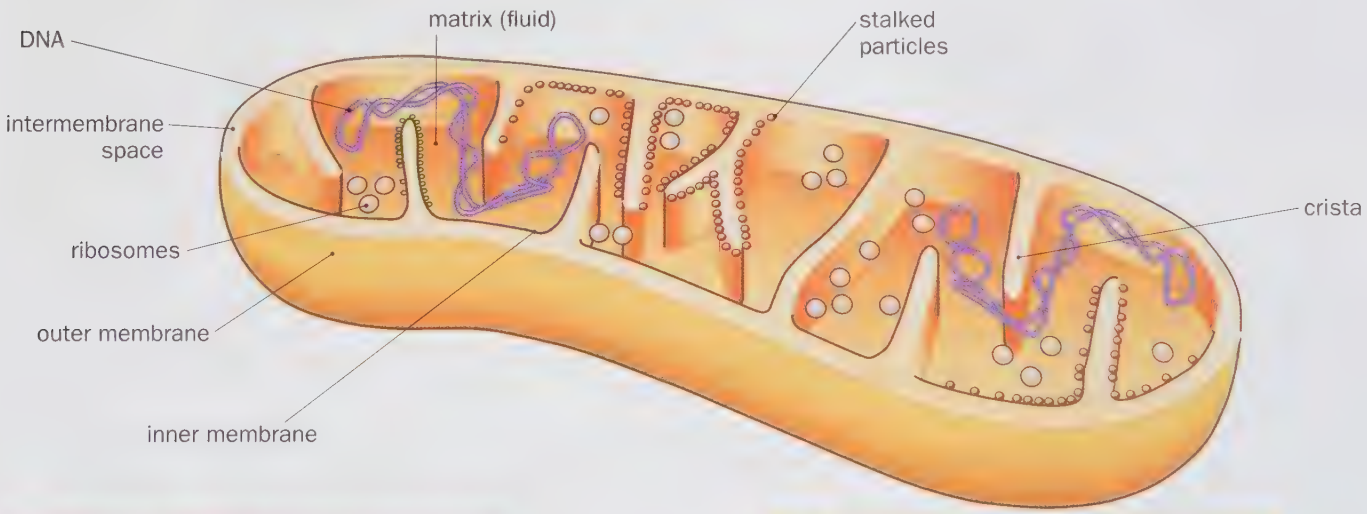
Mitochondria

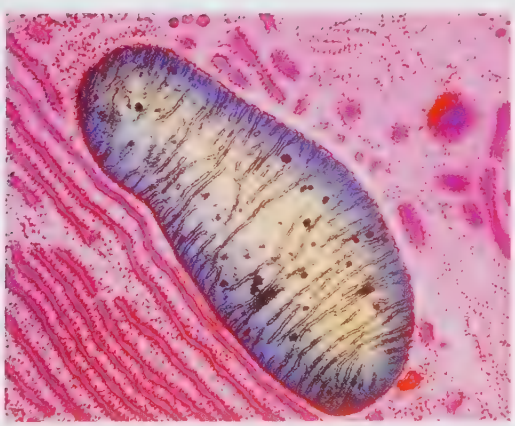
Mitochondria are relatively large organelles found in all eukaryotic cells. They are barely visible under the light microscope, but the electron microscope reveals them as sausage-shaped structures about 1 μm wide and 5 μm long.

Each mitochondrion has a double membrane, the outer one of which controls the entry and exit of materials.
The inner membrane forms many folds called **cristae**.
The surface of each crista is covered in stalked particles, where energy-rich **ATP** (**adenosine triphosphate**) is made.
The mitochondrion is filled with a jelly-like **matrix**, where some of the enzymes used in respiration are found.
The matrix contains ribosomes and loops of DNA, which enables the mitochondria to replicate themselves when the cell divides, so that both daughter cells produced will have enough mitochondria of their own.

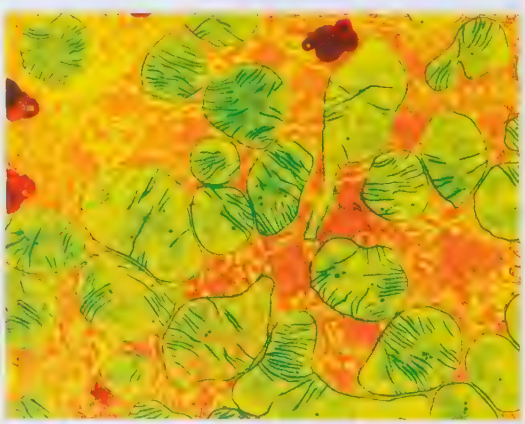
Mitochondria are the sites of **aerobic respiration** in the cell.
They are often described as the 'power plants' of the cell.
Enzymes involved in different stages of cell respiration are located on the cristae and in the matrix.
Details of aerobic respiration are covered in Chapter 17.

Not surprisingly, the numbers of mitochondria present reflect the metabolic activity of a cell. For instance, insect flight muscle and liver cells contain vast numbers of mitochondria.





TEM of a mitochondrion in section



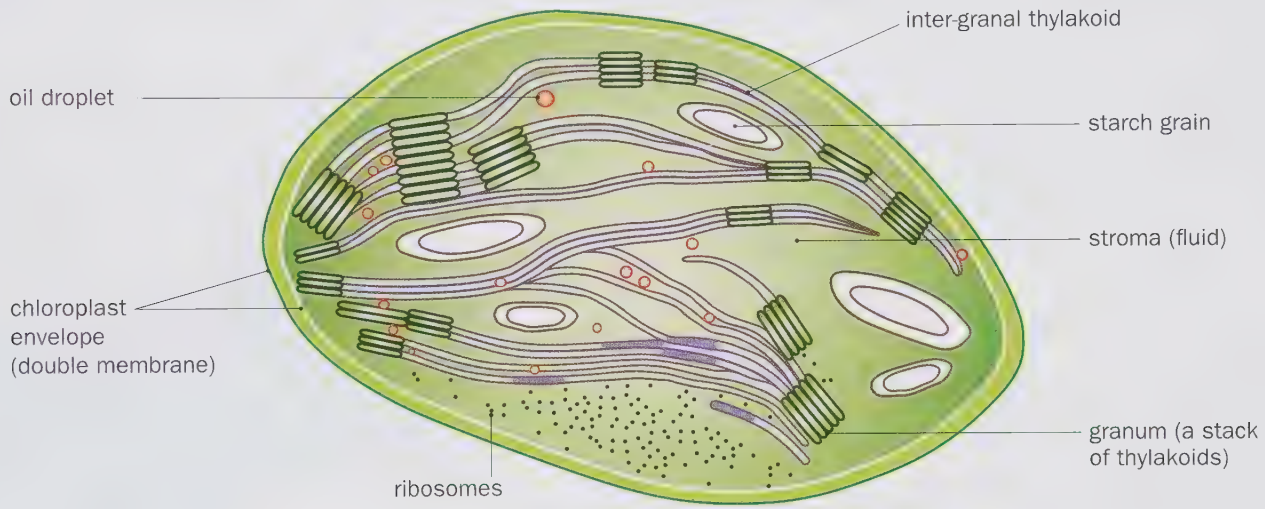
TEM of mitochondria mainly cut in cross section, note the cristae

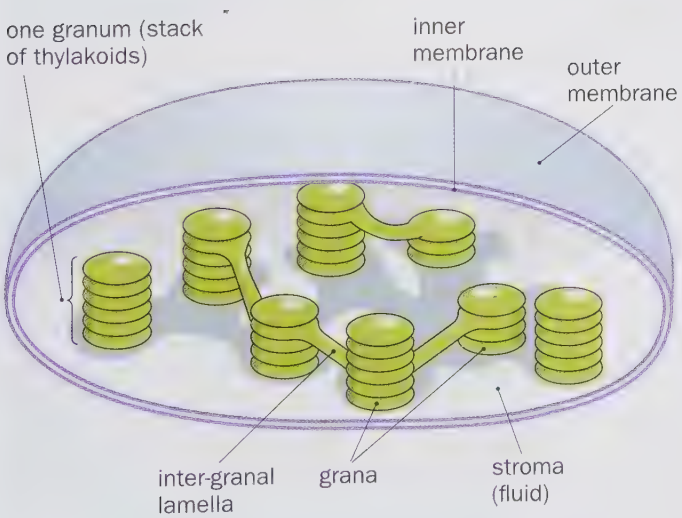
Chloroplasts

Chloroplasts are found inside the photosynthetic tissues of plants and some protoctists. They are particularly abundant in the palisade mesophyll cells of leaves. They belong to a group of organelles called **plastids**, many of which contain pigments.

Chloroplasts are similar in size to mitochondria and, like mitochondria, they have a double membrane – the **chloroplast envelope**. Inside this envelope is the fluid **stroma**, which contains enzymes involved in the reactions of photosynthesis.

Within the stroma is a network of flattened sacs called **thylakoids**. **Grana** (singular **granum**) are formed when many of these thylakoids are stacked together like piles of coins. The chlorophyll molecules, which trap light energy, are located within the thylakoids of each granum. The grana combine the ability to present a large surface area for light absorption with economy of space. Also visible in the stroma are large starch grains, which form a temporary store for the products of photosynthesis. Details of the biochemistry of photosynthesis are covered in Chapter 18.





Three-dimensional representation of the chloroplast structure

TEM of a chloroplast in section

Cytoskeleton

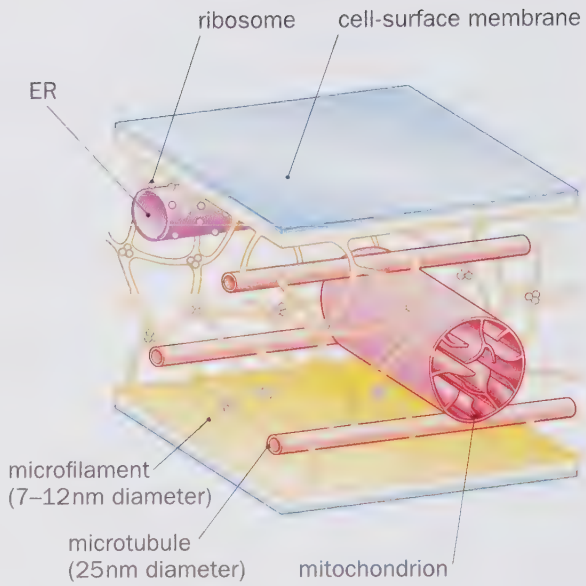
Despite its appearance, the cytoplasm is not a clear, formless blob of jelly.
Throughout the cell there is a complex network of fibrous proteins that make up the cytoskeleton.
However, the cytoskeleton is not a rigid framework since the protein fibres can be built up and broken down in different parts of the cell at the same time.
This means the cytoskeleton will allow movement, as well as giving the cell shape and support.

Microtubules are fine, tubular, unbranched structures.
They run through the cell providing the basis of the supporting scaffolding.
Microtubules are made up of the protein **tubulin**.

Microfilaments are much thinner strands, composed of the protein **actin**.
Because this protein is involved in muscle contraction, it is thought that microfilaments may be linked to cell movements and transport within the cell.
The single-celled *Amoeba* and some white blood cells are capable of this sort of independent movement.

Functions of the cytoskeleton as a whole are:

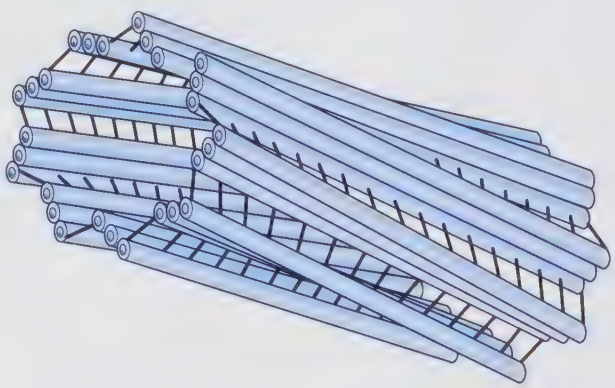
- to provide an internal framework that supports the cell,
- to organise and move organelles within the cell,
- to move the whole cell,
- to construct the spindle during cell division,
- to provide the components of cilia and flagella.



The cytoskeleton has a complex three-dimensional framework

Centrioles, cilia and flagella

Centrioles are two short bundles of microtubules positioned at right angles to each other.
They are located just outside the nucleus in a clear area of cytoplasm called the **centrosome**.
The wall of each centriole is made up of nine triplets of microtubules arranged at an angle.
At times of cell division, they migrate to opposite poles of the cell where they produce the **spindle**.
This arrangement of microtubules assists the movement of chromosomes.

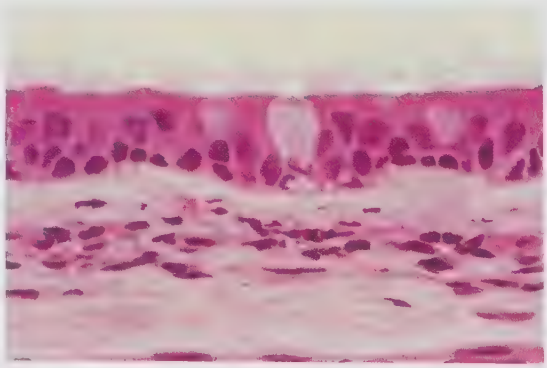


A centriole is a bundle of microtubules

Cilia and flagella are very similar in structure to each other.
Each has a ring of nine pairs of microtubules surrounding two central pairs.
Cilia are much shorter (5–10 μm) and are found in greater numbers.

Flagella often occur singly and can be anything up to 1000 μm long.
They are often used to help cells to move. Many unicellular organisms and the tails of sperm cells have flagella to allow them to move.

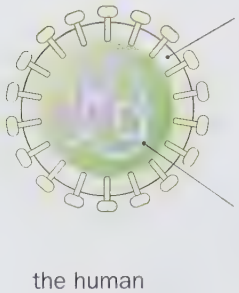
Cilia are often used to move materials about.
For instance, cilia beat to provide a conveyer belt carrying mucus from the respiratory passages up to the throat.



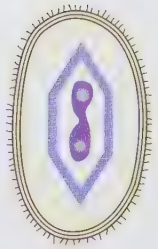
Light micrograph of the ciliated epithelial cells of the respiratory passages

Viruses

Viruses are much smaller than bacteria and are only visible with an electron microscope.
They range in size from 20 nm to 400 nm and do not have a proper cell structure.
As such they are exceptions to the cell theory.
These 'non-cells' have no cytoplasm, no organelles and no chromosomes. Viruses are acellular and non-living.



the human immunodeficiency virus (HIV)

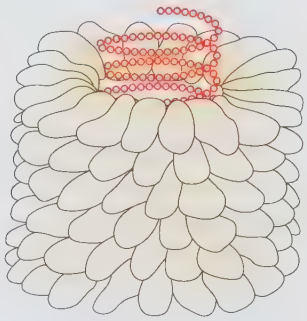

Herpes simplex virus causes cold sores

Viruses can only become active inside a living host cell.
Outside the cell they exist as inert virus particles called **virons**.
When they invade a host cell they are able to take over the cell's metabolic machinery and make new virus particles.
So 'reproduction' is the only characteristic that viruses share with other living organisms.

Viruses consist of

- a core of nucleic acid,
- a protein coat or **capsid**.


bacteriophages are viruses which infect bacteria


tobacco mosaic virus infects tobacco plants

The core of nucleic acid can be RNA or DNA.
The influenza virus and the human immunodeficiency virus (HIV) (see pages 279–80) contain RNA, as do most of the viruses that cause diseases in plants.
Herpes simplex, the virus that causes cold sores, and *Parvovirus*, which causes gastroenteritis, both contain DNA.

As you can see from the diagrams, viruses have distinct structures and come in a variety of shapes and sizes. Because of the absence of cytoplasm, viruses cannot carry out any chemical reactions on their own.
They are totally dependent on the cellular machinery of other cells.
As such viruses are regarded as **parasites**.

It's a take over

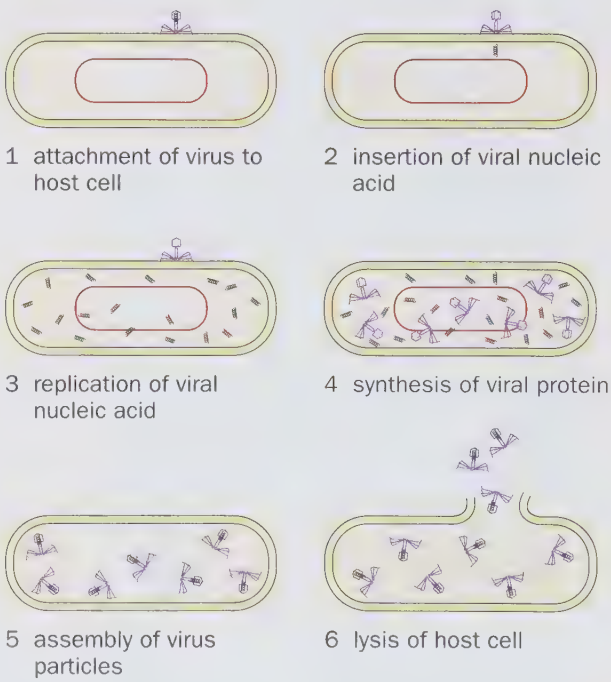
Many viruses cause disease.
Some are even named by the symptoms they produce in their hosts.
For example, tobacco mosaic virus (TMV) produces a distinctive mosaic pattern on tobacco leaves.

The sequence of events that occur when a virus takes over a cell and makes new viral particles is similar in most cases.

The lytic cycle

- The viral nucleic acid enters the host cell. This can occur by the viral membrane joining to the cell-surface membrane of the host cell or, as in the case of bacteriophages, the DNA is injected into a bacterial cell (see page 45).
- The viral DNA is 'read' by the host cell.
- The host cell uses this information to make new viral DNA and new viral protein.
- The viral DNA is surrounded by protein coats and the host cell bursts (**lysis**), releasing the new viruses.

Virus latency is the ability of a pathogenic virus to remain dormant (latent) within a host cell before the lytic cycle continues.



Replication and lysis of the bacteriophage lambda (λ) in a host cell

Levels of organisation

Complex multicellular organisms contain a variety of different cells. During development, cells **differentiate**, developing different structures and shapes. This means that they can carry out different functions.

Cells that differentiate in the same way form a **tissue**, for example cardiac muscle.
So tissues are made up of cells of the same type.
Different tissues combine to form **organs**, such as the heart.
Organs then work together as **systems**, for instance the circulatory system.
All the systems in an individual make up the **organism**, for example you!

Model of an amino acid

More and more complex

What makes up an organism?
We can identify different levels of organisation.
Starting at the lowest level and getting more complex:

- ***Chemical level***: atoms make up important biological molecules, such as carbohydrates, proteins, lipids and nucleic acids. These are needed for the maintenance and metabolism of an organism.

- ***Organelle level***: as you have seen, specialised structures within cells are called organelles. The Golgi body, for instance, is composed of membranes made up of phospholipids and proteins.

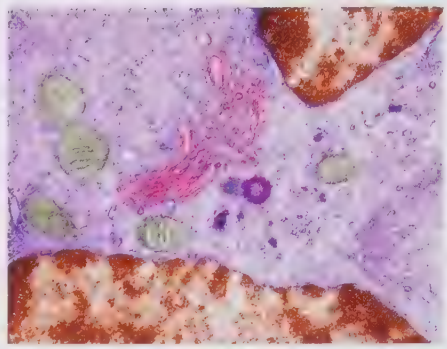
- ***Cellular level***: cells are the basic structural unit of life. Different cells differentiate to carry out particular functions. These specialised cells show division of labour, since they carry out different jobs. For instance, red blood cells transport oxygen.

- ***Tissue level***: a tissue is a collection of specialised cells of the same type, all working together to carry out a particular function. For example, muscle tissue is composed of individual muscle cells. When these cells contract, the muscle tissue contracts.

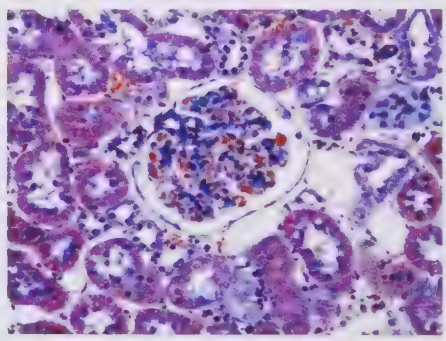
- ***Organ level***: organs may be made up of several tissues. For instance, the heart is made up of cardiac muscle, nervous tissue and connective tissue. Organs are structures in the body with a definite form and function.

- ***System level***: a system involves a number of organs working together to perform a common function. The organs of the digestive system include the stomach, intestines, liver and pancreas. These organs work together to digest and absorb your food.

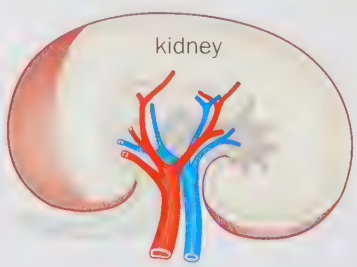
- ***The organism***: all the systems of the body together make up an organism.

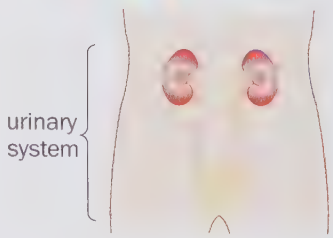


Mitochondria (green) and smooth ER (pink) in kidney cell



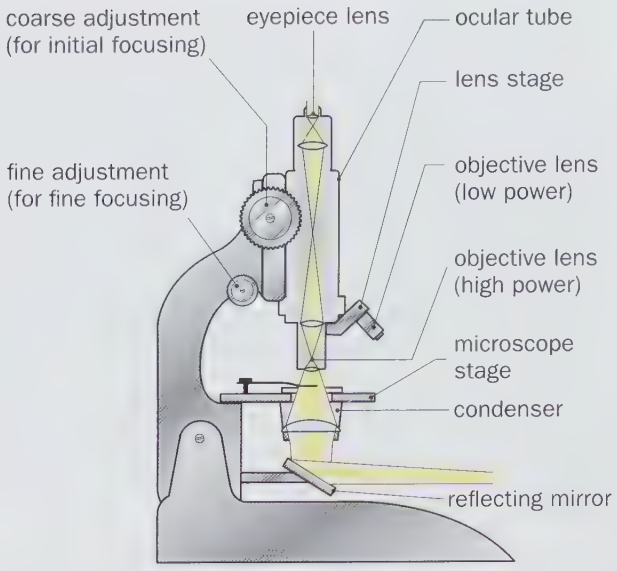
A Bowman's capsule in kidney tissue





The light microscope

The diagram shows the basic structure of a **light** (or **optical**) **microscope**. It is also known as a **compound microscope** because it has two lenses: the **eyepiece lens** and the **objective lens**. These lenses combine to produce a much greater magnification than could be obtained by a single lens. The magnification of each lens is usually written on the eyepiece and objective mountings. So how do you know the total magnifying power of a microscope? You simply ***multiply*** the two magnifications together. A ×10 eyepiece lens and a ×40 objective lens together produce a total magnification of ×400. Light microscopes have the ability to magnify up to ×2000. This is sufficient to observe cells and some of the larger organelles.



A light microscope

Resolving power

The ability to magnify is not the only quality needed of a microscope. It also needs to produce a ***clear*** image showing all the fine detail of the specimen.

This ability is known as **resolving power** and it can be expressed as: 'the minimum distance between two points at which they are still visible as two separate points'.

Two objects close together may appear as a single image when viewed under the light microscope.

Increasing the magnification will only show the objects as ***one*** larger image. The microscope is unable to ***resolve*** the two objects into separate images.

The maximum resolution of a light microscope is 200 nm. So two objects closer together than 200 nm cannot be distinguished as being separate. The reason for this is the nature of light itself.

Poor resolution in this photomicrograph of plant cells taken under the light microscope

High resolution in this TEM

The wavelength of visible light is about 500–650 nm.

Organelles such as mitochondria (about 1000 nm in diameter) are large enough to interfere with light rays.

But ribosomes (20 nm in diameter) are too small to interfere with light rays. So ribosomes cannot be seen under a light microscope whereas mitochondria can.

A general rule is that if two objects are each smaller than half the wavelength of the radiation used to view them, then they cannot be seen as being separate from each other.

So in the case of a light microscope, if each object is smaller than 200 nm, then they will not be seen as separate from each other.

Staining

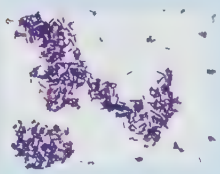
Because cytoplasm is usually transparent, it is necessary to stain microbes and other cells before they can be viewed with a light microscope.

In a **Gram stain test** bacteria are first stained with crystal violet and then washed in a decolourising solution. A counter stain, such as safranin or fuchsine, is then added. **Gram-positive** bacteria retain the violet stain, but **Gram-negative** bacteria do not and look red or pink.

This is due to the difference in the structure of the bacterial cell walls. Gram-positive bacteria have a cell wall containing a thick layer of peptidoglycan, which retains the crystal violet stain.

Gram-negative bacteria have an outer membrane and only a thin layer of peptidoglycan, so do not retain the stain.

During staining, **artefacts** can appear on the slide. These are not actual parts of the specimen, but material that has been introduced in error during the staining process.



Microscopic view of dental plaque showing Gram-positive (purple) and Gram-negative (red) bacteria

Maths skills

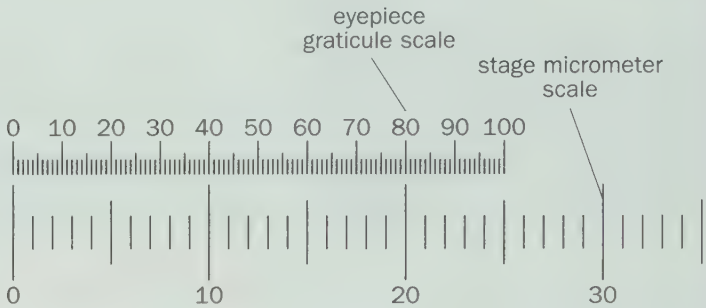
Measuring cells under a microscope

Scale on an eyepiece graticule

The size of cells under a light microscope can be measured using an **eyepiece graticule**.
This is a transparent scale which is placed in the eyepiece of the microscope.
As shown in the diagram, the scale is 10 mm long divided into 100 sub-divisions.
It can be seen in the field of view when looking down the eyepiece of the microscope.
Before it can be used, the eyepiece graticule scale must be calibrated, since each objective lens will magnify to a different degree.
To calibrate the graticule for a particular objective lens a **stage micrometer** is placed on the stage of the microscope. This is a glass slide that also has a scale etched onto it.
The scale is usually 2 mm long and its smallest sub-divisions are 0.01 mm (10 µm).

Calibrating the eyepiece graticule

1 Place the stage micrometer on the stage of the microscope and bring its lines into focus.
2 Move the stage micrometer so that its scale is lined up with the eyepiece graticule scale, as shown in the diagram.
3 10 units on the micrometer scale will be equivalent to 40 units on the graticule scale.
So 1 unit on the micrometer scale will equal 4 units on the graticule scale.
As each unit on the micrometer scale equals 10 µm, each unit on the graticule equals 10 ÷ 4 = 2.5 µm.



Calibrating an eyepiece graticule with a stage micrometer

4 Remember you will need to calculate the scale for different objective lenses by dividing the differences in magnification.
For instance, if an objective lens that magnifies ×40 gives a calibration of 25 µm per eyepiece graticule unit, then an objective lens magnifying ×400 will result in a graticule unit of 25 µm ÷ 10 = 2.5 µm.

Size and magnification

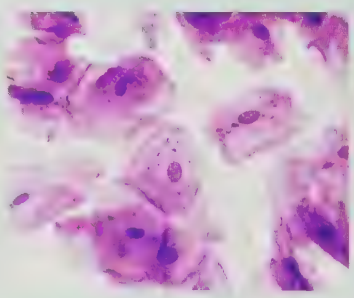
Photomicrographs usually have a statement and magnification, for example ×100.
The cells in the photograph are 1000 times larger than real life.
This means that they have been magnified 1000 times.
To work out the ***actual size*** of a cell, measure the length of one magnified cell in mm.
Let's say that it measures 13 mm.
Now use this formula:

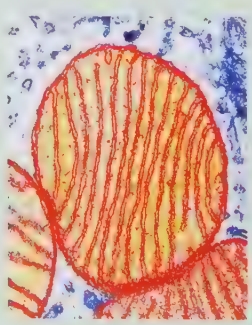
$$\textbf{actual size} = \frac{\textbf{image size}}{\textbf{magnification}}$$

So the actual size of our cell $= \frac{13}{1000} = 0.013\,\text{mm}$

There are 1000 µm in 1 mm. We can convert the answer to micrometres by multiplying by 1000 to give 13 µm.
Measure the image size of the maximum width of the mitochondrion in the electron micrograph; the actual size is 1 µm.
We can use a similar formula to calculate the magnification of an image:

$$\textbf{magnification} = \frac{\textbf{image size}}{\textbf{actual size}} = \frac{\textbf{29 000}}{\textbf{1}} = \textbf{×29 000}$$


Human cheek cells


TEM of a mitochondrion in cross section

The electron microscope

The **electron microscope** was developed in the 1950s.
It relies upon an electron beam instead of light rays.
The image is formed as the electrons are scattered by the biological specimen, rather like light rays are scattered in a light microscope.

The average wavelength of light is about 550 nm, whilst the average wavelength of an electron beam is 0.005 nm.
The shorter the wavelength of the radiation used to produce the image, the greater the resolving power of the microscope.
A good light microscope can resolve two objects that are 200 nm apart.
But an electron microscope can resolve objects that are only 1 nm apart.

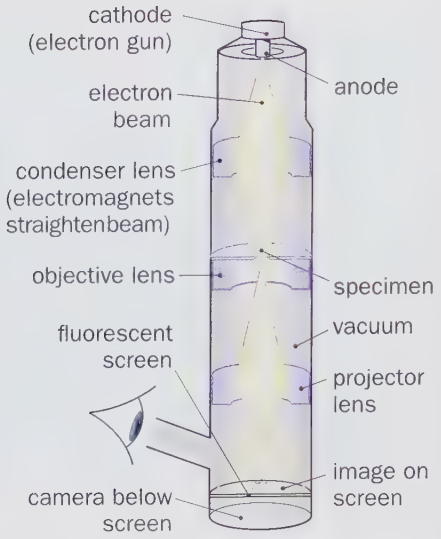
So how does an electron microscope work?

- At the top of the electron microscope column the filament of an electron gun is heated, causing it to emit electrons.
- These negatively charged electrons are attracted to a positively charged electrode called the anode.
  As they move, the electrons are concentrated into a beam by the negatively charged cathode.
- The condenser and objective are electromagnets.
  They straighten the electron beam, focusing it onto the specimen.
- The projector focuses an image of the specimen onto a screen.
- A vacuum has to be maintained inside the microscope otherwise the electron beam would be scattered when it hits air molecules.
- Any specimens to be viewed have to be dead if they are to be placed in a vacuum.
  Skilled preparation is needed to dehydrate and fix the material.

There are two types of electron microscopes:

A **transmission electron microscope** (TEM) passes the electron beam ***through*** the specimen.
The electrons are deflected as they pass through the thin slices of material, and the pattern produced is converted into an image.

A **scanning electron microscope** (SEM) records the electrons that are reflected off the surface of a specimen.
Consequently, thin sections of material are not required and three-dimensional images of intact specimens can be produced.



Electron microscope


TEM in use

Comparison of a light microscope and an electron microscope

	Light microscope	Electron microscope
radiation used	light rays	electron beams
magnification	×2000	×500 000
resolving power	200 nm	1 nm
focused by	glass lenses	electromagnets
biological material	living or dead	dead
size	small and portable	very large and static
preparation of material	quick and simple	time-consuming and complex
cost	relatively cheap	very expensive

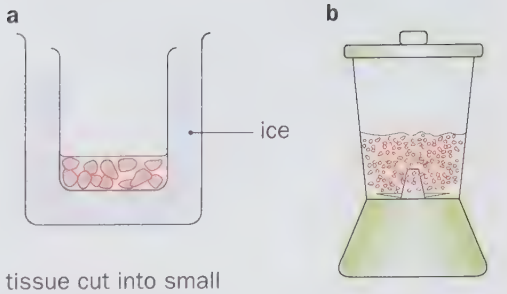

SEM of human head louse (×25)

Cell fractionation

Cell fractionation is a technique used to separate the different parts of cells. Each part can then be studied to determine its structure, using an electron microscope, and its function.

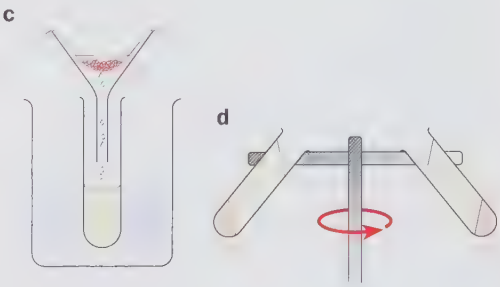
The following steps are involved:

- The tissue to be studied (for example, liver) is cut into small pieces and placed in cold **isotonic** buffer solution (that is, at the same concentration as the liver tissue).
- The pieces of tissue are ground into very small fragments in a type of liquidiser called an **homogeniser** to break open the cells.
- The suspension of cell fragments is filtered to remove large pieces of tissue debris.
- The filtrate is then placed into a centrifuge.



tissue cut into small pieces and placed in cold, isotonic buffer

cells broken open in homogeniser



mixture is filtered to remove cell debris

filtrate is centrifuged

A centrifuge is a machine that can spin tubes of liquid at very high speeds. The spinning exerts a force on the contents similar to, but much greater than, the force of gravity (***g***).
The faster the speed at which the tubes are spun, the greater is this force.

Cell organelles separate out in order according to their density and shape. At slower speeds, larger organelles, such as nuclei, collect at the bottom of the tube and form a pellet of sediment.
Other less dense organelles remain in suspension in the liquid above the pellet, which is called the **supernatant**.
Increasing the speed of the centrifuge results in other organelles separating out from the supernatant.
Centrifuging at different speeds results in new pellets forming, each of which has relatively pure samples of one type of organelle.
You can see from the table that the length of time centrifuging is important, as well as the speed.

	Organelle	Centrifuge setting (*g*)	Time (min)
first to separate out	nuclei	800–1000	5–10
	mitochondria lysosomes	10 000–20 000	15–20
	rough ER	50 000–80 000	30–50
	plasma membranes smooth ER	80 000–100 000	60
last to separate out	free ribosomes	150 000–300 000	>60

In what order would you expect the following organelles to separate out: ribosomes, nuclei and mitochondria?

The buffer solution used is 'isotonic' with the tissue (the same concentration). What might happen to the organelles if the buffer was
a) ***less*** concentrated? b) ***more*** concentrated?

Liver tissue contains many lysosomes. Why do you think this might make the study of liver mitochondria difficult?

Biochemical activity of isolated organelles is soon lost.
What ***two*** features of cell fractionation help to reduce this loss?

A high speed centrifuge in use

Biology at work: Laser microscopes

The use of light microscopes and electron microscopes to look at biological specimens has been fundamental to the advancement of biological research.
Laser illumination has revitalised microscopy in recent years. The **laser confocal microscope** uses laser beams to excite chemical stains which emit fluorescent signals.
These stains can be attached to the DNA in the nucleus of a cell to reveal its structure.

Laser scanning confocal microscope with image of rat kidney tissue on screen

Computer analysis

The fluorescent images that are produced are converted into a format that computers can analyse in depth.
This includes three dimensions but only after electronic colour enhancement.
Different signal strengths are given different computer colours, so a colour-coded image can be developed.

A major advantage of laser imaging is that the images of specimens are extremely clear.
The same images under a light microscope would be blurred.
For thick samples, such as cervical smears, **optical sections** are taken at different depths through a cell nucleus.

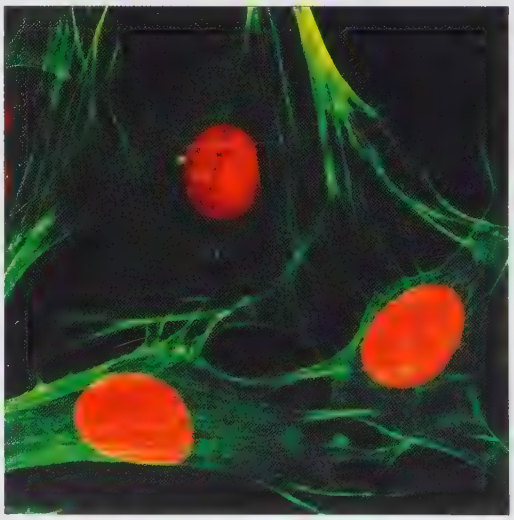
A vertical profile of images is then built up so that all are clearly in focus.
Obtaining an all-round view of the fluorescent signals with this technique is difficult.
This can be overcome by using **three-dimensional laser imaging**.
This involves taking a series of six images of the same nucleus, with each being rotated by 30°.

Lasers and cancer

Laser imaging techniques can be applied to a wide range of cytological studies, including cancer.
The natural fluorochrome known as Schiff's reagent binds to those elements of DNA that are released during the early stages of cancer.
The intensity of staining is proportional to the amount of cancerous growth, allowing quantitative assessment.

This application has revealed a number of novel features specific to cancerous cells, such as the occurrence of 'hot spots' of localised fluorescence in cell nuclei.
The use of laser microscopy enables much clearer viewing of previously known cancer cell abnormalities.
These include the bending and breakdown of the nuclear envelope and the appearance of 'holes' in the nucleoplasm.

The latest application of miniaturised laser microscopy has been in the field of endoscopy: the examination of the gastrointestinal and respiratory tracts. Here it offers an earlier, more accurate, and less invasive form of cancer diagnosis.



Colour-enhanced laser scanning micrograph of kidney epithelial cell tumour

Summary

- All living organisms are made up of units called cells.
- Viruses are non-living and acellular.
- Prokaryotes include bacteria and blue-green bacteria.
- Eukaryotes include plants, animals, fungi and protoctists.
- Eukaryotic cells have a true nucleus. They are relatively large with a high degree of internal organisation.
- Animal and plant cells contain many similar organelles, including a cell-surface membrane, cytoplasm containing mitochondria, lysosomes, endoplasmic reticulum, Golgi bodies, ribosomes, and a nucleus with a nucleolus and chromatin.
- In addition, plant cells have chloroplasts, a large permanent vacuole and a rigid cell wall.
- Animal cells have centrioles, which are not found in plant cells.
- The genetic material of plant and animal cells is found inside the nucleus.
- Mitochondria provide the sites where most ATP is produced. ATP provides the energy for the processes taking place inside the cell.
- Chloroplasts are able to trap light energy as chemical bond energy, which can later be released and used by cells.
- Most cell organelles cannot be seen with a light microscope because of the limit of resolution when using light rays.
- Electron microscopes can be used to see smaller structures in greater detail. They can resolve these small structures because electron beams have a shorter wavelength than light rays.

Questions

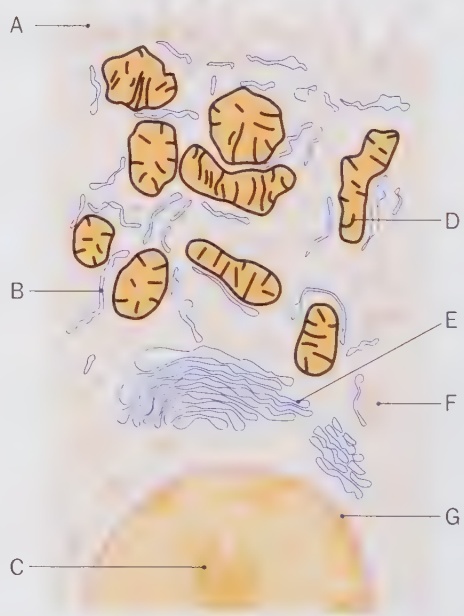
1 Match the structures i)–x) with the descriptions of their functions a)–j).

i) mitochondria
ii) Golgi body
iii) lysosome
iv) ribosome
v) centriole
vi) cell wall
vii) nucleolus
viii) chloroplast
ix) cell-surface membrane
x) nucleus.

a) manufactures ribosomal RNA
b) site of protein synthesis
c) controls the substances that pass into and out of a cell
d) contains the genetic material of the cell
e) converts light energy into chemical energy
f) makes ATP from the oxidation of glucose
g) gives mechanical support to the cell
h) contains digestive enzymes involved in intracellular digestion
i) organises the fibres of the spindle in animal cells
j) modifies proteins after their production.

2 The diagram in the next column shows part of an electron micrograph of a cell.

a) Match the letters A–G with the following labels:
i) cell-surface membrane
ii) vacuole
iii) chromatin
iv) Golgi body
v) crista of mitochondrion
vi) smooth endoplasmic reticulum
vii) pore in nuclear envelope.



b) Give three features that suggest that this is a secretory cell.
c) Why do you think that this is an animal cell?

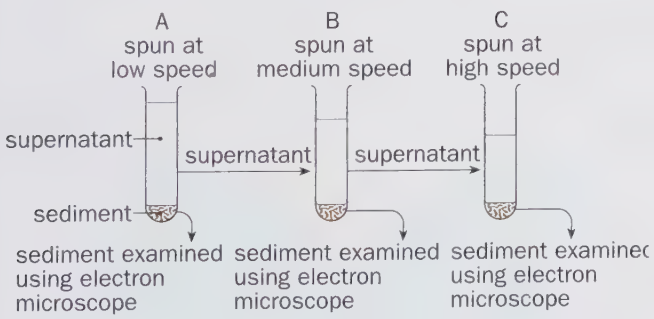
3 The table below refers to structures that may be contained within a bacterial cell, a liver cell, and a palisade mesophyll from a leaf.
Copy and complete the table, placing a tick (✓) in the appropriate box if the structure is present, and a cross (✗) if the structure is absent.

Feature	Bacterial cell	Liver cell	Palisade cell
nuclear envelope			
cell wall			
glycogen granule			
microvilli			
chloroplast			

4 Draw a table to compare the advantages and disadvantages of a light microscope with an electron microscope.

5 Copy and complete the following sentences.
a) Cell organelles that are likely to be abundant at sites of active transport are ___.
b) A cell organelle that is particularly rich in hydrolytic enzymes is the ___.
c) Parts of the endoplasmic reticulum that break off as vesicles are thought to form ___ ___.
d) Microtubules are a component of ___.
e) The organelle that converts light energy into chemical energy is the ___.

6 Liver cells were ground to produce an homogenate. The flow diagram shows how centifugation was used to separate organelles from liver cells.



Drawings of electron micrographs of three organelles separated by the centrifugation are shown below. The drawings are ***not*** to the same scale.

1 

2 

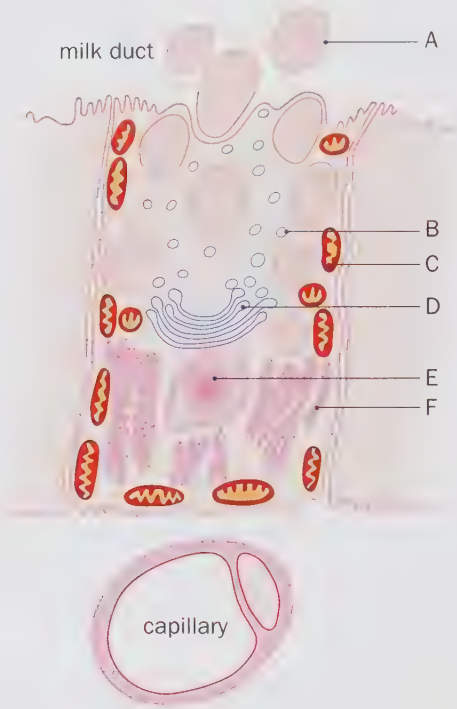
3 

a) Copy and complete the table below.

Electron micrograph	Name of organelle	Centrifuge tube in which the organelle would be the main constituent of the sediment
1		
2		
3		

b) Explain why it is possible to separate the organelles in this way.

7 The diagram (drawn from an electron micrograph) shows a secretory cell from a mammary gland and its relationship to a blood capillary. The globules (A) consist only of triglycerides.



a) Identify the structures labelled C, D, E and F.
b) Radioactivity-labelled amino acids supplied to such cells grown in tissue culture were later found in milk protein (casein) produced by the cells. Give, in correct sequence, the letters of those structures which show the likely route taken through the cell by those amino acids after absorption.

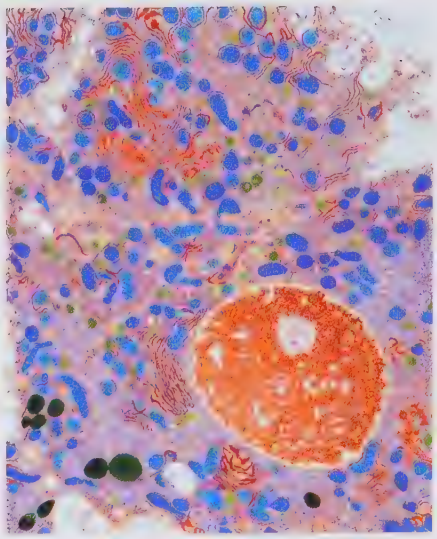
5 Cell membranes and transport

Many of the organelles that we looked at in the last chapter are composed of or surrounded by membranes.

Can you name some of these cell organelles?

Mitochondria, chloroplasts, lysosomes, Golgi bodies, the nucleus and the endoplasmic reticulum are all enclosed by membranes.

In this chapter we will focus on the cell-surface membrane. This acts as a boundary to the cytoplasm and defines the limits of the cell.
It also controls the substances that are able to pass into and out of the cell.



Which organelles can you find in this photomicrograph?

The structure of the cell-surface membrane

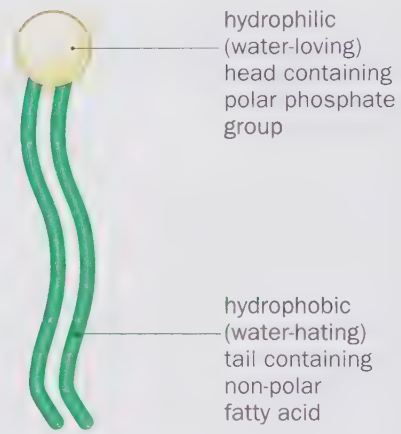
The cell-surface membrane is about 7 nm thick.
Under the light microscope this merely appears as a single line.
However, the development of the electron microscope has made it possible to investigate the detailed structure of biological membranes.

Chemical analysis has shown the membrane to be 75% phospholipid.
In addition, the membrane contains proteins, cholesterol and polysaccharides.
However, it is the phospholipids that form the key elements in the structure.

As you saw in Chapter 1, simple lipids are made up of glycerol and three fatty acid molecules. Lipids are **non-polar** molecules.
In a phospholipid, one of the fatty acids is replaced by a phosphate group.

This phosphate end of the phospholipid molecule is a **polar** molecule.
It has an attraction for other polar molecules such as water.
The other end of the molecule (made up of two fatty acid tails) remains non-polar and will not mix with water.

The polar phosphate heads of the molecule are water-loving (**hydrophilic**), whereas the non-polar tails are water-hating (**hydrophobic**).

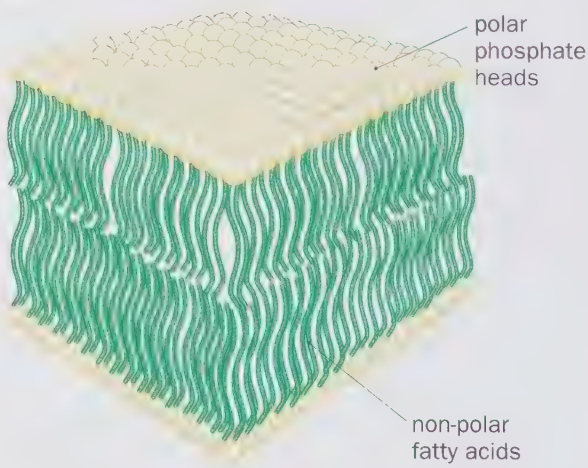


As you saw in Chapter 1, phospholipids can form monolayers and micelles. Perhaps more importantly though, phospholipids can form **bilayers**.

One sheet of phospholipids forms over another.
Can you see in the diagram that the hydrophilic heads point outwards, facing the water, and the hydrophobic tails face inwards?

This phospholipid bilayer is the basis of the structure of the cell-surface membrane and all other membranes.

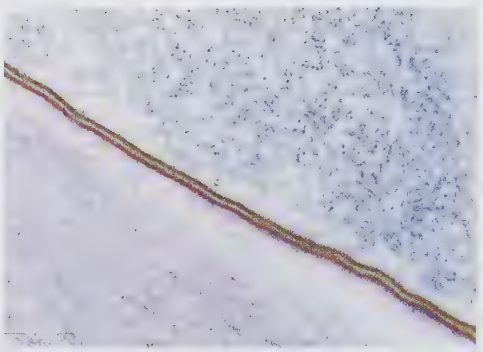
What is meant by the terms hydrophilic and hydrophobic?
How do the hydrophilic and hydrophobic properties of phospholipids help to explain the basic structure of the cell-surface membrane?



A phospholipid bilayer

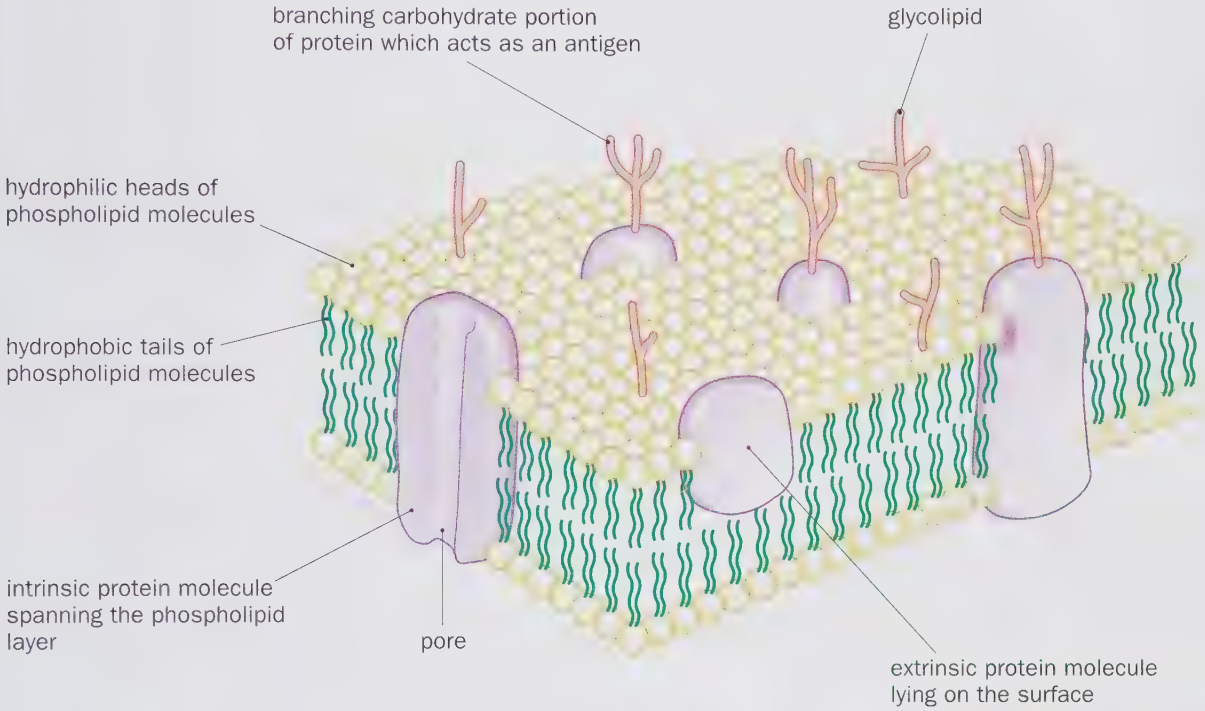
The fluid mosaic theory

The **fluid mosaic theory** was first proposed by Singer and Nicholson in 1972.
With the increased magnification and resolution of the electron microscope, the cell-surface membrane could be distinguished as two black lines, referred to as 'tram lines'.
Singer and Nicholson suggested that the cell-surface membrane was made up of two layers of phospholipids.



Electron micrograph of a cell-surface membrane

Further chemical analysis showed that a large number of different proteins were located in the cell-surface membrane.
It was thought that these proteins 'floated about' in the fluid phospholipid layer.



As you can see, some of the proteins completely span the membrane.
These are called **intrinsic proteins**.
Other proteins are found only on the inner surface or on the outer surface of the membrane.
These are known as **extrinsic proteins**.
Just like the phospholipids that make up the bulk of the membrane, the proteins have polar and non-polar regions.
Weak hydrogen bonds between the polar regions of the proteins and the phospholipids keep the membrane stable.

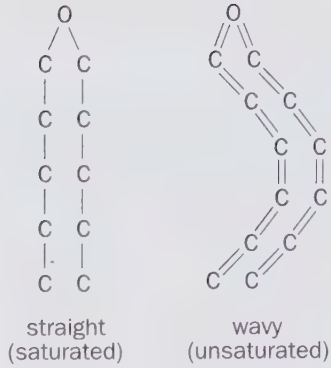
The fluid mosaic model for the cell-surface membrane has been described as 'a number of protein icebergs floating in a sea of lipids'.

Freeze-fracture TEM of the surface of a plasma membrane showing the double membrane system

How fluid is fluid?

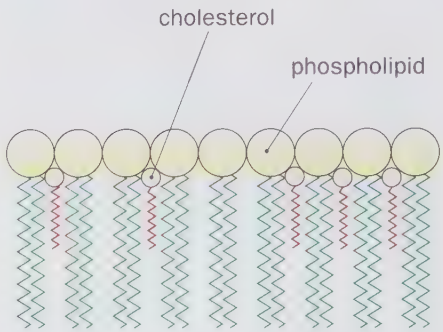
As you saw in Chapter 1, fatty acids can be saturated or unsaturated.
Some phospholipids, then, will have saturated fatty acid tails.
Other phospholipids will have unsaturated fatty acid tails.

- The more unsaturated fatty acids present in the phospholipid bilayer, the more fluid the membrane.
- The shorter the fatty acid tails, the more fluid the membrane.
- The greater the steroid content of the membrane, the ***less*** fluid it is.
  Cholesterol is a steroid that makes up about 20% of the lipids found in animal cells, but is rarely found in plant cells.
  Cholesterol can fit between the phospholipid molecules, increasing the rigidity and stability of the membrane as a whole.
- An increase in temperature increases the fluidity of the membrane.



Fatty acids

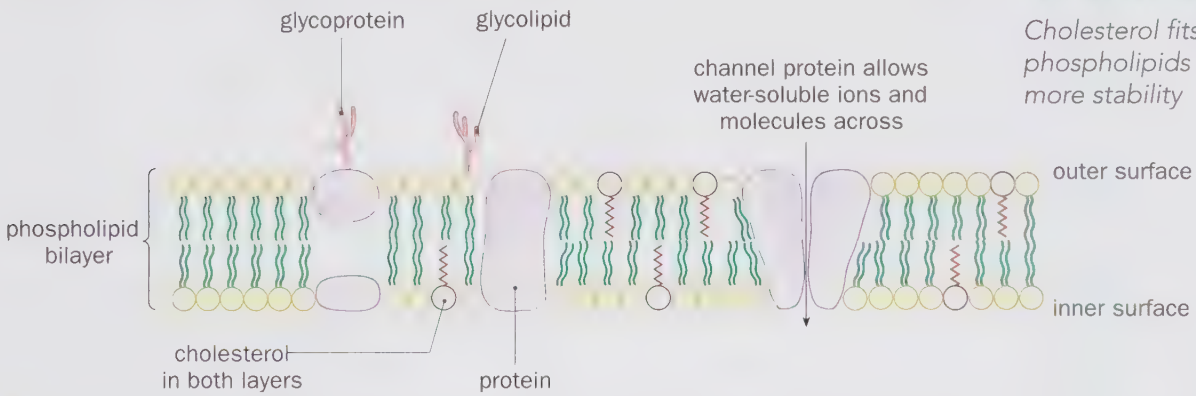
Glycolipids make up about 5% of the lipids found in the membrane.
These are lipids that have combined with polysaccharides.
They are usually found in the outer layer of the membrane,
and are thought to play a role in cell-to-cell recognition.



Cholesterol fits between the phospholipids giving the membrane more stability

What about the proteins?

Thousands of different protein types appear in membranes.
It is the proteins that give each type of membrane its own important functions.



- **Carrier proteins** and **channel proteins** assist and control the movement of water-soluble ions and certain molecules across the membrane.
  They can maintain different concentrations of ions on either side of the membrane.
  This can maintain an electrochemical gradient between the inside and the outside.
- Other proteins are involved in **active transport**.
  This involves the use of energy from ATP to ferry certain ions and molecules across the membrane, against a concentration gradient.
  A good example is the sodium–potassium pump, where a specific protein uses energy from ATP in nerve impulse transmission.
- **Receptor proteins** recognise and bind with specific molecules outside the cell, such as hormones and drugs (see page 331).
- Some **enzymes**, such as ATPase, are located within membranes.
- A **glycoprotein** is a combination of a protein and a polysaccharide.
  As with glycolipids, they stick out from the surface of some membranes like antennae and are important in cells recognising each other.
  They act as sites of cell communication (**cell signalling**).
  Some glycoproteins act as **antigens**.

Diffusion

You soon notice if someone has put on too much aftershave or perfume. The molecules of aftershave spread out in all directions of the room.

Molecules and ions in a gas or in a liquid are always on the move. This tendency to spread out through the gas or liquid is called **diffusion**.

Diffusion is the movement of molecules (or ions) from a region of high concentration to a region of lower concentration until they are spread out evenly.

'Oh no it's aftershave man'

What happens if you drop a crystal of dye into a beaker of water?

First the crystal starts to dissolve, forming a region of high concentration.
Then the molecules of dye diffuse out in all directions.
Eventually the molecules are spread evenly throughout the water.

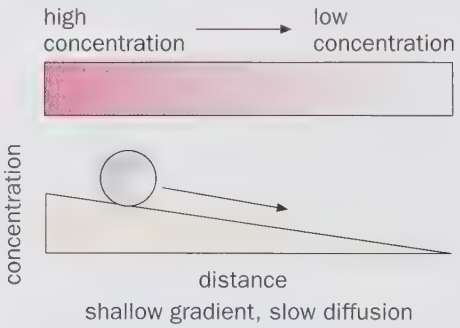
Can you think of any examples of diffusion in living organisms?

Oxygen diffuses across the air sacs of the lungs into the blood.
Carbon dioxide diffuses out of the blood in the opposite direction.
Some digested food diffuses across the gut wall into the blood.
Mineral salts diffuse from water in the soil into root hairs.

What affects the rate of diffusion?

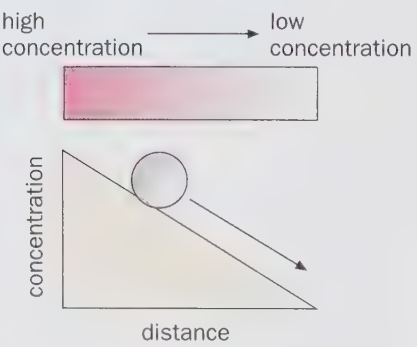
- The greater the difference in the concentration of a substance in two areas, the faster the rate of diffusion.
  This difference in concentration is known as the **concentration gradient**.

  Can you see from the diagram that the rate of diffusion is directly proportional to the concentration gradient?



shallow gradient, slow diffusion

- Small particles tend to diffuse faster than larger particles.
- Diffusion takes place more quickly through thin membranes because there is only a short diffusion pathway.
  Think about the air sacs (alveoli) in the lungs.
- Diffusion is quicker if the membrane has a large surface area, as in the case of the spongy mesophyll cells of a leaf, for instance. They provide a huge surface area over which diffusion can take place.
- The shorter the distance between two regions, the faster the rate.
- The number of protein channels and carrier molecules there are in the membrane.
  The more there are, the faster the rate (see facilitated diffusion page 88).
- An increase in temperature increases the rate of diffusion because the particles will have greater kinetic energy.
  In effect, this increase in particle movement is quite small.



steep gradient, rapid diffusion

Facilitated diffusion

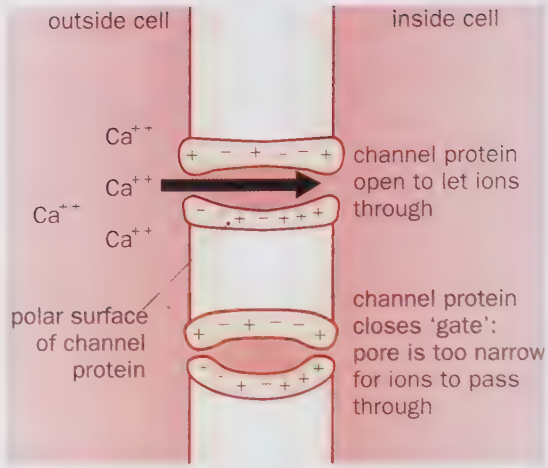
This special form of diffusion allows faster movement.
It involves the use of proteins to assist or ***facilitate*** diffusion.
There are two main types of protein involved:
channel proteins and carrier proteins.

Charged ions, such as Na^+, K^+, Ca^{2+} and Cl^+, cannot diffuse easily across the non-polar centre of the phospholipid bilayer.
Channel proteins open up spaces or pores across the membrane, and so allow entry or exit. These pores are lined with polar groups allowing charged ions to pass through.

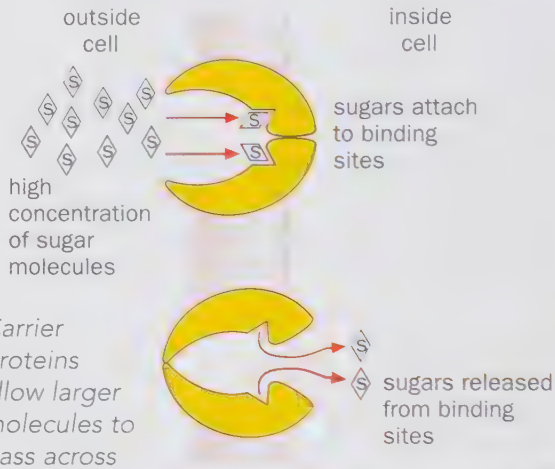
Usually each channel protein is ***specific*** for one type of ion.
That is, each protein will only let one particular ion through.
The channel proteins can also open or close their pores, acting like gates depending upon the cell's needs.

Carrier proteins are more sophisticated in the way they work.
They are able to allow the diffusion across the membrane of larger polar molecules such as sugars and amino acids.
A particular molecule attaches to the carrier protein at its particular binding site.
This causes the carrier protein to change its shape.
As it does so it 'delivers' the molecule through the membrane.

Carrier proteins and channel proteins both increase the rate of diffusion down a concentration gradient.
It is important to remember that neither requires ATP from respiration. Each is a ***passive*** process.



Channel proteins act like 'gates', which open to let ions through



Diffusion and surface area

As a cell increases in size, its surface area to volume ratio decreases.
You can prove this with a simple exercise.
Look at the drawings of the four 'cells' A–D.
Copy and complete the table below to work out the surface area to volume ratio.

Maths skills

	Cube A	Cube B	Cube C	Cube D
surface area of one face	$1\,cm \times 1\,cm = 1\,cm^2$			
surface area of cube	$6 \times 1\,cm^2 = 6\,cm^2$			
volume of cube	$1\,cm \times 1\,cm \times 1\,cm = 1\,cm^3$			
ratio: surface area to volume	$6\,cm^2/1\,cm^3 = 6:1$			

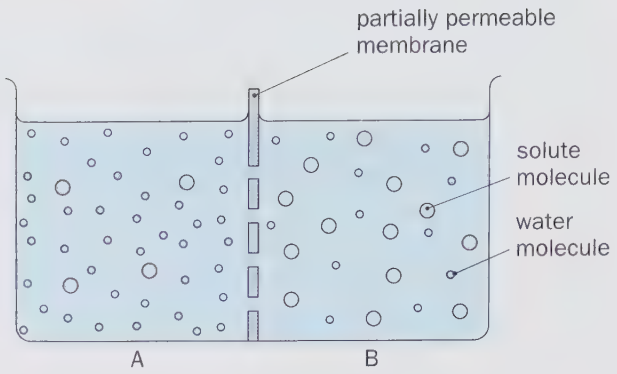
As the cell increases in size there is less surface area in proportion to its volume.
This means that, ***relatively***, there is less surface area of cell membrane over which diffusion can occur. But some cells are able to increase their surface area, as is the case with microvilli in epithelial cells.

Osmosis

The cell-surface membrane separates the cell contents from the surrounding environment.
As you have seen, the membrane allows some molecules to pass through but not others.
We say that it is a **partially permeable membrane**.

Look at the diagram.
It shows two solutions separated by a partially permeable membrane.
(Remember that a solution consists of solute molecules dissolved in a solvent, for example sugar molecules dissolved in water.)

Which solution, A or B, is the most concentrated?
Which solution contains the most water molecules?



The solute molecules are too large to pass through the pores in the membrane.
But the water molecules are small enough to pass through.
Since A has a greater concentration of water molecules than B, there will be movement of water molecules through the membrane, from solution A into solution B.
This movement of water molecules is called **osmosis**.

Osmosis is the movement of water molecules from a region of high concentration to a region of lower concentration through a partially permeable membrane.

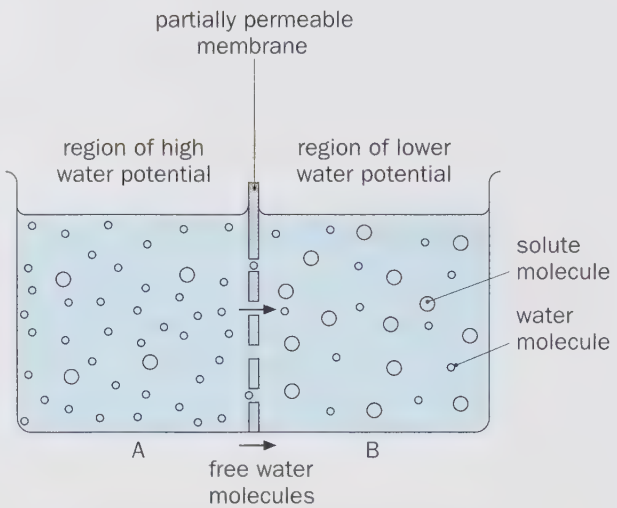
A matter of potential

A 'weak' solution will have a high concentration of water molecules.
These 'free' water molecules will have a tendency to move about.
We say that the solution has a **high water potential**.

Pure water has the highest water potential at zero.
So all lower water potentials will have negative values.

A 'concentrated' solution will have a lower concentration of water molecules.
There will be fewer free water molecules moving about.
We say that this solution has a **low water potential**.
By low water potential we mean a potential that is more negative.

Water will move out of cells with a high water potential into cells with a lower water potential.
Perhaps we should give a more accurate definition of osmosis.



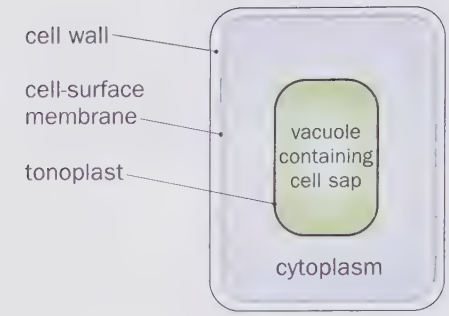
Osmosis is the movement of water molecules from a region of high water potential to a region of lower water potential through a partially permeable membrane.

Water potential is expressed as Ψ, the Greek letter psi.

Osmosis in plant cells

Can you make a simple but accurate drawing of a plant cell?

- Surrounding the cell is the cell wall.
  The cell wall is freely permeable, allowing all molecules in and out.
- Flush against the cell wall is the cell-surface membrane.
  As you know, this is a partially permeable membrane.
- Inside the cell-surface membrane is another membrane – the **tonoplast**.
  The tonoplast separates the contents of the vacuole from the cytoplasm.



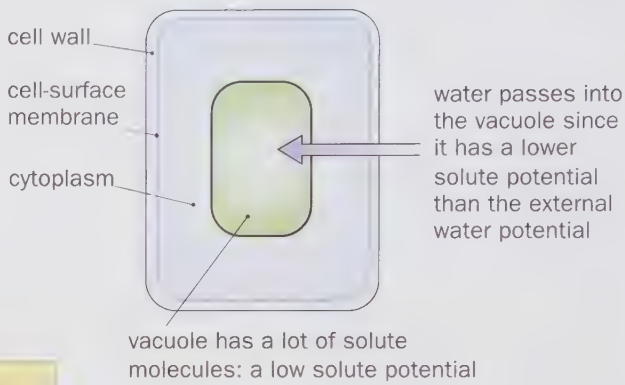
Solute potential (Ψ_s)

The concentration of dissolved substances inside the cell is called the **solute potential**.
This value is ***always*** negative because the forces of attraction between the solute molecules and the water molecules ***reduce*** the movement of the water molecules.

(Remember that the water potential of pure water is zero. So anything that reduces the ability of water molecules to move must ***lower*** the potential, that is, make it ***more*** negative.)

The ***more*** solute molecules present, the ***lower*** the water potential.
The ***fewer*** solute molecules present, the ***higher*** the water potential.

Solute potential (Ψ_s) is a measure of the reduction in water potential due to the presence of solute molecules.



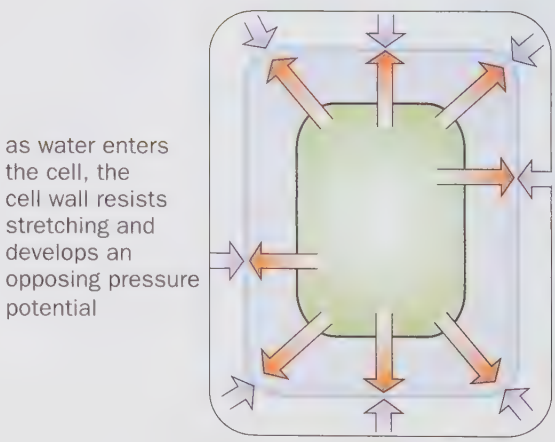
Pressure potential (Ψ_p)

Water enters a plant cell if the solute potential inside the cell is lower than the water potential outside the cell.

As water passes into a plant cell, the cell contents start to swell. But this does not go on indefinitely.

Soon the cellulose cell wall starts to become stretched.
It starts to physically resist the swelling caused by the influx of water.
The pressure that the cell wall develops is called the **pressure potential**.
The pressure potential is usually, though not always, positive.

Pressure potential (Ψ_p) is the pressure exerted on the cell contents by the cell wall and cell membrane.



At any time, the water potential of a plant cell is the sum of the solute potential and the pressure potential:

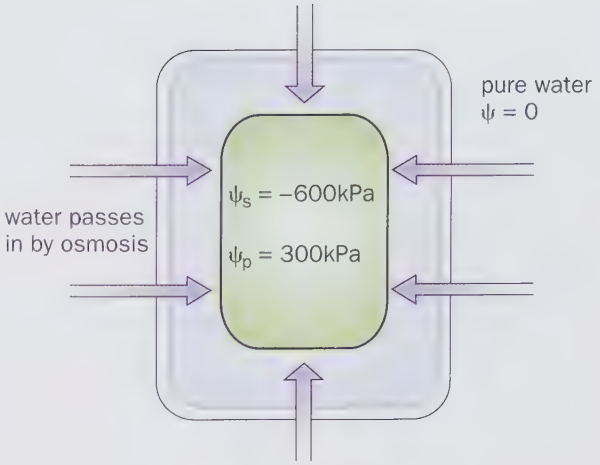
$$\text{Water potential } (\Psi) = \text{solute potential } (\Psi_s) + \text{pressure potential } (\Psi_p)$$

Turgidity

What happens if you put a plant cell into distilled water or a weak solution (sometimes called a **hypotonic** solution)?

The water potential inside the cell will be ***lower*** than the water potential of the external solution.
So water enters the cell by osmosis.

This influx of water causes the cell to swell.
The contents press against the cell wall, producing a pressure potential.
As more water enters, the pressure potential rises until it is equal (and opposite) to the solute potential.
The water potential is now zero.
No more water can enter the cell.
A cell in this state is said to be **turgid**.



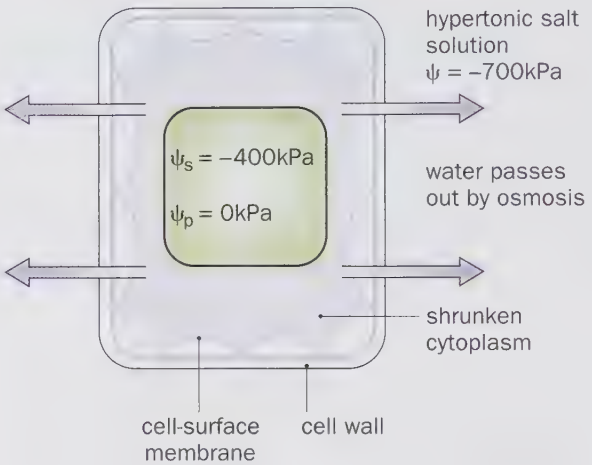
Plasmolysis

What happens if you put a plant cell into a strong solution (sometimes called a **hypertonic** solution)?

In this case, the external solution has the lower water potential.
So water passes out of the cell by osmosis.

As water leaves the cell, the cell-surface membrane starts to shrink away from the rigid cell wall.
The pressure potential is now zero, and the cell is **flaccid**.

As more water leaves the cell, the cell contents continue to shrink.
The cell-surface membrane peels away from the cell wall.
A cell in this condition is **plasmolysed**.



Turgid cells are firm because they are full of water.
They give mechanical support to many soft plant stems and keep them upright.
If these cells lose water, they become flaccid.
They are no longer firm and cannot give the same support.
As a result, the plant stems **wilt**.
But watering them will enable them to regain their turgidity!

What would happen if a plant were placed in **isotonic** solution, that is, a solution with the ***same*** water potential as that of the cells?
There would be no net movement of water in either direction.
The cells would be in equilibrium.

Maths skills

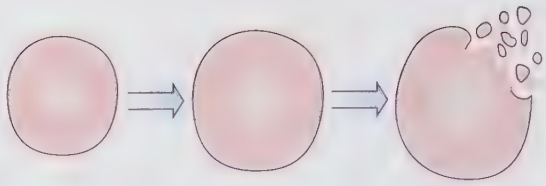
Try to predict the water movement in these cells:

A	B
ψ_s = –300kPa	ψ_s = –400kPa
ψ_p = 100kPa	ψ_p = 300kPa

A	B
ψ_s = –600kPa	ψ_s = –700kPa
ψ_p = 100kPa	ψ_p = 200kPa

A	B
ψ_s = –400kPa	ψ_s = –600kPa
ψ_p = 200kPa	ψ_p = 300kPa

Osmosis in animal cells

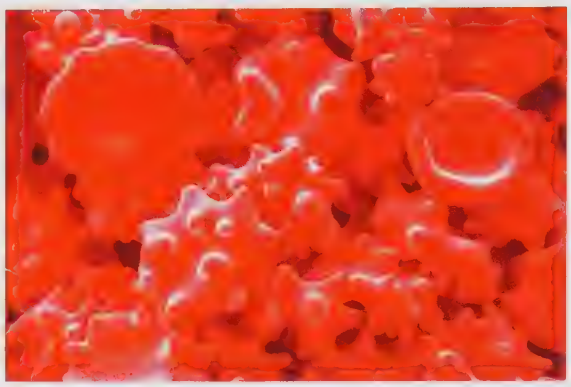


red blood cell | when placed in water the cell swells up ... | ... and bursts! (haemolysis)

What do you think would happen if you put some animal cells (let's say red blood cells) into distilled water?

You can see in the diagram.
Water enters the cells by osmosis, the cells swell and burst.
The reason is that animal cells have no cell wall to limit the expansion of the cell.

The cells have a low water potential.
The water outside has a high water potential of zero.
So there is an inflow of water into the cells by osmosis.
Without a cell wall to stop the expansion of the cell, water keeps entering the cell until it bursts.
When this happens to red blood cells it is called **haemolysis**.



These red blood cells are crenated

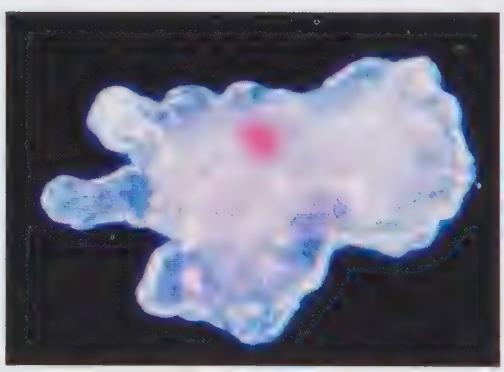
What do you think would happen if the red blood cells were put into strong salt solution (a hypertonic solution)?

This time the solute potential of the external solution is more negative than that inside the cells.
This time water will diffuse out of the cells, so they will shrink.
Red blood cells look crinkled, or **crenated**, like those in the photograph to the right.
This can happen in cases of severe dehydration.

We have blood plasma and tissue fluids that are isotonic with our cells (they have the same solute potential).
Our kidneys are able to maintain a constant water level in our bodies.
This example of homeostasis is called **osmoregulation**.

Amoeba stays in shape

Amoeba is a single-celled organism (one of the Protoctist group).
Some species of *Amoeba* live in freshwater and some are marine.



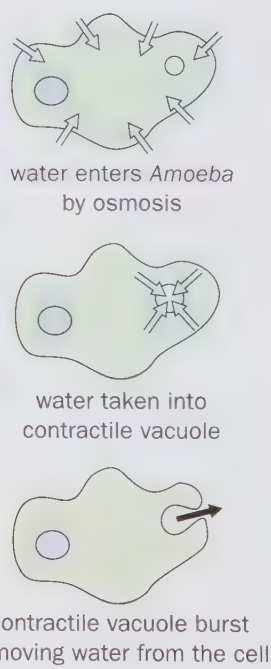
Amoeba

What problem could *Amoeba* have when living in freshwater?
Water is continuously entering *Amoeba*, because its cell has a negative solute potential.

So why doesn't *Amoeba* burst?

Inside its cell *Amoeba* has an organelle called a **contractile vacuole**.
Water accumulates inside the contractile vacuole.
When it is full the vacuole passes to the cell membrane and releases the water to the outside.
A new contractile vacuole then starts to fill up.

Why do you think that the species of *Amoeba* that live in the sea do not have contractile vacuoles?



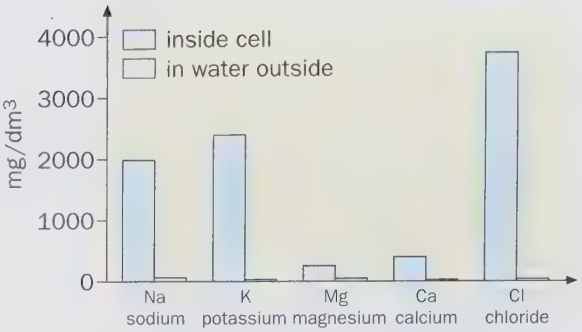
water enters *Amoeba* by osmosis

water taken into contractile vacuole

contractile vacuole burst removing water from the cell

Active transport

Look at the histogram showing the concentration of ions inside and outside the cells of a freshwater alga. Can you detect any pattern?



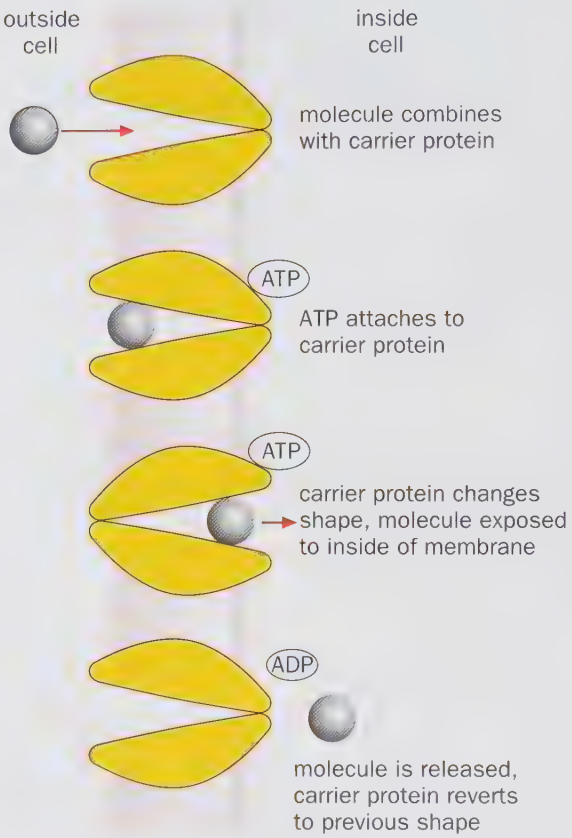
The ions are present in far higher concentrations inside the cell than they are on the outside. There must be some mechanism at work in the membrane, otherwise the ions would diffuse out of the cell.

The cell is able to accumulate molecules and ions against a concentration gradient. This involves the use of energy provided by ATP from respiration. So the process at work is called **active transport**.

Active transport is the uptake of molecules or ions against a concentration gradient using energy from respiration.

How does it work?

There are specific carrier proteins present in the cell membrane. As in **facilitated diffusion**, each carrier protein can combine with a particular molecule or ion.
But unlike facilitated diffusion (which is a passive process), active transport needs an input of energy.
This energy is provided by ATP made in cellular respiration.



- The molecule or ion combines with a specific carrier protein.
- ATP transfers a phosphate group to the carrier protein on the inside of the membrane.
- As a result, the carrier protein undergoes a change of shape, which carries the molecule or ion to the inside of the membrane.
- The molecule or ion is released to the inside of the membrane and the carrier protein reverts to its original shape.

Due to the energy needed for active transport, cells involved in the process:

- tend to contain many mitochondria,
- have a high rate of respiration.

Their ability to take up molecules or ions against a concentration gradient is affected by temperature, oxygen concentration, and the presence of poisons such as cyanide, all factors that affect the rate of respiration.

Can you name any processes that involve active transport?

Here are a few:

- nerve impulse transmission,
- muscle contraction,
- absorption of amino acids in the gut,
- absorption of mineral salts by plant roots,
- protein synthesis,
- excretion of urea by the kidney.

Co-transport is the linked, simultaneous transport of one substance across a membrane, coupled with the simultaneous transport of another substance across the same membrane in the same direction.
For example, facilitated diffusion is coupled with active transport in the absorption of amino acids and glucose by cells lining the mammalian ileum (see page 156).

Endocytosis and exocytosis

So far, we have looked at the ways in which the cell-surface membrane is able to transport individual molecules and ions.
There are processes where the cell can transport large quantities of material (solids or liquids) ***into*** the cell (**endocytosis**) or ***out*** of the cell (**exocytosis**).
Endocytosis and exocytosis are both active processes requiring ATP as an immediate source of energy.

Endocytosis

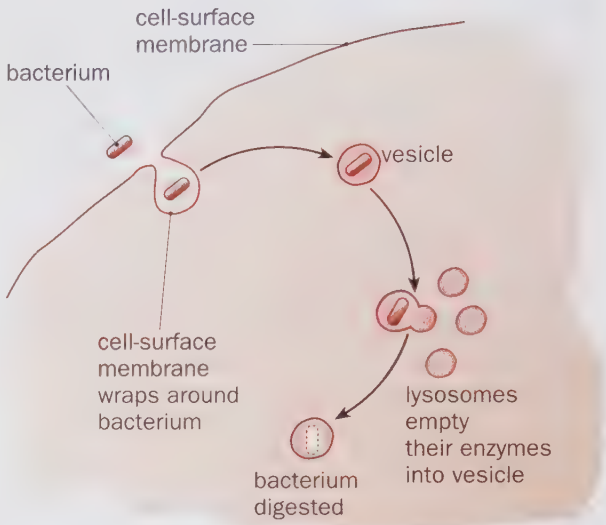
During endocytosis, the cell wraps the cell-surface membrane around the material and brings it into the cytoplasm inside a vesicle.

There are two main types of endocytosis.

- **Phagocytosis** – solid material is taken into the cell in a vesicle. Lysosomes fuse with the vesicle, emptying their enzymes into it. The enzymes digest the material and the products are absorbed into the cytoplasm.

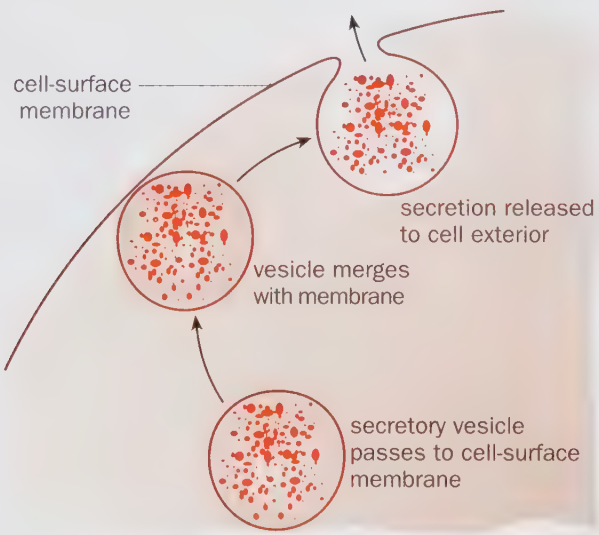
White blood cells, called **phagocytes**, remove bacteria and cell debris by phagocytosis.
Amoeba engulfs its food by phagocytosis. The food is taken into the cytoplasm inside a food vacuole. Lysosomes release digestive enzymes into the vacuole and the soluble products are absorbed.

- **Pinocytosis** – sometimes called 'cell drinking', is similar to phagocytosis but in this case liquid material is taken into the cell. The vesicles formed during pinocytosis can be extremely small. A human egg cell can take up nutrients from surrounding cells by pinocytosis.

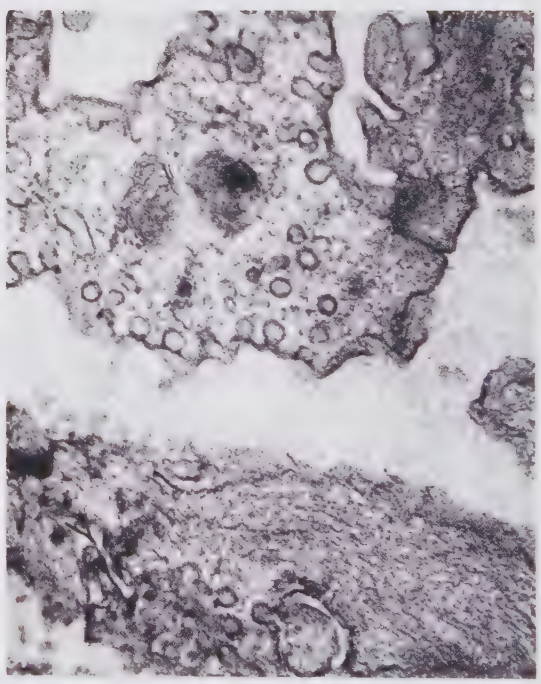


Exocytosis

Exocytosis is the reverse of endocytosis.
It is the passage of materials ***out*** of the cell.
Often this material is a useful **secretion**, as in the case of digestive enzymes, hormones or mucus.



Secretory vesicles carry their contents to the cell-surface membrane. The vesicle merges with the membrane and the secretion is released.



Electron micrograph showing pinocytosis and vesicles (×150 000)

Biology at work: Cryopreservation

The freezing process

Water cooled to below its freezing point without the formation of ice is said to be **super-cooled**.
In the cells of most organisms, only a proportion of the water is converted to ice. The removal of liquid water increases the concentration of solutes in the remaining solution.
Adding a **cryoprotectant**, such as glycerol, reduces this effect and also reduces the amount of ice formed during cooling.

Biological membranes act as efficient barriers to ice crystals.
External freezing of the cell causes its interior to have a lower concentration than the surrounding solution.
Water must therefore be lost from the cell to maintain equilibrium.
This loss can occur in two ways:

- water can move osmotically from the cell to the hypertonic solution around it,
- liquid water can be removed by the formation of intracellular ice.

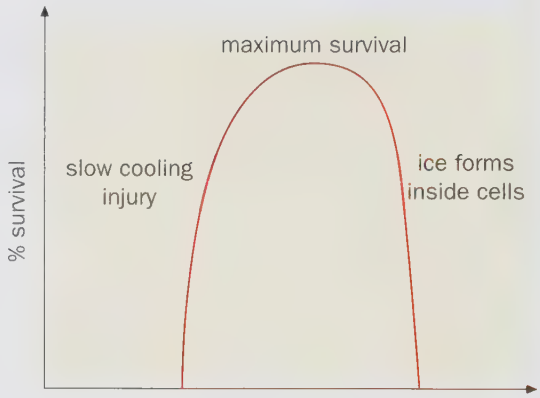
The direct observation of cells during freezing and thawing is now possible using a specialised microscope called a **cryomicroscope**.

A cryomicroscope

This can be used to show that cell survival following freezing and thawing is determined by two potentially damaging processes:

- the effect of concentrated solutions at slow rates of cooling,
- the formation of intracellular ice at faster rates.

Maximum survival varies for different cell types, and is determined by the extent to which the two damaging processes overlap.
Slow freezing rates of $10\,°C\,min^{-1}$ allow sufficient water to leave the cell during the progressive freezing of the extracellular fluid.
Look at the graph. Survival rate increases with increasing cooling rate to an optimum, whilst solute effects are minimised.
The optimum is reached just as the first ice crystals appear.



Effect of cooling rate on survival of a representative biological cell

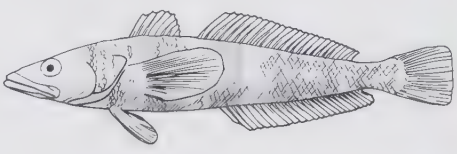
In Antarctic waters

The Antarctic Ocean is the coldest ocean in the world, with average temperatures as low as $-1.87\,°C$. The Antarctic toothfish has evolved to survive temperatures as low as $-2.2\,°C$. It contains a natural antifreeze substance, a type of glycoprotein molecule, which acts as a cryoprotectant. The glycoprotein molecule does not bind to water molecules but restricts their ability to move freely.
The production of this antifreeze substance needs a lot of energy.

The Antarctic toothfish has developed 'neutral buoyancy' or weightlessness in water. This means it does not have to use up energy to float.

- It has a skeleton made of cartilage and has hollow vertebrae. As a result, the density of the skeleton is less than if it was made of bone.
- It also has large amounts of lipids filling the fat cells under the skin and in its muscle. The lipid is less dense than water.

These adaptations aid buoyancy without excessive energy use.
The saved energy can be used to make antifreezing substances.



An Antarctic toothfish which lives, feeds and reproduces on the ocean floor

Effects of low temperatures upon biological membranes

A cell's response to freezing and thawing is determined by the properties of its cell-surface membrane.
The membrane is composed of both lipid and protein components.

The consistency of the lipid bilayer is affected by heat.

At low temperatures the fatty acids of the **phospholipids** are in a relatively rigid, crystalline state.

At higher temperatures the fatty acids assume a more random fluid structure.

The temperatures at which these changes occur is largely determined by the composition of the fatty acid molecules.

The more double bonds a fatty acid molecule contains, the greater the degree of **unsaturation,** and the lower the temperature at which the lipid becomes ***fluid***.

Once cooled, membrane lipids become solid and so the membrane proteins are no longer free to move.
The membrane becomes less ***dynamic***.

At rapid rates of cooling, there is insufficient time for the proteins to migrate.
They become set in a configuration similar to that found at normal temperatures.

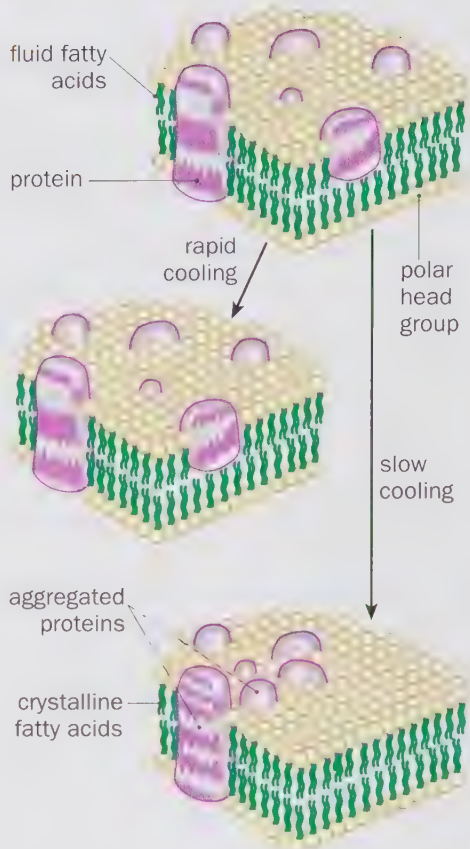
During slow cooling, a gradual change in state occurs, with proteins being pushed into areas of still-fluid membrane.
This results in an aggregated distribution of proteins.

Certain important membrane processes will occur only if the membrane is sufficiently fluid.
Examples include membrane synthesis, transport, and the activity of membrane-bound enzymes.

Living organisms have a semi-fluid lipid composition at normal temperatures.
This is achieved by regulating the amount of unsaturated fatty acids.

This knowledge has been used to develop artificial **cryoprotectant** substances and techniques for use in medical and veterinary applications.
These include the preservation of human bone marrow cells for transplantation to cancer patients, and the storage of sperm and ova for in-vitro fertilisation.

Differences between cells account for some of the difficulties experienced in the preservation of organs.
Each of the organ's cell types has a specific cooling rate required for optimum survival.
Exceptions are human skin grafts and heart valve grafts where there is no need to reconnect blood vessels.



Effects of slow and rapid cooling on the fluid mosaic model of membrane structure

Human bone marrow being placed in a controlled rate freezer

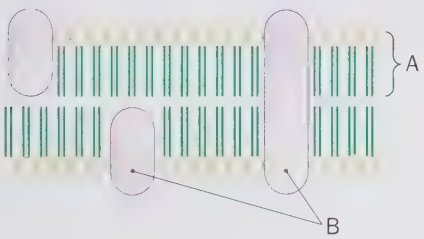
A human aortic valve being prepared for presentation

Summary

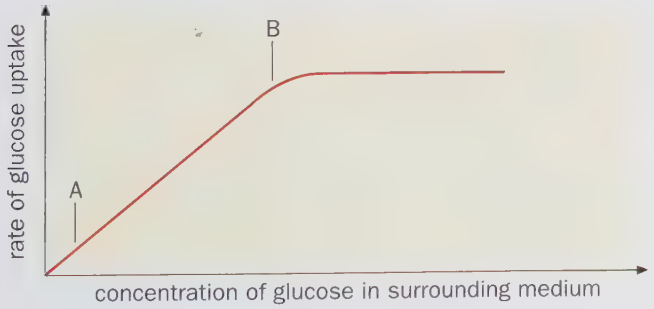
- The cell-surface membrane around a cell is made up of phospholipids and proteins.
- The cell-surface membrane regulates the movement of molecules and ions into and out of the cell.
- Diffusion is the movement of molecules or ions from a region of high concentration to a region of lower concentration until an equilibrium is reached.
- The cell-surface membrane is thin and provides a large surface area over which diffusion can occur.
- Facilitated diffusion increases the rate of diffusion. It involves the use of carrier and channel proteins present in the cell-surface membrane.
- Osmosis is a specialised form of diffusion. It can be defined as the diffusion of water from a region of higher water potential to a region of lower water potential across a partially permeable membrane.
- The ability of a cell to take in water is known as the water potential (Ψ). This can be determined by solute potential (Ψ_s) and the pressure potential (Ψ_p).
- Active transport is the movement of molecules or ions across the cell-surface membrane against a concentration gradient. This involves the use of energy in the form of ATP from respiration.
- Pinocytosis and phagocytosis are different forms of endocytosis. Pinocytosis involves droplets of fluid being taken into the cell. Phagocytosis involves solid particles being taken in.
- Exocytosis occurs when substances are passed out of the cell.
- Co-transport involves the coupling of more than one transport process, for example facilitated diffusion and active transport.

Questions

1 a) Name the molecules labelled A and B.
b) Why is this model described as being ***fluid***?
c) Give two functions of the molecules labelled B.

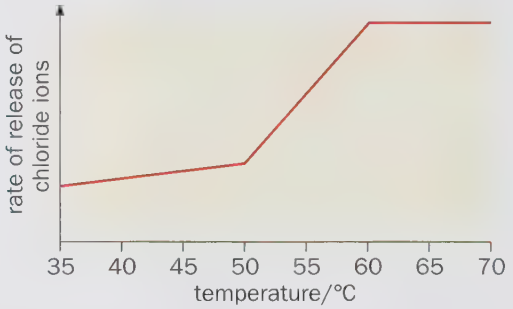


2 The graph shows how the rate of uptake of glucose by red blood cells depends upon the concentration of glucose outside the cells.



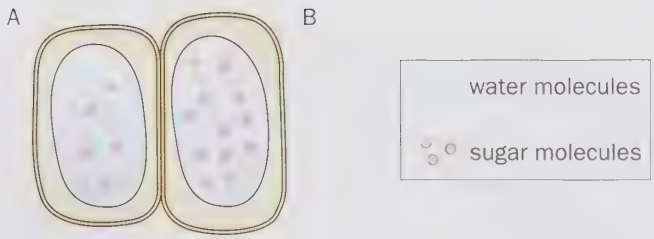
a) i) What limits the rate of uptake of glucose from the surrounding medium between points A and B on the graph?
ii) What evidence from the graph supports your answer?
b) What do you think is limiting the rate of uptake of glucose after point B?

3 The effect of temperature on the permeability of cell membranes can be investigated using fresh carrot. When the carrot discs are placed in water there is a slow release of chloride ions from the vacuoles of the carrot cells.
A number of sets of equal-sized discs were cut and placed into water at different temperatures, from 35 °C to 70 °C. The graph shows the release of chloride ions over this temperature range.



a) Why is it necessary to wash the discs before placing them in water at different temperatures?
b) Explain the increase in the rate of release of chloride ions between the temperatures
i) 35 °C and 45 °C
ii) 50 °C and 60 °C.
c) What assumption is made about the cell wall of carrot cells in this investigation?

4 a) Give a definition of osmosis.
b) i) Using the diagram at the top of the next page, which cell has the higher water potential?
ii) Which cell has the higher solute potential?
iii) In which direction will the water move?

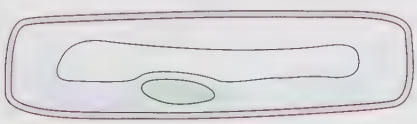


c) i) In cell A the solute potential (Ψ_s) = −1400 kPa, the pressure potential (Ψ_p) = 600 kPa. What is the water potential (Ψ)?
ii) In cell B the Ψ_s = −2000 kPa, the Ψ_p = 800 kPa What is the Ψ?
iii) What will be the Ψ at equilibrium?

5 Some plant tissue was placed in distilled water until the cells became fully turgid.
It was then placed into concentrated sucrose solution until the cells became plasmolysed.
The table shows some of the values of the potentials of the cells.

Conditions of cell	Potential (kPa)		
	Water potential	Solute potential	Pressure potential
fully turgid	0		+300
plasmolysed		−500	

a) Copy the table and complete the missing values.
b) The diagram shows a turgid plant cell.
Draw the same cell in a plasmolysed condition.

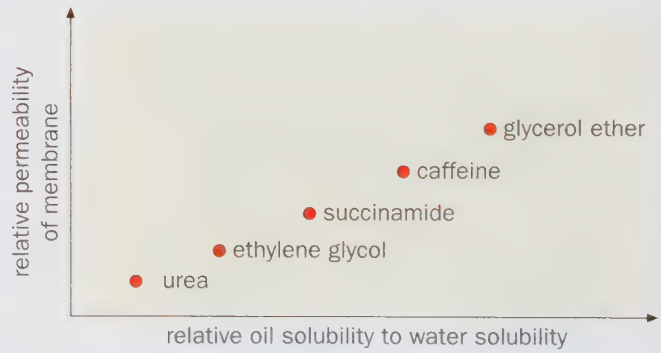


6 Measurements were made of the rate of uptake of substances P and Q across the cell-surface membrane of some cells. The cells were placed in different concentrations of either P or Q and the results are shown in the table.

Concentration (mmol dm^{-3})			Rate of uptake (μmol hour^{-1})	
External (E)	Internal (I)	(E−I)	P	Q
25	50		0	20
50	50		0	40
75	50		12	60
100	50		24	68
125	50		36	70
150	50		48	71

a) Copy the table and complete the column E minus I (E − I).
b) From the data in the table, what is the condition that is essential for the transport of P to take place?
c) Describe the relationship between the concentration gradient and the rate of uptake of P.
d) What process is responsible for the type of transport shown by P?
e) i) What type of transport is shown by Q?
ii) Give one piece of evidence to support your answer.
iii) Apart from Q, name two other molecules associated with the membrane that are needed for this process to take place.
f) i) Compare the increase in the rate of uptake of Q between 25 and 50 mmol dm^{-3} (external concentration) with the increase between 125 and 150 mmol dm^{-3}.
ii) Explain the reason for this difference.

7 a) Draw a simple diagram to show the arrangement of phospholipid molecules in the cell-surface membrane.
b) The graph shows the results of an investigation into the movement of various molecules across the membrane.

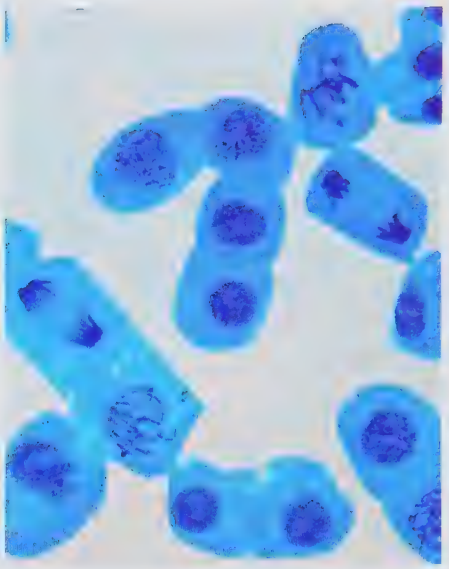


The graph shows an important property of cell-surface membranes.
i) Describe this property.
ii) Explain how the structure of the cell-surface membrane determines this property.
c) Use only information in the graph to explain why:
i) urea has to be actively excreted,
ii) it would be a disadvantage for a cell to excrete a waste molecule having a higher relative oil to water solubility than urea.

6 Cell division

One of the main principles of the cell theory states:
'New cells are formed by the division of pre-existing cells.'
Since all living things are able to grow and reproduce, then the cells of all living organisms must be able to reproduce themselves.

As you have seen in Chapter 4, most of your body cells become specialised in order to perform particular functions.
The cells in your pancreas secrete the hormone insulin, whilst those in your brain transmit electrical impulses.
Once you are fully grown, little cell division occurs in your body, apart from your skin and gut cells, and the cells that form gametes.
In fact, any other incidences of cell division in a fully grown adult may result in a cancer.
Tumours can develop if things go wrong in tissues where cell division is normally very active.



Cells at various stages of cell division

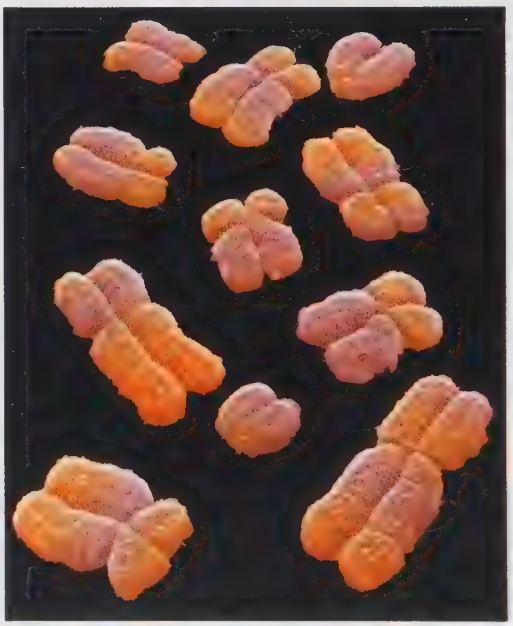
Cells increase in number by **cell division**.
The parent cell divides and passes on genetic material to the daughter cells.
As you already know, this genetic material (DNA) is found inside the nucleus.
The most important part of cell division concerns events inside the nucleus.

Chromosomes

Each chromosome is a single thread-like structure made up of a long molecule of DNA combined with histone protein.
The DNA molecule is made up of many small sections called **genes**.
When the cell is not dividing, the chromosomes are not visible and are dispersed throughout the nucleus as **chromatin**.

When the cell is about to divide, each chromosome condenses and becomes visible.
Each chromosome appears as ***two*** threads.
Each of the threads is called a **chromatid** and they are joined together at a point called the **centromere**.

Where does the second thread come from?
Shortly before cell division occurs, each DNA molecule makes a copy of itself.
So the one thread of DNA becomes two identical threads, the chromatids.
An **allele** is one form of a gene.
Since the chromatids are identical, the form of the alleles for the genes will be the same in the two chromatids.



SEM of human chromosomes

Numbers of chromosomes

Look at the table.
It shows the number of chromosomes found in different plant, animal and fungal species.

Different species have different chromosome numbers.
Can you see any other pattern?

Notice that they are all ***even*** numbers.
This is because chromosomes occur in pairs.

What about size? Do bigger organisms have more chromosomes?
No. There is no relationship between the size of an organism and the number of chromosomes that it possesses.

Species	Number of chromosomes
fruit fly	8
broad bean	12
onion	16
locust	24
lily	24
yeast	34
cat	38
mouse	40
human	46
potato	48
chimpanzee	48
horse	64
dog	78

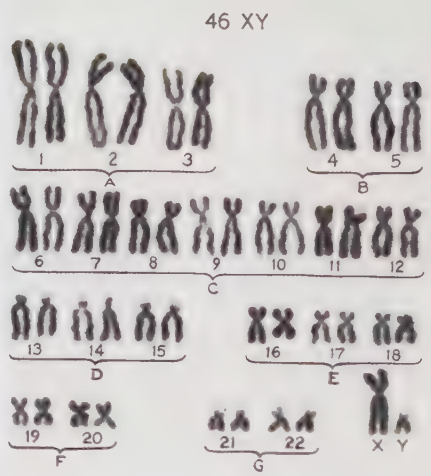
Pairs of chromosomes

Look at the chromosome preparation made from a human male.
This sort of photograph is called a **karyotype**.
It is made by cutting out the chromosomes from a photograph and putting them in their pairs.

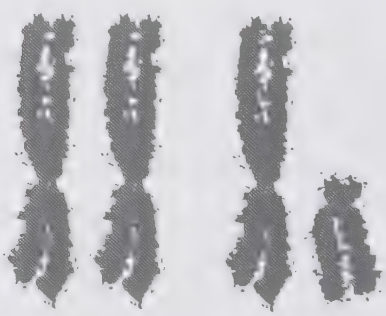
Can you see that:

- They are not all the same size.
  They have been arranged in decreasing order, starting with the biggest.
- They have been put into matching pairs according to their size and shape.
  These are called **homologous pairs** of chromosomes.
  Each homologous pair is given a number.
- The last pair of chromosomes displayed are the **sex chromosomes**.
  All the other chromosomes are termed **autosomes**.
  We can quickly identify the sex of an individual by looking at the sex chromosomes.
  In females, the two sex chromosomes are alike and are termed X chromosomes.
  In males, there is one X chromosome but the other is much shorter and is called the Y chromosome.

Human cells have 46 chromosomes – that's 23 homologous pairs.
This total number of chromosomes is called the **diploid number** (***2n***).
Gametes (sex cells) have ***half*** the normal diploid number.
This is called the **haploid number**, shown as (***n***).
So human sperms will have 23 chromosomes and human eggs will have 23 chromosomes.



Human male karyotype

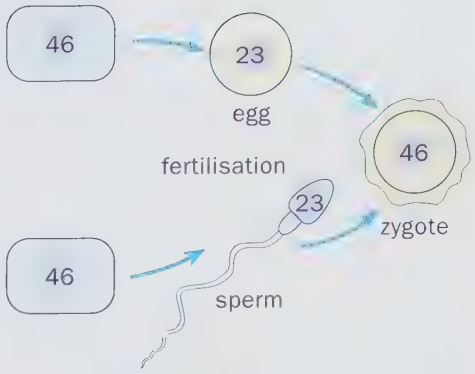


female sex chromosomes

male sex chromosomes

Halving and doubling

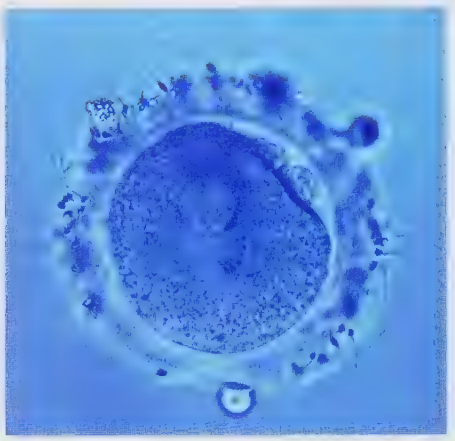
So why is it important that gametes are haploid?
What would happen if a diploid sperm fertilised a diploid egg?
That would be 46 sperm chromosomes and 46 egg chromosomes, giving 92 chromosomes in the fertilised egg – double the normal number!



Normally cell division produces identical daughter cells, each with a full complement of chromosomes.
There has to be a special type of cell division that produces gametes with a haploid chromosome number.
So at fertilisation a diploid **zygote** (fertilised egg) is produced.

Half your chromosomes came from your father in the sperm.
These are called the **paternal** chromosomes.
The other half came from your mother in the egg.
These are called **maternal** chromosomes.
Each homologous pair is made up of one paternal and one maternal chromosome.

The zygote divides by mitosis, producing diploid cells with identical chromosomes.
These chromosomes must therefore carry the same genes and alleles as those found in the zygote.
This is why you have inherited characteristics from each of your parents.
Half your alleles came from your father and half came from your mother.

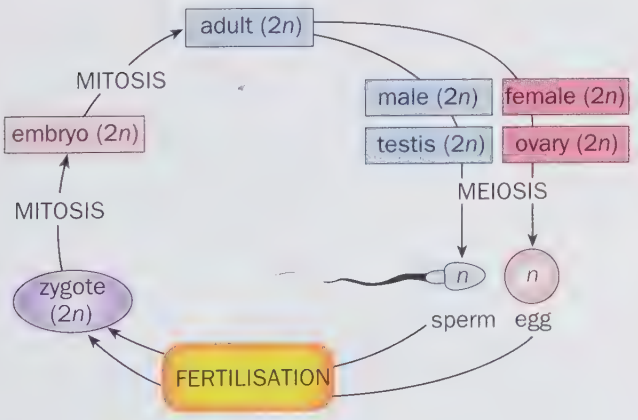


A fertilised human egg or zygote

The dividing cell

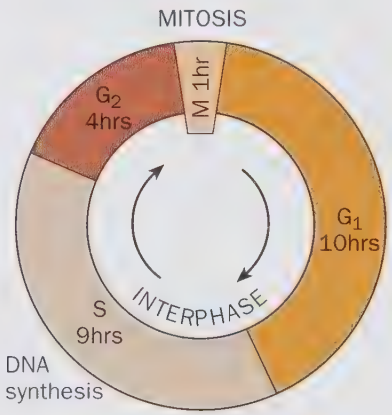
Cell division involves the division of the nucleus and then the division of the cytoplasm.
There are ***two*** types of cell division:

- **Mitosis**, which produces two daughter cells that are genetically identical to the parent cell.
- **Meiosis**, which produces four genetically different haploid cells. Meiosis, unlike mitosis, involves two consecutive divisions.



Mitosis occurs during growth and asexual reproduction when it is important that each daughter cell has the same chromosomes as the parent cell and the same genes.

Meiosis occurs during sexual reproduction, when it is important that haploid gametes are produced.



*The cell cycle consists of mitosis (the **M phase**) and a period between divisions called **interphase**.*
*Interphase is divided into three **growth phases**:*
***G_1 phase** a period of rapid cell growth when new organelles are synthesised.*
***S phase** when the amount of DNA in the cell doubles.*
***G_2 phase** when the centrioles replicate and microtubules start to construct the spindle.*

Stages in mitosis

The function of mitosis is to increase the number of cells that are genetically identical to the parent cell.

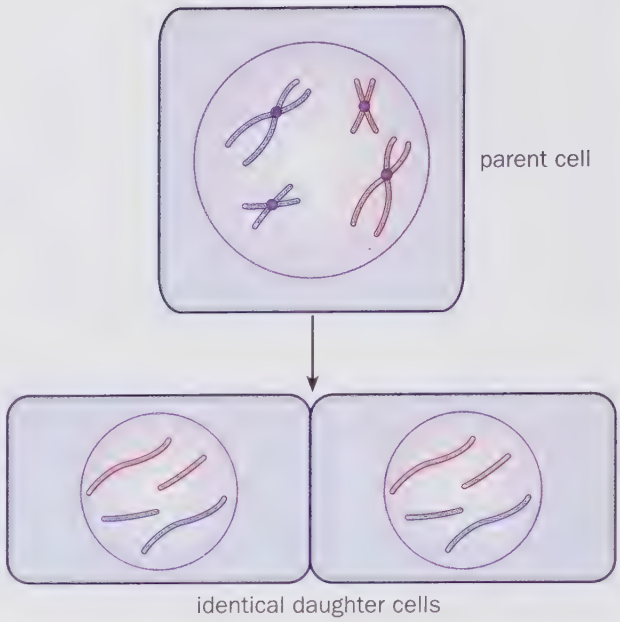
Mitosis occurs during growth and asexual reproduction.
A fertilised egg divides many times by mitosis to form an embryo.
But so do the cells in your skin, bone marrow and in a healing wound.
In flowering plants, growth is greatest at the shoot and root tips.
Here mitosis occurs in particular areas called **meristems**.

The use of time lapse photography with a microscope enables us to observe mitosis as a continuous process.
However, for convenience, mitosis can be divided into a number of stages when particular events take place.

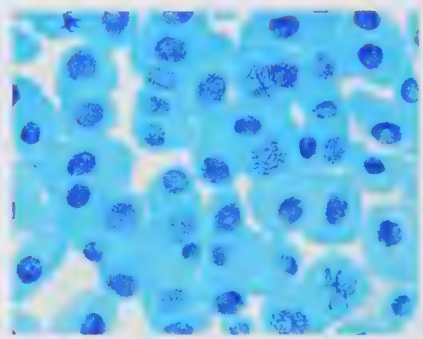
If you look at a section of root tip under the microscope, most cells will not be dividing.
This stage between divisions is known as interphase.
It is sometimes called the 'resting stage' but in actual fact the cell is metabolically active.
During interphase:

- the amount of DNA in the nucleus doubles,
- new organelles, for example mitochondria, are made.

No chromosomes are visible at interphase because the chromosome material, chromatin, is dispersed throughout the nucleus in a diffuse form.



At mitosis the parent cell divides to produce two daughter cells with identical chomosomes



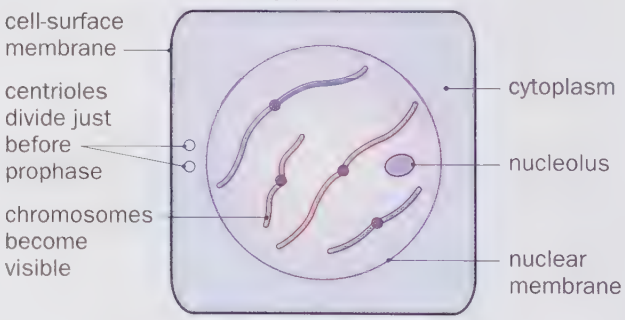
Cells at various stages of mitosis

Prophase

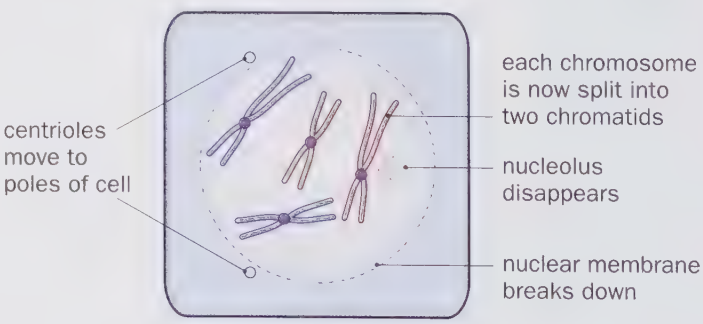
Prophase is the longest stage in mitosis.

- The chromosomes become visible as long thin threads.
- The chromosomes start to coil up and become shorter and thicker.
- In animal cells, the centrioles divide and move to opposite ends (**poles**) of the nucleus.
- Protein microtubules develop from each centriole, forming **spindle fibres**. Some of these extend from pole to pole. In plant cells, there are no centrioles and the **spindle** forms independently.
- Towards the end of prophase, each chromosome can be seen to consist of two chromatids held together by a centromere.
- At the end of prophase, the nucleolus disappears and the nuclear envelope (consisting of nuclear membranes) breaks down.
The chromosomes now lie free in the cytoplasm.

Early prophase



Late prophase



Metaphase

Metaphase is a relatively short stage in mitosis.
Consequently, it can be difficult to observe on a slide section.

- The chromosomes move towards the **equator** of the spindle.
- Here they attach themselves to a spindle fibre by means of a centromere.

Anaphase

Anaphase is also a short stage in mitosis.

- The centromeres holding each pair of chromatids together divide.
- The free chromatids move to the poles, centromere first.
  This movement results from the contraction of the spindle fibres.
  As they shorten, they pull the chromatids apart.

Telophase

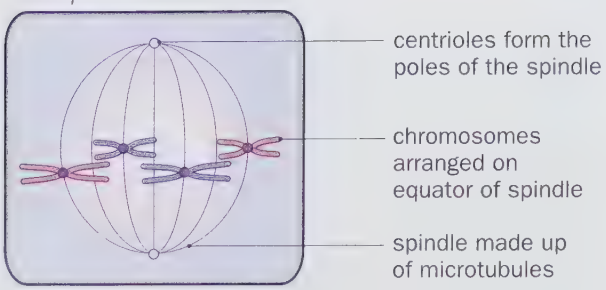
- The chromatids have now reached the poles and can be regarded as distinct chromosomes.
- This final stage of mitosis can almost be regarded as the reverse of prophase.
  The nuclear envelope forms around each group of chromosomes and the nucleolus reappears.
  The chromosomes uncoil to form diffuse chromatin.
- The cytoplasm divides by a process called **cytokinesis**.

Cytokinesis

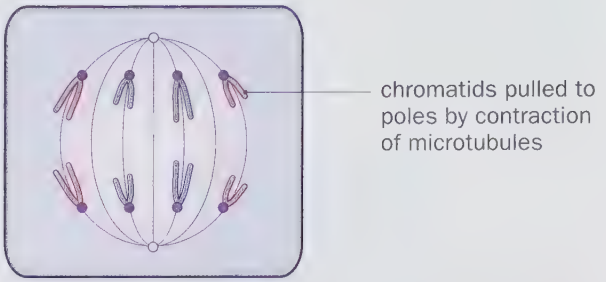
Division of the cytoplasm often starts during telophase.
In animal cells, the centre of the cell 'pinches in' to form a **division furrow**.
This forms in the same plane as the equator.
The furrow forms due to the contraction of a ring made up of two proteins, actin and myosin.
As the division furrow deepens, the cell-surface membrane on each side joins up and two separate cells result.

Cytokinesis is different in plant cells.
First, vesicles produced by the Golgi body collect on the equator.
These vesicles contain carbohydrates, such as pectins and hemicelluloses.
The vesicles fuse together to form a **cell plate**.
The cell plate eventually stretches right across the cell, forming the **middle lamella**.
Cellulose builds up on each side of the middle lamella to form the cell walls of two new plant cells.

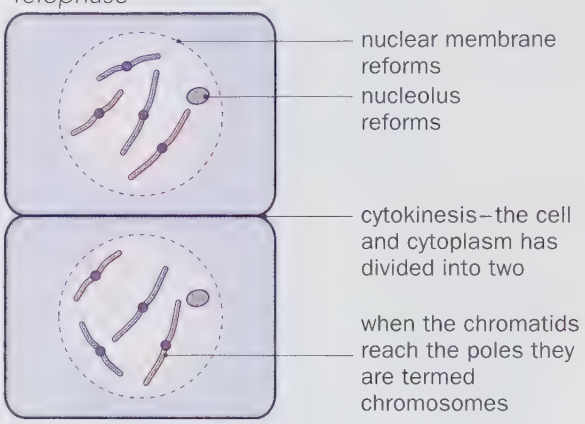
Metaphase



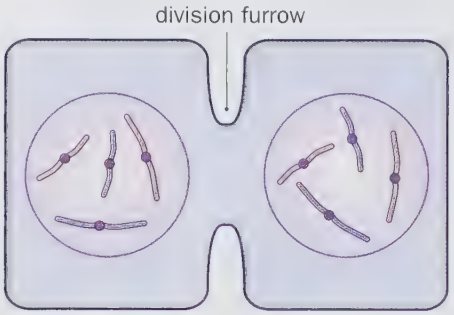
Anaphase



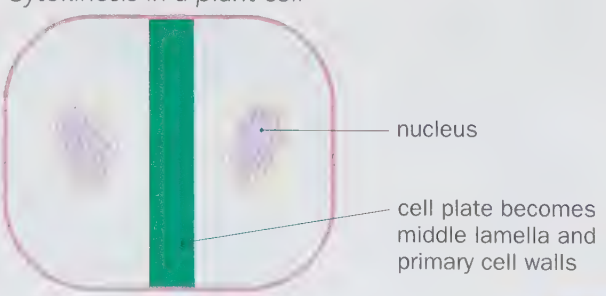
Telophase



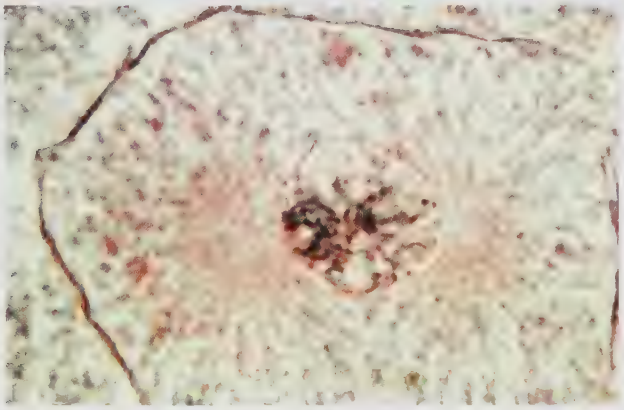
Cytokinesis in an animal cell



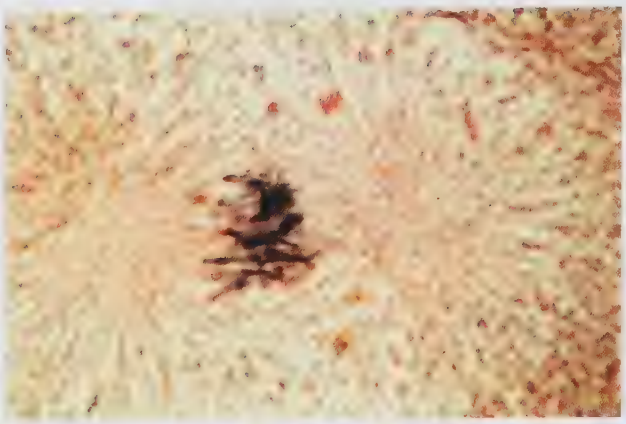
Cytokinesis in a plant cell



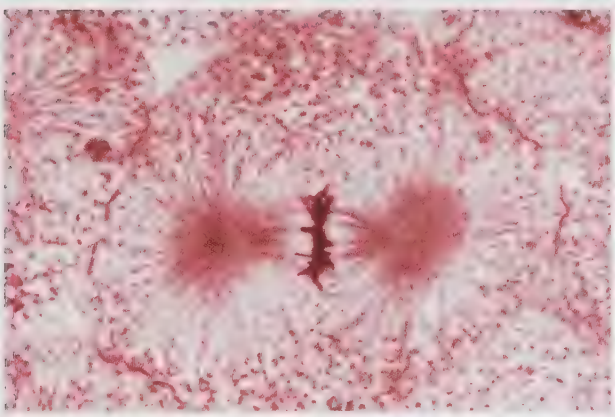
Photomicrographs of stages in mitosis



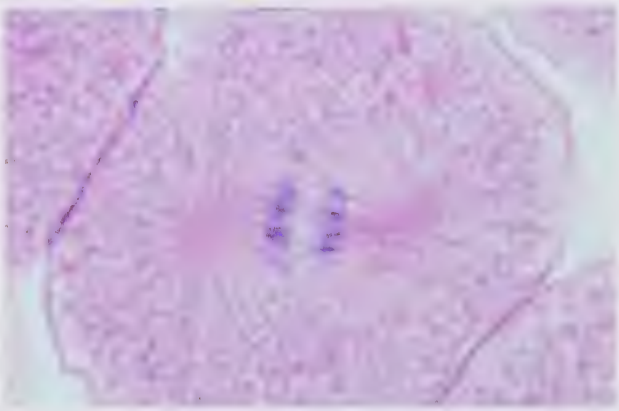
Prophase: chromosomes become visible as chromatin condenses



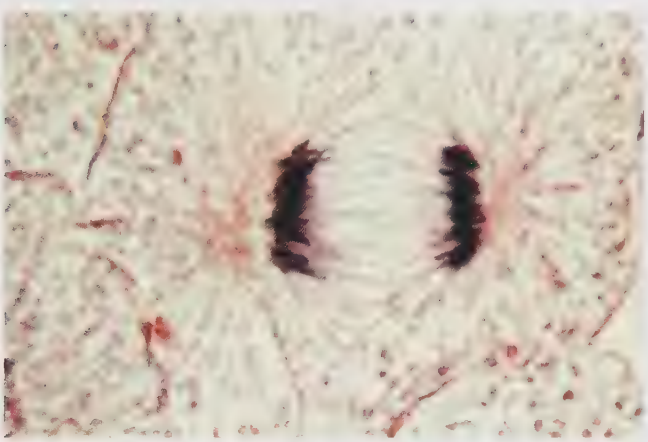
Late prophase: fully organised chromosomes, split into chromatids, move to the centre of the cell. The spindle fibres are well formed



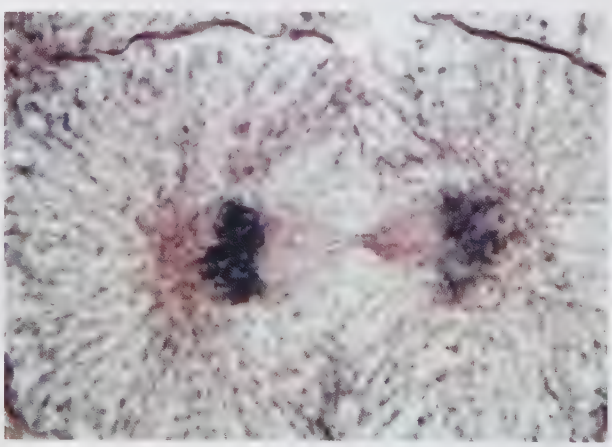
Metaphase: the spindle fibres are well formed from microtubules.The centrioles are located at the poles of the spindle and the chromosomes arrange themselves on the equator



Early anaphase: the chromatids separate and start to move to their respective poles



Late anaphase: the chromatids reach the poles drawn by the contractile microtubules in the spindle



Telophase: the chromatids once they reach the poles are fully organised chromosomes. A cell membrane forms between the two cells (cytokinesis)

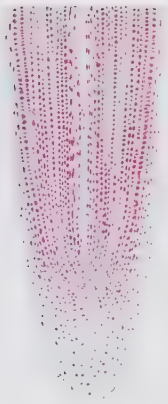
These stages of mitosis are shown from photomicrographs of whitefish

Significance of mitosis

Mitosis produces cells that are an ***exact*** copy of the parent cell. These daughter cells have the same number of chromosomes and are genetically identical to the parent cell.

Mitosis is important for the following processes:

- **Growth**
  As multicellular organisms grow, the number of cells making up their tissues increases.
  The new cells must be identical to the existing ones.
  Growth by mitosis takes place over the whole body in animals.
  In plants, growth is confined to certain areas called meristems.
- **Repair of tissues**
  Damaged cells must be replaced by identical new cells.
  Your skin cells and the cells lining your gut are constantly dying and being replaced by identical cells.
- **Asexual reproduction**
  Asexual reproduction results in offspring that are identical to the parent. Mitosis occurs when unicellular organisms, such as yeast and *Amoeba*, reproduce.



Mitosis takes place first behind the root tip

Mitosis can also produce new offspring in multicellular organisms. *Hydra* is a primitive animal that lives in freshwater habitats. It produces 'buds' that eventually break away and form new individuals identical to the parent.

Budding in Hydra

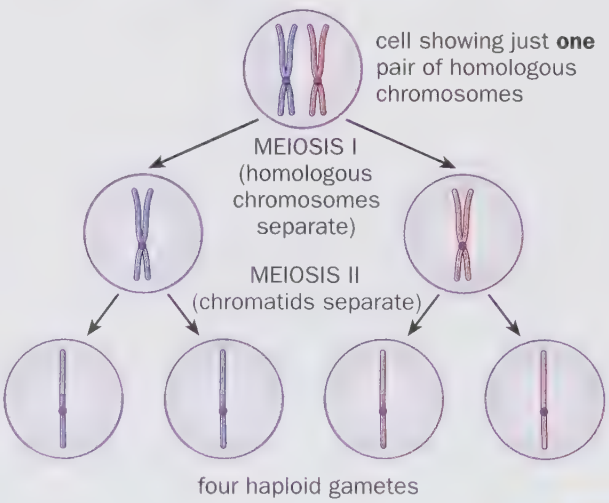
In flowering plants, organs such as bulbs, corms, tubers, rhizomes and runners are produced by mitotic division. When they separate from the parent they form a new individual. Mitosis can result in the production of large numbers of offspring in a relatively short period of time. But there is little or no variation between each individual. As you will see, mitosis of cells grown in tissue culture has important applications in both genetic engineering and biotechnology.

Micropropagation: plants grown in tissue culture

Binary fission occurs in prokaryotic cells. This involves replication of the circular DNA (nucleoid) and of plasmids, and division of the cytoplasm into two daughter cells each with a single copy of the circular DNA and a variable number of **plasmid** copies.

Meiosis

Meiosis is a type of cell division that is vital for sexual reproduction and takes place in the reproductive organs. It results in the formation of gametes with half the normal chromosome number.



So haploid sperms are made in the testes and haploid eggs are made in the ovaries. In flowering plants, haploid gametes are made in the anthers and ovules.

In contrast to mitosis, meiosis produces cells that are not genetically identical so it has a key role in producing new genetic types, that is, it results in **genetic variation**.

Stages in meiosis

Meiosis involves ***two*** divisions of the cell.
These two divisions are termed meiosis I and meiosis II.
As with mitosis, the cell is said to be in interphase when it is not dividing.
During interphase the DNA content of the cell doubles and new cell organelles are formed.

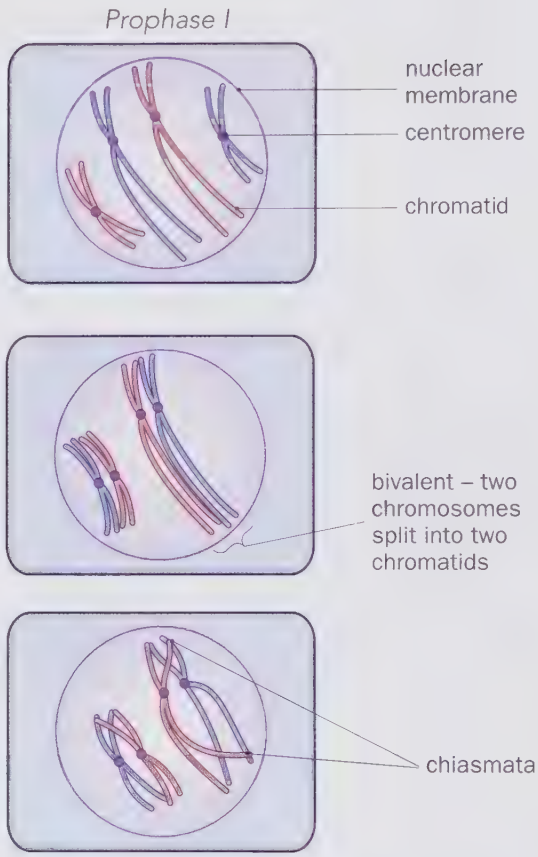
Meiosis I

Prophase I

- The chromosomes condense and are seen to have split into two chromatids.
- As in prophase of mitosis, the chromosomes shorten and thicken by coiling.
- One of the most important features of prophase I of meiosis is that the paternal and maternal chromosomes come together in homologous pairs.
- This pairing of the chromosomes is called **synapsis**.
  Each homologous pair of chromosomes is called a **bivalent**.
  So a bivalent consists of ***four*** strands:
  two chromosomes, each split into ***two*** chromatids.
- As the chromosomes pair up, they shorten and twist around each other. This causes a tension, and sections of chromatid may break off and exchange with corresponding sections of a different chromatid. The points where this exchange of chromatid material occurs are called **chiasmata** (singular **chiasma**). This swapping of chromatid material is called **crossing over**.
- Towards the end of prophase I, the nucleolus disappears and the nuclear envelope breaks down.

Prophase I is the longest and most complex stage in meiosis.
The key events are:

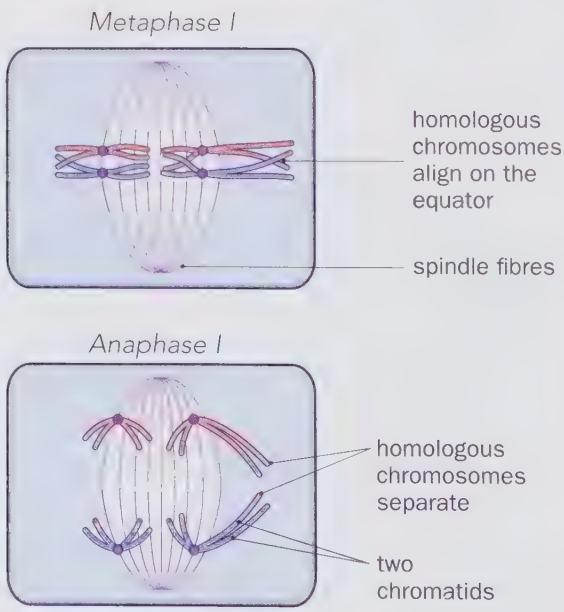
- the pairing of homologous chromosomes (bivalents),
- the exchange of chromatid material at chiasmata (crossing over).



Metaphase I

- At the start of metaphase I, the spindle will have formed.
- As in mitosis, the chromosomes assemble on the equator of the spindle.

How does the arrangement of chromosomes differ from that at metaphase of mitosis? The key difference in meiosis metaphase I is that the chromosomes are joined in homologous pairs (bivalents).



Anaphase I

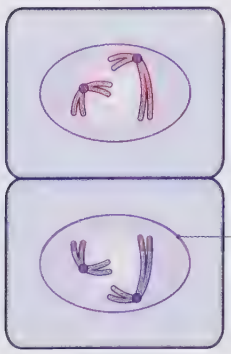
- At anaphase I the ***chromosomes*** in each bivalent separate. Compare this with anaphase of mitosis, when the ***chromatids*** separate.
- As a result of anaphase I, each pole receives only ***one*** of each homologous pair of chromosomes.
- As in mitosis, the contraction of the spindle fibres pulls the homologous chromosomes apart.
  Each pole receives a ***haploid*** number of chromosomes.

Telophase I

- The chromosomes reach the opposite poles of the spindle.
- The nuclear envelope forms around each group of ***haploid*** chromosomes.
- Usually the chromosomes stay in their condensed form and meiosis II follows on straight away.
- Cytokinesis occurs to produce two haploid cells.

What has happened to the number of chromosomes in each cell at the end of meiosis I?

Telophase I



Meiosis II

Prophase II

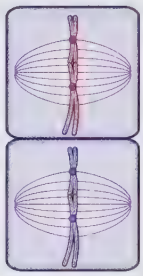
- The new spindle fibres develop at right angles to the old spindle.

Metaphase II

- The separate chromosomes arrange themselves on the equator of the spindle.
- Each chromosome attaches to a spindle fibre by means of its centromere.

How is this different from the arrangement of chromosomes at metaphase I of meiosis?

Metaphase II



Anaphase II

- The centromeres divide.
- The spindle fibres contract to pull the two chromatids to the poles, centromere first.

How is this different from the events that take place at anaphase I of meiosis?

Anaphase II

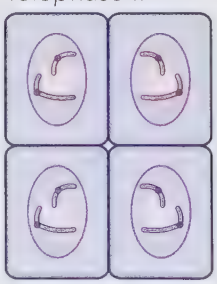
Telophase II

- On reaching the poles, the chromatids lengthen and become indistinct.
- The spindle disappears and the nuclear envelope reforms.
- Cytokinesis takes place, resulting in four haploid cells, each with a different genetic make up.

Can you identify the ***two*** main ways in which the genetic make up of these cells has been changed.

Try looking back over the events that take place during meiosis on these two pages.

Telophase II



Meiosis and genetic variation

There are two main ways in which genetic variation occurs at meiosis.

Random segregation of chromosomes
This is also referred to as 'independent assortment' and 'independent segregation'.

At metaphase I the pairs of homologous chromosomes arrive at the equator.
They arrange themselves in a random order on the equator.

Look at the diagram.
It shows two cells with just ***two*** homologous pairs of chromosomes ($4n$).

What do you think determines whether the blue chromosome or the red chromosome is uppermost?
The answer is that it relies on chance.

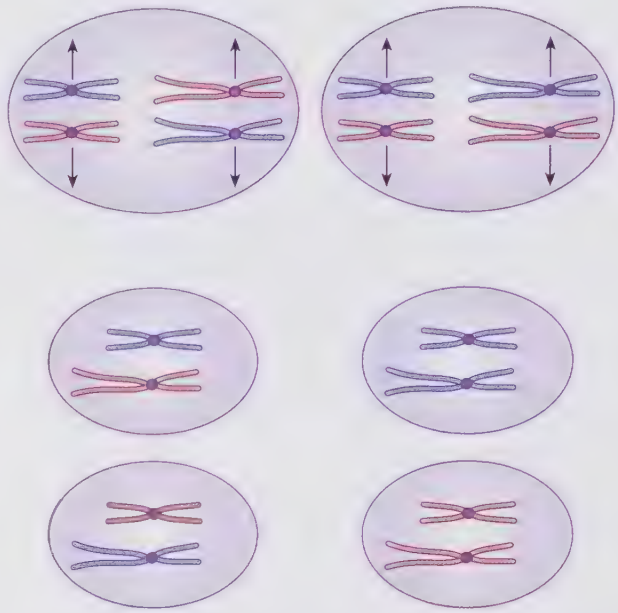
The diagram shows that there are ***two*** ways that a pair of chromosomes can arrange themselves on the equator.

As a result, it is possible to produce ***four*** different types of gamete.

Our example had just two pairs of chromosomes.
But a human cell contains 23 pairs of chromosomes.

Can you work out how many possible types of gamete could be produced?
The answer is 2^{23} – that's over 8 million different types of gamete!

And during fertilisation ***any*** male gamete can join with ***any*** female gamete.
So thousands of millions of new genetic combinations are possible!



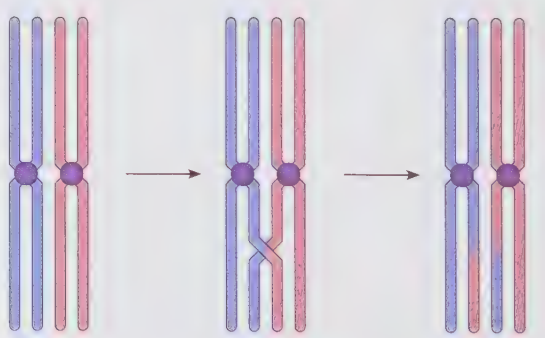
Random arrangement of homologous chromosomes at metaphase I of meiosis

Crossing over

During prophase I of meiosis the homologous chromosomes come together in pairs.
Each chromosome is divided into two chromatids.
The homologous chromosomes twist around each other.
This creates a tension, which may cause breaks to occur along the length of the chromatids.

During crossing over, corresponding fragments of chromatid may get swapped over.
This 'cutting and sticking' of chromatids means that genetic material is exchanged.
In this way, new genetic combinations are produced and variation in the gametes is increased.

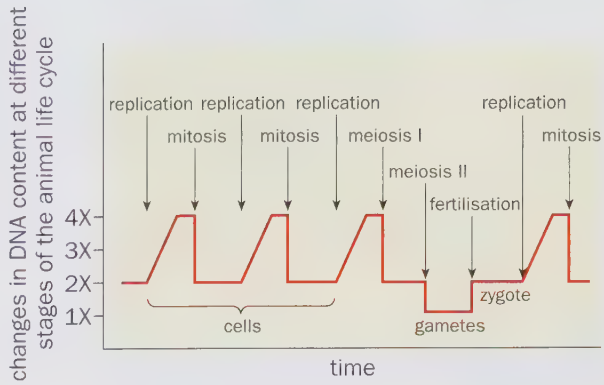
So, together with random segregation of chromosomes, crossing over can produce an enormous amount of variation, both in the gametes and the resulting offspring.



Cell division and DNA

As you have seen, the number of chromosomes can change at different stages of cell division.

The chromosomes are made up of DNA and histone proteins. So it should not be a surprise to you that the DNA content of a cell changes at different stages of mitosis and meiosis.



Look at the diagram.
The normal amount of DNA in this diploid cell is $2x$.

What happens to the amount of DNA just before mitosis?
The amount of DNA doubles (to $4x$) due to replication.

At the end of mitosis I, the DNA content is back to $2x$.
This is because the $4x$ content has been distributed equally between the two daughter cells.

At the interphase of meiosis, the DNA content again doubles (to $4x$).
What do you think happens to the DNA content after meiosis I?

The amount of DNA halves because each homologous chromosome separates into each daughter cell.

What do you think happens after meiosis II?

The DNA content of the cell is now down to $1x$.
This is because the chromatids have separated, effectively halving the DNA content.

After fertilisation the DNA content is back to $2x$, because the haploid gametes (each $1x$) have joined together.

Differences between mitosis and meiosis

The differences between mitosis and meiosis can be summarised in the form of a table.

Mitosis	Meiosis
one division	two divisions
the number of chromosomes remains the same	the number of chromosomes is halved
homologous chromosomes do not pair up	homologous chromosomes pair up to form bivalents
chiasmata do not form and crossing over never occurs	chiasmata form and crossing over occurs
daughter cells are genetically identical	daughter cells are genetically different from the parent cells
two daughter cells are formed	four daughter cells are formed

Stem cells

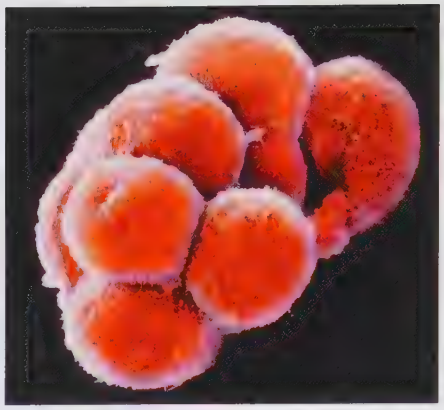
Most cells become specialised to carry out a particular function.
We say they have become **differentiated**, and in animals this process is irreversible, so when, for instance, skin cells divide the daughter cells produced will be skin cells. There are, however, some cells that remain undifferentiated and can develop into any type of cell, these are called **stem cells**.
Stem cells have the ability to divide indefinitely and they have the potential to differentiate into specialised cell types. (See also gene expression on page 388.)

Embryo stem cells

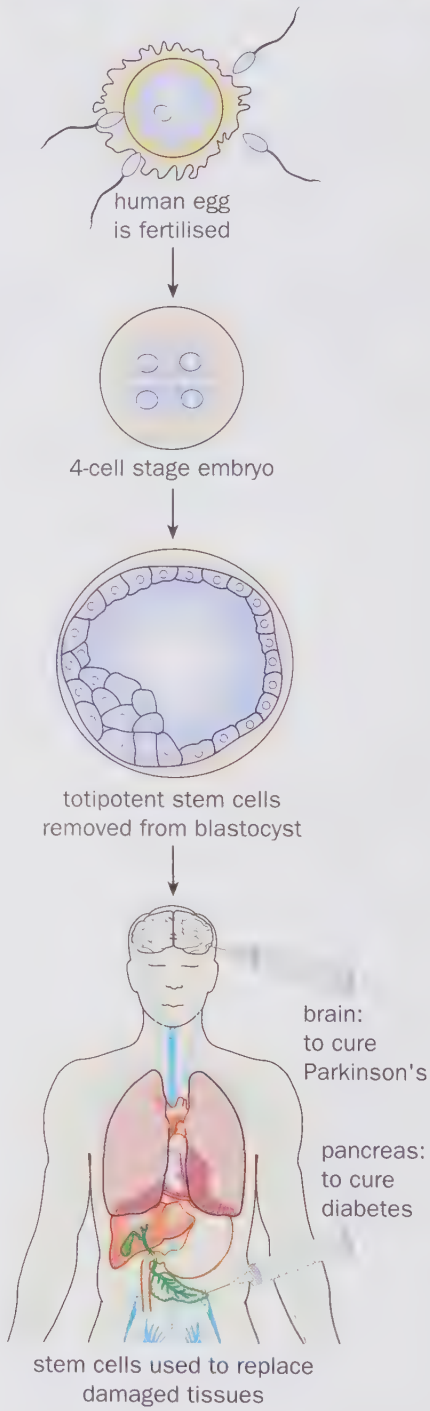
In humans a fertilised egg or zygote has the potential to form any of the many cell types of the body.
The zygote soon starts to divide by mitosis producing a mass of small identical and undifferentiated cells called a **blastocyst**.
These early embryos are made up of tiny stem cells which are **totipotent** and have the ability to differentiate into any cell type from instructions in their DNA.
A blastocyst consists of a ball of cells and the outer layer of cells eventually forms the placenta.
However, the cells of the inner layer have already lost some of their ability to differentiate.
They are already destined to form the different cell types needed in the future (with the exception of the placenta).
These cells are known as **pluripotent** embryonic stem cells.
Early embryos have been created by **in-vitro fertilisation** (**IVF**) in order to help infertile couples.
Unused embryos have been used to create stem cells, with the consent of the donor couple.
These totipotent stem cells can be differentiated into any cell type for medical use.
However there is a debate as to whether it is ethical to use these embryos in this way.
Embryonic cell research is currently allowed in the UK, but under stringent guidelines set out by the government.

Adult stem cells

Once cells have matured and specialised they can no longer develop into other cells, they lose their totipotency. Red blood cells, which carry oxygen in animals, and xylem vessels, which transport water in plants, are so specialised that they lose their nuclei once they mature. Only a few totipotent cells exist in mature animals. These are called **adult stem cells** and can be extracted from certain tissues in the body, for example the bone marrow. It is thought that most organs have a small number of undifferentiated stem cells which can be used to repair and replace damaged tissue. These stem cells are termed **multipotent**, since they can differentiate into their own type of cells, for example blood cells and skin cells. There are no ethical issues in the clinical use of these cells, however they are difficult to locate and hard to grow in culture.
The potential for the use of stem cells in medicine could include the transplant of tissue grown from stem cells into damaged tissue. Stem cells extracted from bone marrow have already been used successfully to treat leukaemia (cancer of the white blood cells). Other diseases that could possibly be treated include Parkinson's, motor neurone disease, Alzheimer's, type 1 diabetes, muscular dystrophy and osteoporosis. Since the tissues could be grown from the patient's own adult stem cells there is no risk of rejection.
(See page 410 for plant stem cells in tissue culture.)



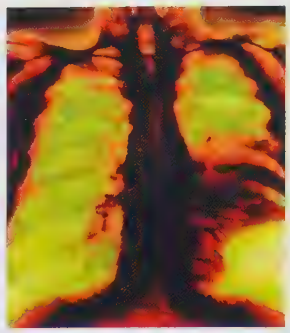
Early human embryo



Biology at work: The biology of cancer

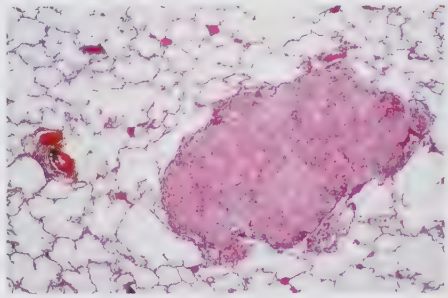
According to the World Health Organisation (WHO) cancer is one of the world's leading causes of death, and in 2012 there were 8.2 million deaths from all forms of cancer worldwide. It is, however, not one single disease but a group of diseases, all resulting from uncontrolled mitotic cell division.

There are over 200 different types of cancer, and it affects more than one in three of the UK population at some time in their lives. The likelihood of developing cancer increases with age, with the risk roughly doubling with each decade beyond the age of 30. In the UK, breast cancer is the most common cancer overall, and amongst men prostate cancer is the most common.



X-ray of human chest showing cancer of the lung

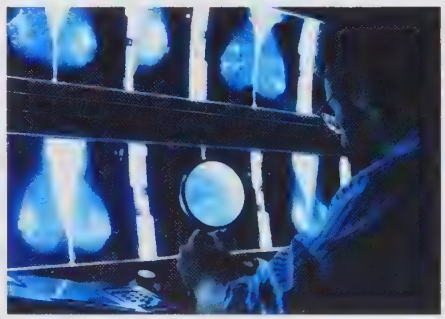
Cell division is controlled by genes, and normally stops when enough cells have been made to perform a particular task.
If these genes undergo **mutation**, they form **oncogenes**.
Cell division runs out of control and the cells simply continue to divide. This results in the development of a tumour, which is a group of abnormal cells that divide more rapidly than the normal surrounding cells. Cancer can result if mutations occur in **tumour suppressor genes** (or **antioncogenes**). The loss of these genes is thought even more important than the activation of oncogenes in the formation of some forms of cancer. The process of methylation plays an important part in the loss of tumour suppressor genes and the activation of oncogenes (see page 396).



Human lung tissue invaded by a malignant tumour (pink, right)

Tumour cells usually lack differentiation. This means that they do not carry out the specialised function of the cells in their host tissue. They interfere with the normal activity of these cells, for example restricting the blood flow to them. At this point a small tumour may not be producing any noticeable symptoms, but cancerous cells may be breaking free and may be transported around the body in the bloodstream. If this happens then secondary tumours (called **metastases**) may develop in a variety of other body tissues.

Tumours that behave in this way are called **malignant**.
Some tumours remain inactive and are relatively harmless.
These inactive growths are called **benign** tumours.



A radiographer studying mammograms

What factors cause the mutation of genes?

Although mutations occur spontaneously, smoking and excessive exposure to strong sunlight are two activities known to increase the likelihood of mutation.
There is also a range of **carcinogenic** (cancer causing) agents to which you could be exposed, for example asbestos, ionising radiation and benzene.
Research has shown that increased levels of the female reproductive hormone oestrogen is linked to the incidence of breast cancer.

Treatment

Each specific type of cancer has its own set of treatment methods.
Surgery is also sometimes carried out to remove cancerous tissue, and this is often combined with chemotherapy (powerful cancer-killing medication) and radiotherapy (the controlled use of high energy X-rays).
These therapies inhibit cell division in the tumour. However, they can have unpleasant side effects owing to their effect on other tissues.

Biology at work: Cancer by infection

As you know the genes that cause cancer are called oncogenes.
These are the mutated forms of the genes that normally regulate cell division.
The rate of mutation can be increased by a number of factors, for example a virus infection.
Viruses usually carry oncogenes or regulatory genes that can become oncogenes.
An example of one such virus is the **human papillomavirus** (**HPV**), a serious form of which is responsible for cervical cancer.

Cervical cancer

Cervical cancer is one of the most common forms of disease in women, with 3000 cases diagnosed each year in the UK.
A successful screening programme has led to a decline in the number of women dying from cervical cancer.
Originally, the disease was thought to be caused by a sexually transmitted infection.
A small sub-group of 'high risk' viruses known as HPV16 and HPV18 need to be present before cervical cancer will develop.
The specific gene sequence is known for this virus, as are the identities of the two oncogenes labelled E6 and E7.
It seems likely that the development of the cancer starts when these two oncogenes bind to the proteins known to control cell division in cervical epithelial cells.

Screening

The early stages of cervical cancer occur in surface cells.
This has two useful implications, which have aided the development of an effective screening programme.

1 Cervical cells can be scraped off during a **smear test** and later identified under a microscope.

2 As the cancer remains superficial, it is possible to remove it using a simple surgical technique.

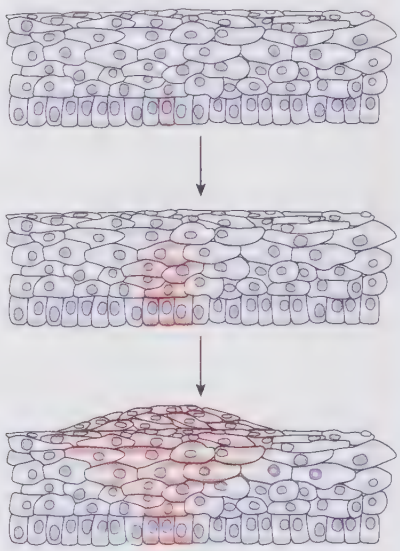
Smear test cells are classified according to their appearance.
These can range from 'normal' to 'invasive cancer'.
If necessary, this diagnosis is later confirmed by taking a small tissue sample or **biopsy**.

Regular repetition of the screening reduces the incidence of false negatives.
Up to 15% of pre-cancerous cells can be missed in the initial smear.

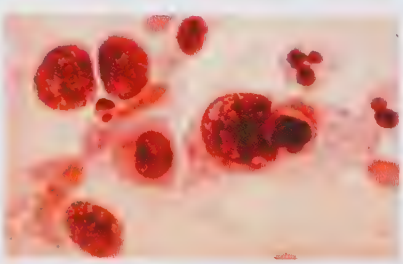
Treatment

In its early stages, cancerous tissue can be destroyed with heat, by using laser treatment, or by the technique of cryosurgery, which uses extreme cold.
More advanced cases, where the cervical cancer has spread to the organs of the pelvis, usually require radiotherapy.

The genetic organisation of HPV16. Each segment represents a gene. The gene segments labelled E6 and E7 encode proteins with oncogenic potential



The establishment of an HPV requires the infection of a basal stem cell in the surface layer of cells (top). It is this layer that is scraped off in a smear test. If left untreated the cancer spreads upwards (middle) until the whole tissue is infected (bottom)



Cervical biopsy reveals a pre-cancerous cell is present with enlarged cell nucleus and abnormal shape (centre)

Summary

- There are two types of cell division: mitosis and meiosis.
- Mitosis occurs during growth and repair of cells and during asexual reproduction.
- The daughter cells produced at mitosis are genetically identical to the parent cell.
- At interphase, the DNA content of the nucleus doubles.
- The main stages of mitosis are prophase, metaphase, anaphase and telophase.
- Meiosis occurs during the formation of gametes (and of spores in plants like mosses and ferns).
- During meiosis, four haploid cells are produced, each being genetically different.
- It is important that gametes are haploid so that at fertilisation a diploid zygote is formed.
- During meiosis, chromosomes come together in homologous pairs.
- Meiosis has two divisions and exchange of chromosome material results in variation.
- Cancers can develop from the uncontrolled division of cells.
- Stem cells have the ability to divide indefinitely and the potential to differentiate into specialised cell types.

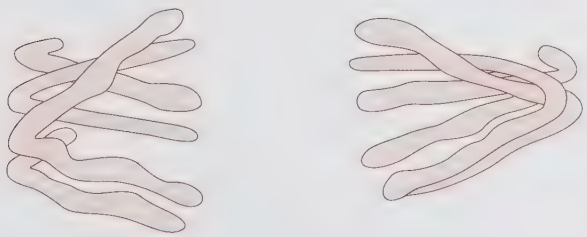
Questions

1 a) Copy and complete the table to show which processes take place during mitosis, and which take place during the first division of meiosis.
Use a tick (✓) if you think that the process does occur and a cross (✗) if you think that it does not.

Process	Mitosis	First division of meiosis
homologous chromosomes pair		
crossing over		
chromatids separate		

b) Why do you think that mitosis is a suitable type of cell division for repairing tissues but meiosis is not?

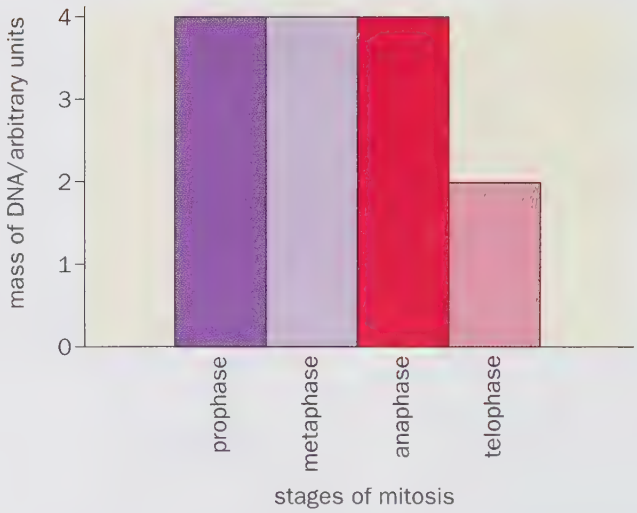
2 a) Explain why root tips are suitable material to show stages in mitosis.
b) When preparing root tips to show stages in mitosis, why is it important to:
i) stain the tissue,
ii) pull the tissue apart with a needle and gently apply pressure to the cover slip?
c) Look at the drawing showing a cell undergoing mitosis.



i) Which stage of mitosis is shown in the drawing?
ii) Using evidence visible in the drawing, give one reason why this cell is not in the first division of meiosis.

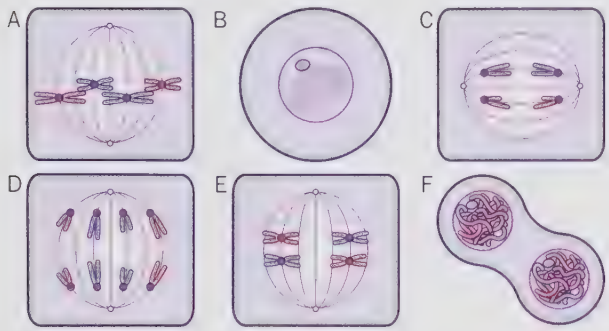
3 a) Make a table to show the differences between mitosis and meiosis.
b) Explain the advantages and disadvantages for organisms in which meiosis takes place during the life cycle, compared with those that only reproduce by mitosis.

4 a) Explain two ways in which meiosis contributes to genetic variation.
b) The bar chart shows the amount of DNA present in one cell of an organism at different stages of mitosis.



How many units of DNA would you expect to be present in one cell of this organism
i) at the end of the first division of meiosis,
ii) in one of the gametes formed as a result of meiosis?

5 The diagrams show stages in cell division in an organism.

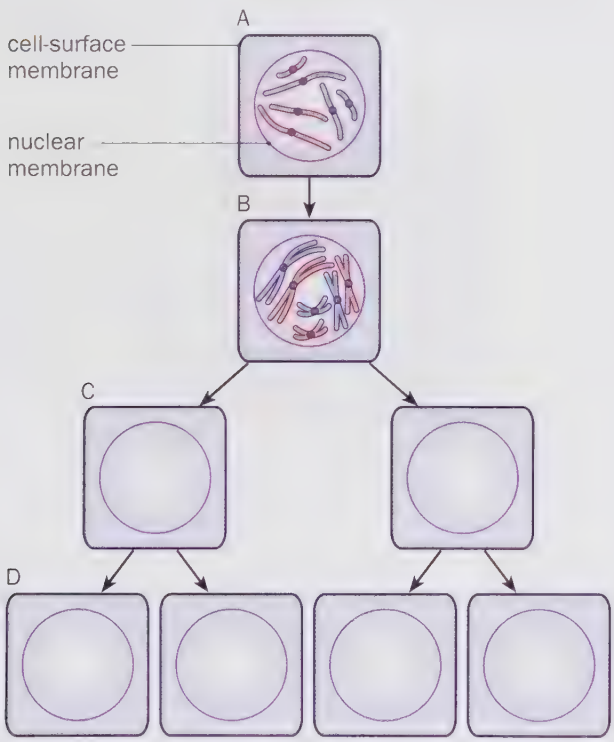


a) Match the letter of each diagram A–F with the correct description of each of the stages below. (You may use each letter once, more than once, or not at all.)
   i) metaphase of mitosis,
   ii) anaphase II of meiosis,
   iii) cell normally increases in size,
   iv) DNA replicates,
   v) bivalents become arranged at equator.

b) What is the main significance of mitosis in the growth of organisms?

c) Explain two ways in which meiosis contributes to genetic variation.

6 The diagram shows meiosis in an animal cell.



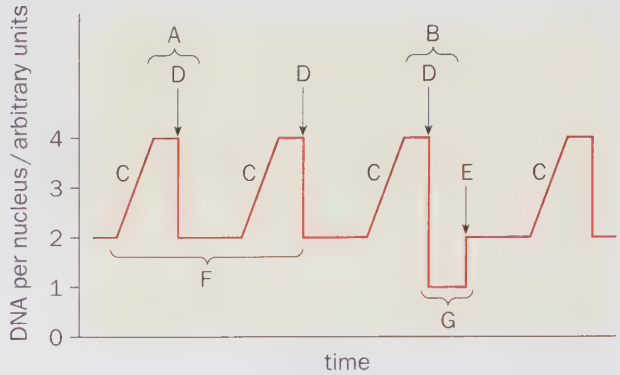
a) What is the diploid number of chromosomes in this cell?

b) Where do you think this cell could be found in an animal?

c) What is the stage of cell division shown at B? Give a reason for your choice.

d) Copy and complete the cells at C and D drawing in the chromosomes that could be found in them.

7 The diagram shows the quantity of DNA, measured in arbitrary units, during several cell divisions in animal tissue.

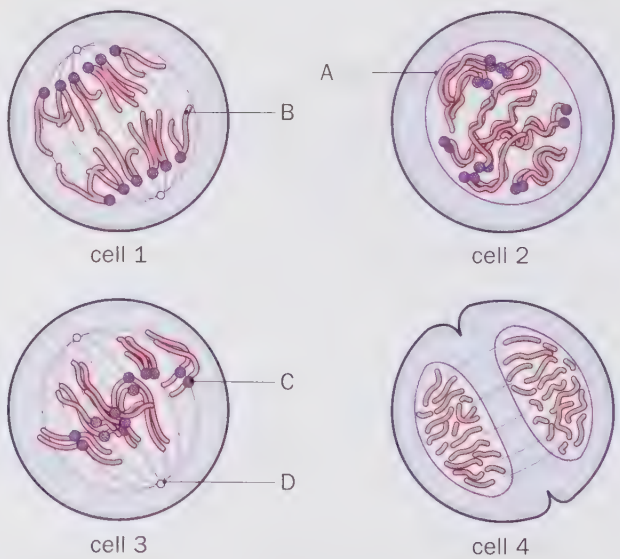


a) Name the type of cell division occurring at A and B. Give reasons for your answer.

b) What do you think is occurring at the points marked C, D and E?

c) What type of cell is produced at F and G?

8 The diagram shows four animal cells at different stages of mitosis.



a) Name the structures labelled A, B, C and D.

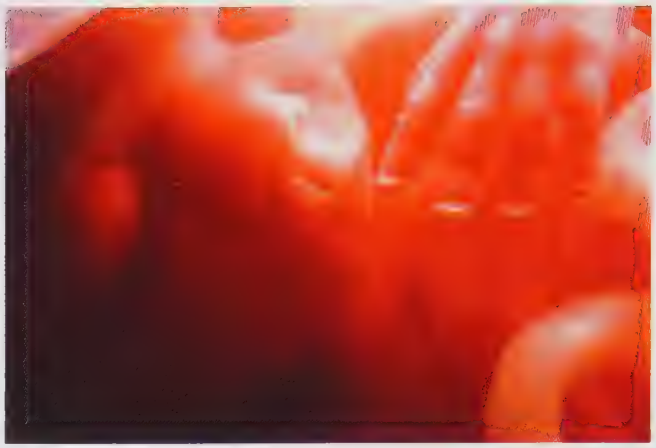
b) i) Name the stages of division shown by cells 1 and 3.
   ii) Use the number of each cell to arrange the stages in the correct sequence in mitosis.

c) How does mitosis maintain genetic stability in an organism?

7 Reproduction

The human embryo starts its life sexually neutral.
As it grows it develops into either a male or a female.
Male embryos have an X and a Y chromosome.
A gene on the Y chromosome codes for the production of factor SRY, which stimulates the development of the testes, so the embryo develops into a boy.
Female embryos have two X chromosomes and so do not have the gene that codes for SRY.

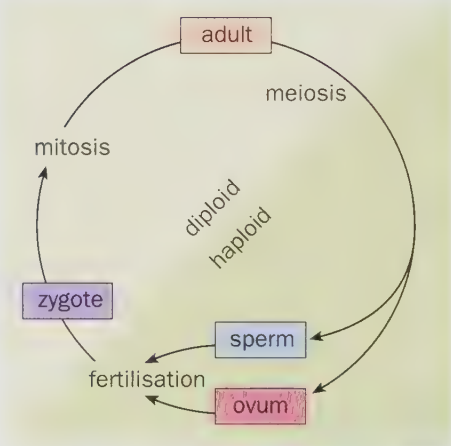
Until recently it was thought that this was the only genetic factor that determined the sex of the embryo.
However, recent research has found another gene that is thought to code for a protein that stimulates the development of female characteristics.
This gene has been named *Wnt-4* and it is hoped that further research may give more insight into the problems of infertility that some women experience.



Sexual reproduction

Animals have diploid body cells and haploid sex cells, or **gametes**.
Body cells with the full chromosome number are made by mitosis.
Haploid gametes with half the chromosome number are made by meiosis.
At fertilisation a haploid sperm fuses with a haploid egg to produce a diploid fertilised egg. This then divides many times by mitosis to eventually grow into a new individual.

In flowering plants, there are two distinct phases of the life cycle: the diploid **sporophyte** and the haploid **gametophyte**.
The diploid sporophyte produces haploid **spores** by ***meiosis*** (the diploid chromosome number is halved).
The spores develop into the haploid gametophyte, which then produces haploid gametes by ***mitosis*** (the chromosome number stays the same).

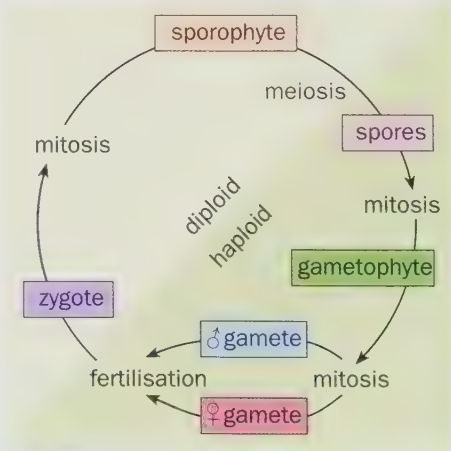


The life cycle of most animals

In plants such as mosses and ferns, the main plant is the diploid sporophyte.
It produces haploid spores by meiosis, which are genetically different.
A spore grows into the gametophyte, which produces haploid gametes by mitosis. These gametes will be genetically identical.
What has happened in flowering plants is that the water-dependent gametophyte stage in the life cycle has become much reduced.
The dominant stage in the life cycle is the sporophyte.

Sexual reproduction in flowering plants and in animals results in offspring that show **variation**. This is the result of:

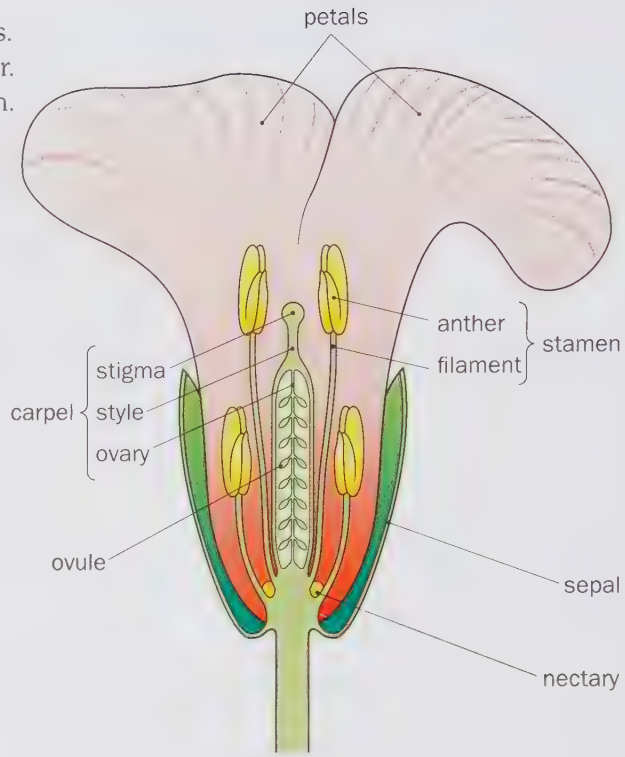
- random segregation of chromosomes at metaphase I of meiosis,
- crossing over during prophase I of meiosis,
- random fertilisation of gametes,
- random mating between organisms of the same species.



The life cycle of most plants

Flower structure

Most adult flowering plants are diploid.
Meiosis occurs within their reproductive tissues to make haploid spores.
The male spores are the **pollen grains**, which are made in the anther.
A pollen grain has a tough wall around it that is resistant to desiccation.
So pollen grains can be transferred from one flower to another without drying out.
At pollination, each pollen grain produces two male gametes by mitosis.
The female spores are the **ovules**, which are made in the ovary.
The female gamete, or **egg nucleus**, develops inside an ovule.
Can you identify the sporophyte and gametophyte phases of development?



Flowers vary in structure depending on their method of pollination.
Basically though, each flower consists of fours sets of modified leaves.
The **sepals**, **petals**, **stamens** and **carpels** are attached to the swollen end of the stem, called the **receptacle**.

- The sepals are the outermost ring of structures.
  They are usually green and leaf-like and their main function is to protect the flower when it is in bud.
  Sometimes, the sepals are coloured and indistinguishable from the petals, as in tulips and lilies.
- Inside the sepals is a ring of colourful petals called the **corolla**.
  Petals of insect-pollinated flowers usually have scent and make **nectar** in order to attract insects.
- Immediately inside the petals are the male parts of the flower, called the stamens.
  Each stamen consists of an **anther** attached to the receptacle by a long stalk called a **filament**.
  The pollen is made inside the anther in four pollen sacs by a process involving meiosis.
  The pollen sacs eventually split to release the pollen.
- In the centre of the flower are one or more carpels.
  These are the female parts of the flower containing the ovules.
  Each carpel is made up of a **stigma** connected to the **ovary** by a long **style**.
  The ovules are made inside the ovary and contain an egg cell formed by a process involving meiosis.
  After fertilisation, the ovules eventually form the **seeds**.

Insect pollination in the foxglove

The structure of any flower is related to its method of pollination.
Insect-pollinated flowers have colourful petals.
They are scented and they have nectar, to attract insects.
Flowers that are pollinated by the wind do not need any of these.
Thus, wind-pollinated flowers, such as grasses, tend to be dull and have no scent. They often dispense with petals altogether, so that the anthers and stigmas are exposed to the wind.

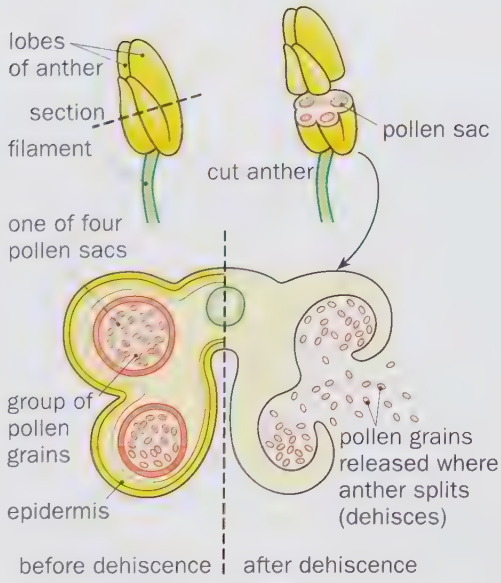
Insect-pollinated flowers have:	Wind-pollinated flowers have:
• colourful, scented petals and nectar to attract insects • anthers and stigmas inside the flower for insects to rub against • small amounts of sticky pollen that can easily stick to insects' bodies	• anthers hanging outside the flower to catch the wind • large, feathery stigmas which catch pollen grains in the air • lots of smooth, light pollen that can easily be blown by the wind

Pollination

Pollination is the transfer of pollen grains from the anther to a stigma of a plant of the same species.
Self-pollination occurs when pollen is transferred from the anther to a stigma of the same flower, or to a different flower but on the same plant.
Cross-pollination occurs when pollen is transferred to a stigma of ***another*** plant of the same species.
Cross-pollination may be carried out by insects or by the wind, and flowers can become highly adapted to either mechanism.

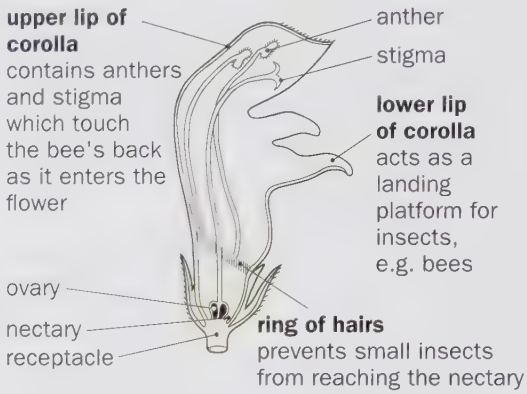
When an anther is ripe, it splits open along its length and opens out. This process is called **dehiscence** and it releases the pollen so that pollination can occur.

Pollination is needed in order to bring the two male gametes (inside a pollen grain) near to the female gamete so that fertilisation can occur.



Insect pollination

Look at the diagram of the white deadnettle (*Lamium album*).
You can see a number of adaptations for insect pollination.
The dead nettle is pollinated by bees, which land on the lower lip of the corolla tube.
Bees have long tongues that are able to reach the nectary at the base of the corolla tube.
The bees feed upon the sugary nectar made in the nectary.
The anthers are positioned in such a way that sticky pollen from them will brush against the bee's back as it pushes its head down the corolla tube.
When the bee enters another flower, it brushes some of the pollen against the ripe stigma and cross-pollination is achieved.
In the white dead nettle, the anthers ripen before the stigma, a condition known as **protandry**.
The tip of the stigma opens to expose the receptive surface some time after the anthers have released their pollen.
This prevents the bee from accidentally self-pollinating the flower.
Lots of separate dead nettle flowers are clustered together to form a more conspicuous **inflorescence**, which is easier for bees to see.

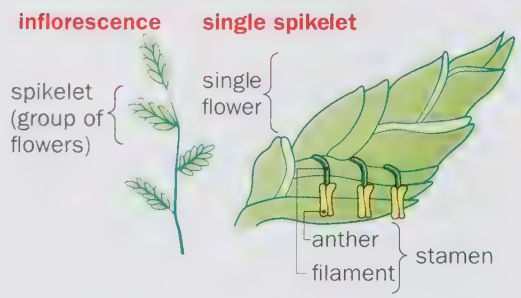


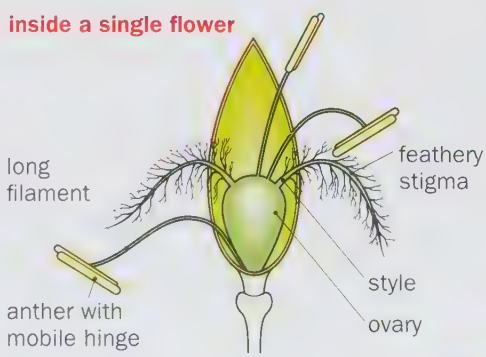
Section of a white dead nettle flower

Wind pollination

Grasses and cereals are all pollinated by the wind.
Rye grass (*Lolium perenne*) has many features typical of a wind-pollinated flower.
The flowers are small, green and inconspicuous compared with an insect-pollinated flower.
They have no scent and no nectar.
The anthers hang outside the flower so the wind can blow away the large quantities of small, smooth and light pollen that they produce.
The feathery stigmas are also positioned outside the flower.
They act like a net, providing a large surface-area for catching pollen grains that get blown into them.

Wind pollination can waste a lot of pollen, which is why the anthers produce so much.
But the fact that plants such as grasses and cereals grow in close proximity to one another means that cross-pollination is more likely to occur.





Mechanisms to ensure cross-pollination

Self-pollination can be an advantage to plants that are scattered over a wide area.
However, it is a form of inbreeding that can reduce the amount of variation in the population and its potential for evolutionary change.
Also, any undesirable recessive characteristics will tend to persist in the population.
Cross-pollination is less reliable than self-pollination but results in far greater genetic variation in a population (outbreeding).
It is not surprising that mechanisms have evolved that make cross-pollination more likely.

Self-pollination will not occur if the anthers and the stigma of a flower mature at different times.
As you saw in the white dead nettle, protandry is the condition where the anthers ripen before the stigma is able to receive pollen.
Protogyny is the condition where the stigma matures before the anthers, as in the bluebell.

A **dioecious** species is one in which individual plants either have all male flowers or all female flowers.
Clearly, self-pollination is impossible in these species.
Willow is an example of a dioecious plant but in general the number of completely dioecious species is few.

White dead nettle flowers are protandrous: the anthers ripen before the stigma

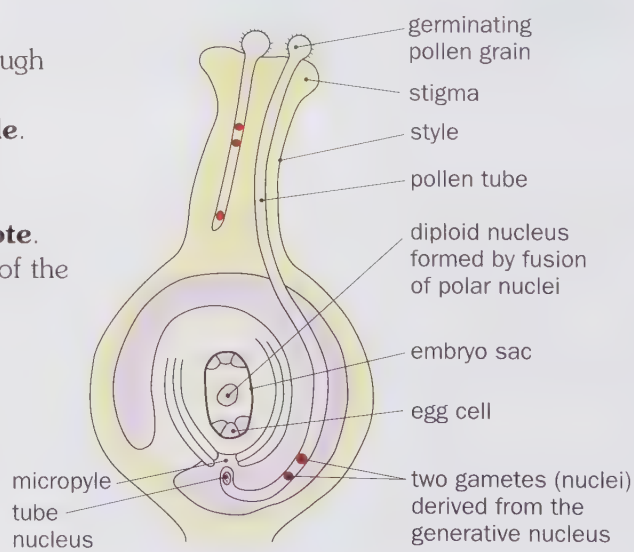
Fertilisation

In flowering plants the female gamete is protected within the ovary.
The only way that the male gamete can reach it is by means of a **pollen tube**.

If a pollen grain lands on the stigma of a plant of the same species, it absorbs water, swells and splits open.
The germinating pollen grain grows a pollen tube down the style.
It is thought to grow in response to chemicals secreted by the ovary.
At the tip of the pollen tube are three nuclei.
The **tube nucleus** precedes the other two and controls the growth of the pollen tube.
Behind the tube nucleus are two haploid male gametes, which have originated from the division of a generative nucleus.

SEM of pollen grains germinating on a stigma

The pollen tube secretes enzymes as it grows, digesting its way through the loosely-packed cells of the style.
When it reaches the ovary, it enters an ovule through the **micropyle**.
The tube nucleus disintegrates and the tip of the pollen tube bursts.
The two male gametes are released into the **embryo sac**.
One male gamete fuses with the egg nucleus to form a diploid **zygote**.
The other male gamete fuses with the diploid nucleus at the centre of the embryo sac to form a triploid **endosperm nucleus**.
So a **double fertilisation** occurs, a process that only occurs in flowering plants.



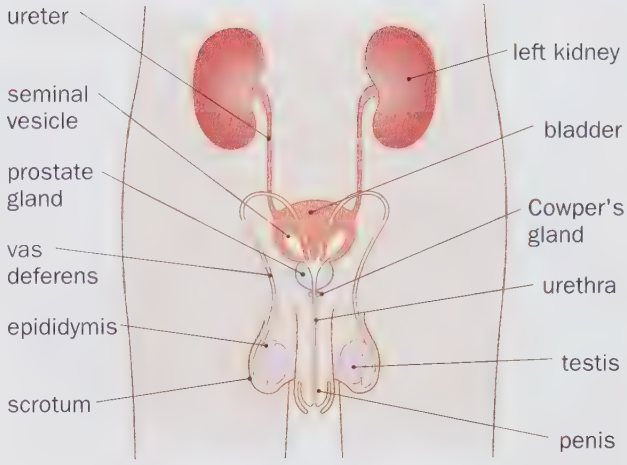
The ovule forms the seed and the endosperm nucleus forms the food store inside the seed.
The zygote eventually develops into an embryo and grows into a new plant.
The ovary wall forms the fruit but the other floral structures wither.

Human male reproductive system

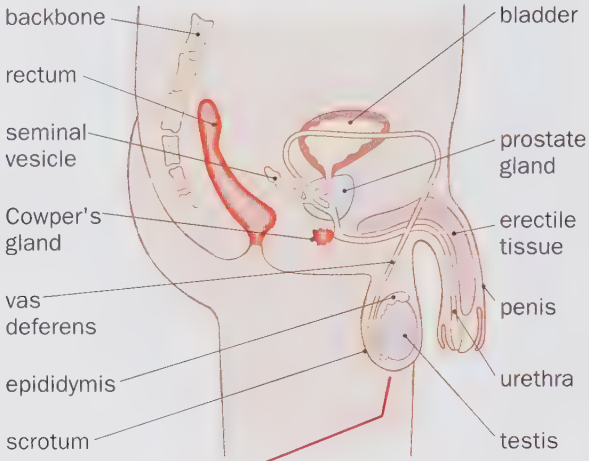
The organs that are responsible for producing gametes are called the **gonads**.
In male mammals, the gonads are the **testes** (singular **testis**).
The testes produce the male gametes, the **spermatozoa** (sperm).
The male hormone **testosterone** is also made in the testes.

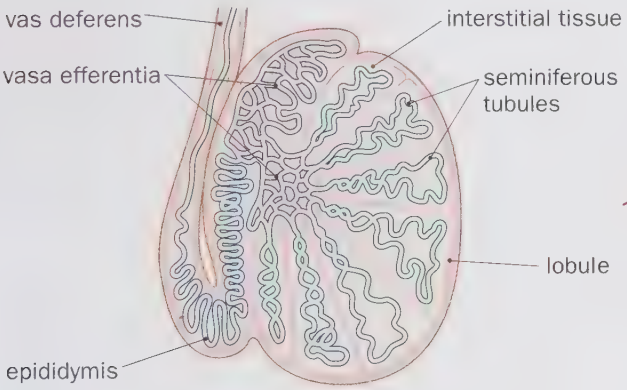
The testes develop inside the abdomen and descend into a sac of skin called the **scrotum** just before birth.
The temperature in the scrotum is about 3 °C cooler than the temperature within the body, giving the optimum conditions for sperm production.

Each testis is an oval structure, about 5 cm long.
Each testis is divided up into many compartments called **lobules**.
Each lobule contains a number of tightly-coiled tubes called **seminiferous tubules**.
The seminiferous tubules are lined by the **germinal epithelium**, which is made up of cells called **spermatogonia**.
The spermatogonia are the cells that divide to form the sperm.
The seminiferous tubules merge together to form a network of tiny tubes called the **vasa efferentia**, which in turn join up to form a 6 m-long coiled tube lying just outside each testis, called the **epididymis**.
The epididymis empties into the **vas deferens**, a tube which carries the sperm out of the testis to the **urethra**.
Sperm is stored in both the epididymis and the vas deferens.



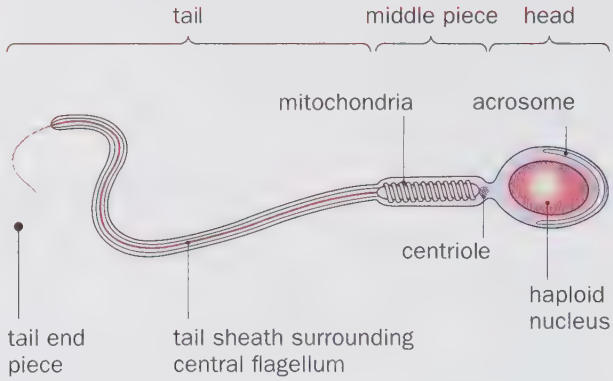
Male reproductive system





Section of human testis

A number of glands also have ducts joining the urethra.
The **seminal vesicles**, **Cowper's glands** and the **prostate gland** secrete fluids that nourish the sperm and make it alkaline.
This neutralises any acidic urine present in the urethra and combats the acid environment in the vagina, which is hostile to sperm.
The resulting mixture of sperm and these secretions is called **semen**.
The semen passes along the urethra and out of the penis during copulation.



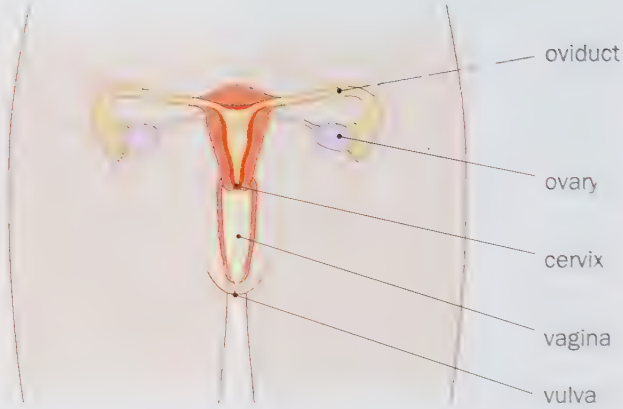
A human sperm

The mature sperm is about 60 μm in length and well adapted to swimming to the egg. The mitochondria provide the energy from respiration to keep the tail beating. The acrosome contains proteases to digest a pathway through to the egg cell and deliver the haploid sperm nucleus.

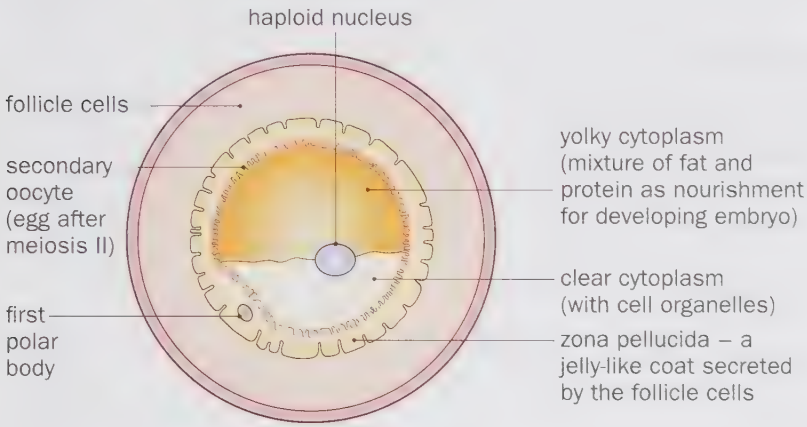
Human female reproductive system

In female mammals, the gonads are called the **ovaries**.
They produce the female gametes called **ova** or eggs.
The ovaries also make female hormones.

Each ovary is an oval structure about 4 cm long, attached to the abdominal cavity by ligaments.
Eggs are formed from the germinal epithelium on the outside of the ovary, which is made up of actively dividing cells called **oogonia**.
Close to each ovary is an **oviduct** or **Fallopian tube**.
The funnel-like opening of this tube has a fringe of finger-like **fimbriae**. This feathery fringe is lined with cilia, which collect the **secondary oocytes** (which form the ova) when they are released from the ovary.



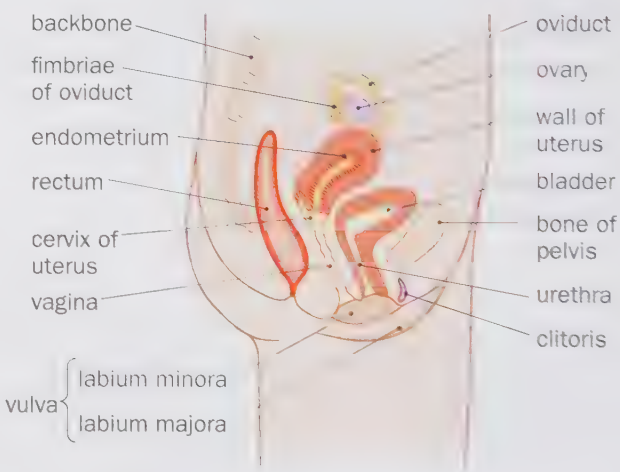
Female reproductive system



A human secondary oocyte (diameter approximately 120 μm)

The oviducts are two muscular tubes lined with cilia.
The egg is swept along the oviduct by a combination of ciliary action and muscular contractions of the wall.
The oviducts open into the **uterus** or womb.
This is pear-shaped and is about 5 cm wide and 8 cm long.
Most of the uterus wall is composed of smooth muscle, called the **myometrium**.
The lining of the womb is called the **endometrium**.
It is well supplied with blood and is the part of the womb into which the embryo implants during pregnancy, and which is shed during **menstruation**.

The uterus opens into the **vagina** through a ring of muscle called the **cervix**.
The vagina is a muscular tube, which opens to the outside through the **vulva**.
The vulva consists of two outer folds of skin, the **labia majora**, which surround two inner, more delicate folds, the **labia minora**.
Enclosed within the labia is a small body of erectile tissue called the **clitoris**.
This is equivalent to the penis in a male. It is sensitive and swells with blood when sexually stimulated.



Side view of female reproductive system

Gametogenesis

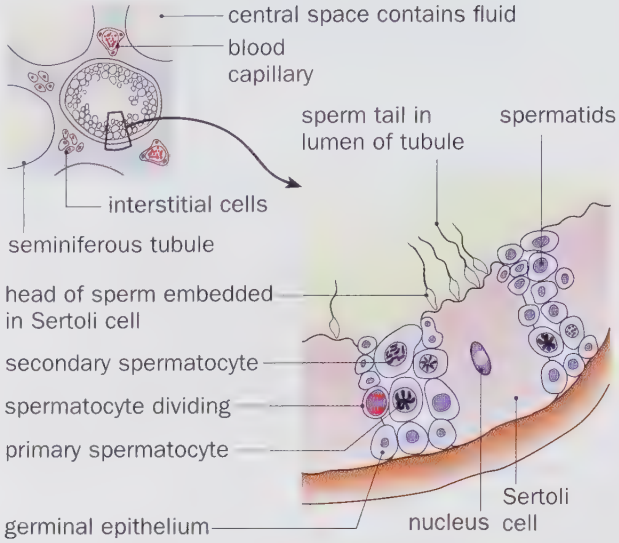
Gametogenesis is the term given to the production of gametes in the gonads.
Spermatogenesis is the formation of sperm in the testis and **oogenesis** is the formation of eggs in the ovary.
These two processes involve meiosis to produce haploid gametes.
It is important that the gametes are haploid so that at fertilisation the normal diploid number is re-established.

Spermatogenesis

Spermatogenesis is the process by which sperm are produced in the seminiferous tubules.
The diploid spermatogonia in the germinal epithelium divide many times by mitosis to produce **primary spermatocytes**.
These then undergo meiosis to form haploid **secondary spermatocytes**, which develop into **spermatids**, eventually forming mature sperm in the space inside the seminiferous tubule.

In the wall of the seminiferous tubule there are large **Sertoli cells**.
These secrete a fluid, which nourishes the spermatids and protects them from the immune system of the male as they mature into sperm.

Around each seminiferous tubule are groups of **interstitial cells**.
Their function is to secrete testosterone, the male sex hormone.
Testosterone controls the development of secondary sexual characteristics in a male at puberty.
The hormone is also important in stimulating the cells of the seminiferous tubule, particularly Sertoli cells, during spermatogenesis.



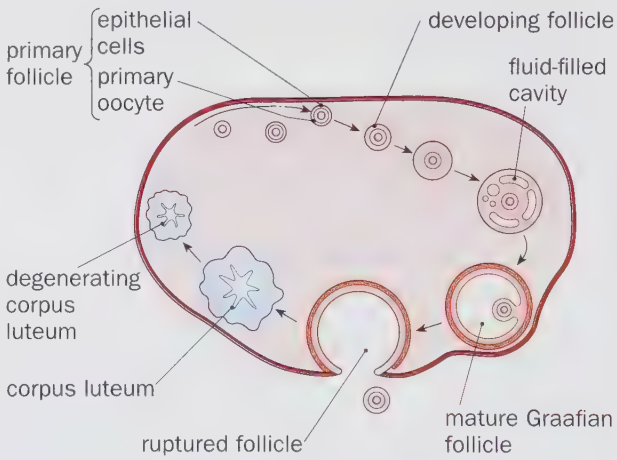
Stages in the development of human sperm

Oogenesis

Oogenesis is the process by which eggs are produced in the ovary.
The process starts in the fetus when the oogonia lining the germinal epithelium divide to form **primary oocytes**.
The germinal epithelium also divides to form **follicle cells**, which surround the primary oocytes forming **primary follicles**.
So at birth, a baby girl will already have about a million primary follicles. The primary oocytes will have started to divide by meiosis, but the process stops at prophase I.

At puberty, hormones produced by the pituitary stimulate the follicles to develop further.
Each month, several follicles start to develop but usually only one matures into a fully developed **Graafian follicle**.

First the primary oocyte completes its meiotic division to form a secondary oocyte and a small **polar body**.
The follicle cells around the secondary oocyte grow and a number of fluid-filled spaces form.
The mature Graafian follicle migrates to the surface of the ovary.
Eventually the follicle bursts and the secondary oocyte surrounded by some follicle cells is released, a process known as **ovulation**.
The second division of meiosis to form a mature ovum will only occur if a sperm penetrates the secondary oocyte.
After ovulation, the remaining follicle cells develop into the **corpus luteum**, which secretes the hormone **progesterone**.



Development of a follicle in the human ovary

The menstrual cycle

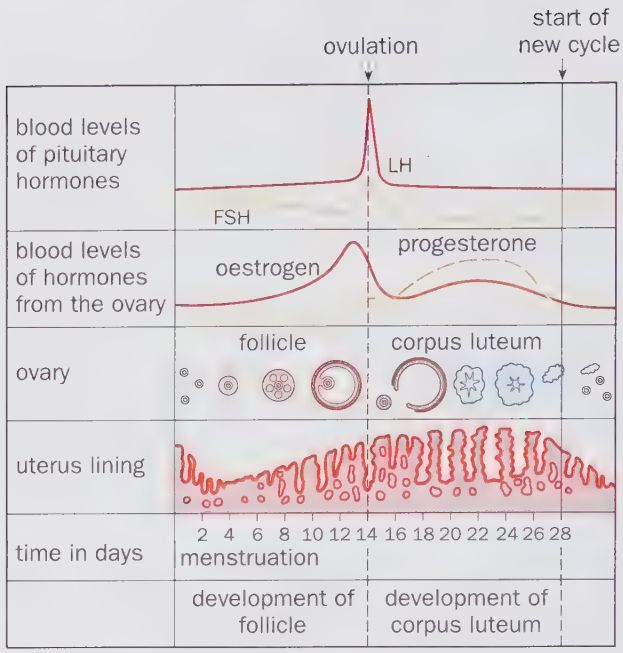
Human females usually produce one mature egg each month, from the start of puberty (around 12–14 years).
The menstrual cycle lasts about 28 days and is controlled by hormones. It involves the production and release of an egg (ovulation), and the preparation of the uterus to receive the egg if it becomes fertilised (**implantation**).

Menstruation is usually taken as being the start of the cycle. This is the breakdown of the lining of the uterus (the endometrium) and the release of blood and cells through the vagina.

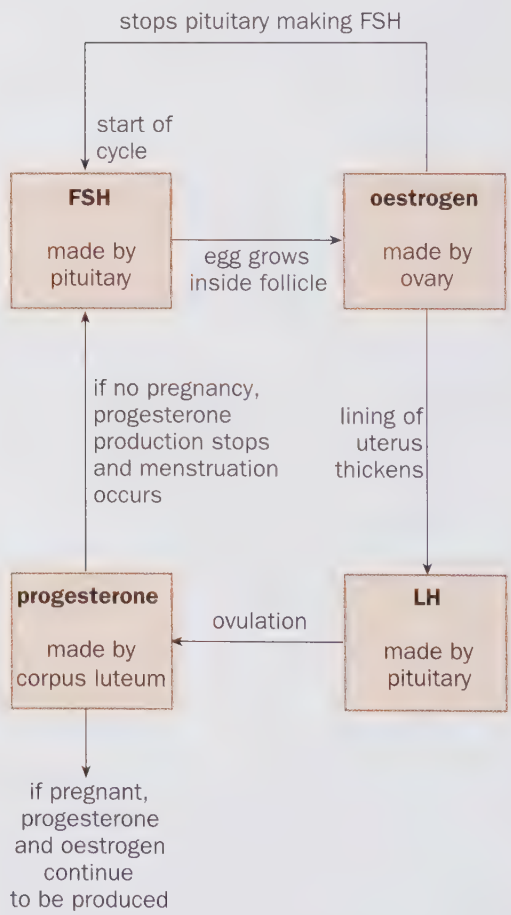
The events of the menstrual cycle and its hormonal control can be summarised as follows:

- The anterior pituitary gland in the brain secretes **follicle stimulating hormone** (**FSH**).
  This is carried in the blood to the ovary, where it stimulates the development of one or more Graafian follicles.
- As the follicle matures, it starts to produce the hormone **oestrogen**.
  Oestrogen has two main effects.
  First, it causes the repair and thickening of the endometrium.
  Secondly, it inhibits the production of FSH by the anterior pituitary gland, preventing further follicles from ripening and causing it to secrete a second hormone, **luteinising hormone** (**LH**).
- The surge of LH into the bloodstream occurs on about day 12.
  This triggers ovulation, which is the release of a secondary oocyte when the Graafian follicle bursts, about 14 days into the cycle.
  LH also stimulates the remaining follicle cells in the ovary to form the corpus luteums, which secretes the hormone progesterone.
  The corpus luteum and the ovary still secrete a reduced amount of oestrogen.
- Progesterone, along with oestrogen, inhibits the production of both FSH and LH by the anterior pituitary, an example of negative feedback.
  Progesterone and oestrogen stimulate the further growth and blood supply of the endometrium.
- If a pregnancy occurs, the corpus luteum is stimulated by hormones released by the developing embryo.
  Progesterone and oestrogen levels remain high, the womb lining stays intact and FSH production is still inhibited.
  If there is no pregnancy, then the corpus luteum starts to degenerate and the levels of progesterone and oestrogen fall.
  FSH is no longer inhibited and starts to be secreted by the pituitary.
  The endometrium breaks down, resulting in menstruation, and the cycle starts again.

Why is it important that FSH is inhibited if a pregnancy occurs?
What are the two effects of the reduction in the secretion of oestrogen and progesterone by the corpus luteum?
Why is it important that the endometrium is at its thickest at the time of ovulation?



Changes occurring during the human menstrual cycle



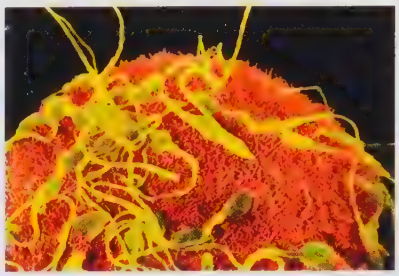
Hormonal control of ovulation and menstruation

Fertilisation

In order that fertilisation can take place, the sperm has to travel from the seminiferous tubule of the male to the oviduct of the female.
The sperms are stored in the epididymis on the outside of each testis.
Here they are mixed with secretions that make them active.
They are moved by muscular contractions of the epididymis into the vas deferens.
As we have mentioned, other secretions from the seminal vesicles, Cowper's glands and the prostate gland are added to the sperm to eventually form semen.

SEM of human sperms

Sexual arousal results in the penis of the male becoming erect.
This is the result of an increase in the blood supply to the spongy tissue of the penis.
In this condition the penis can be inserted into the female's vagina.
Sexual stimulation may eventually result in waves of intense pleasure for both partners, known as **orgasm**.
In the male, the semen is forced out of the penis by powerful contractions of the urethra. This is called **ejaculation**.
About 2–6 cm^3 of semen is ejaculated into the vagina of the female during copulation.

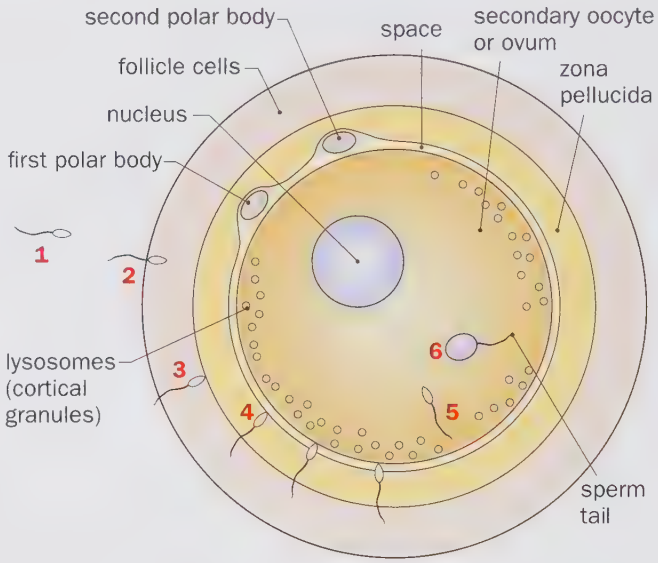


SEM of sperms on the surface of a secondary oocyte (×1600)

The sperm are deposited at the top of the vagina near the cervix. From here they use their tails to swim through the cervix and up through the uterus to the oviducts.
The sperm can remain viable for up to 2 days.
The alkaline semen helps to neutralise the acid fluid in the vagina and uterus.
The semen also contains hormones called **prostaglandins**, which stimulate the uterus and oviducts to contract and assist the sperm on their journey.
However, of the millions of sperm in one ejaculation, only a few hundred will complete the journey to the oviducts.

If ovulation has recently occurred, then there will be a secondary oocyte in the oviduct. This can remain viable for up to 24 hours.
The secondary oocyte is surrounded by the follicle cells and a clear membrane called the **zona pellucida**.
Proteases released from the acrosomes of a number of sperm digest a pathway through the follicle cells and the zona pellucida.
Eventually one sperm succeeds in passing through the outer layers and penetrating the cell-surface membrane of the secondary oocyte.

Instantly the zona pellucida thickens and separates from the surface of the secondary oocyte.
It forms a barrier impenetrable to other sperm.
At the same instant, the secondary oocyte undergoes its second meiotic division to form the mature egg.
The male nucleus of the sperm fuses with the egg nucleus, producing a diploid zygote or fertilised egg, which will develop into the embryo and then into a fetus.



1 sperm approaches secondary oocyte

2 acrosome reaction releases enzymes that digest a pathway through to secondary oocyte

3 a second acrosomal enzyme digests a pathway through the zona pellucida

4 the membrane of the sperm head fuses with the membrane of the oocyte. The lysosomes release enzymes into the secondary oocyte. These enzymes cause the zona to thicker and separate from the oocyte. The zona now forms a barrier the passage of any other sperms

5 only one sperm enters the oocyte. The nucleus of the secondary oocyte undergoes meiosis II and forms an ovum and a secondary polar body

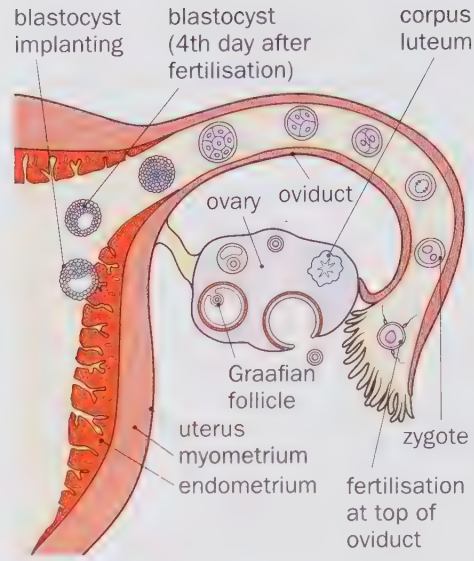
6 the male nucleus fuses with the female nucleus to form a diploid zygote. This is fertilisation

Implantation

After fertilisation, the zygote starts to divide by mitosis, forming a ball of cells called the **blastocyst**.
It takes the blastocyst 3 days to reach the uterus.
The outer layer of cells of the blastocyst is known as the **trophoblast**.
This is able to embed in the endometrium, a process called implantation.

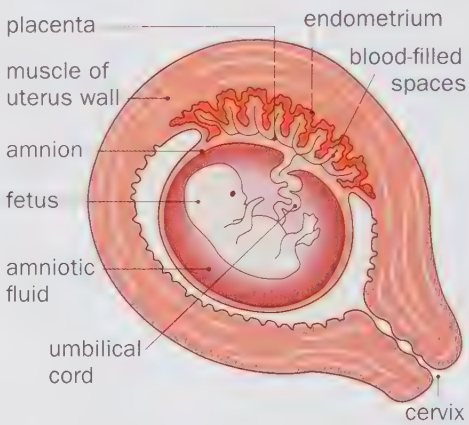
The trophoblast develops into two membranes – the **chorion** and the **amnion**.
The chorion grows a number of finger-like processes called **chorionic villi,** which increase the surface-area for the absorption of nutrients from the uterine wall.
The chorion also secretes **human chorionic gonadotrophin** (**hCG**), which prevents the degeneration of the corpus luteum.
Detection of this hormone in the urine is the basis of most pregnancy tests.

The chorionic villi eventually form part of the **placenta**, which is attached to the fetus by the **umbilical cord**.
The amnion forms a complete sac around the developing fetus and the surrounding amniotic fluid acting as a shock absorber to protect the fetus from damage, keep its skin moist, provide room for movement, and act as a heat buffer.



The placenta

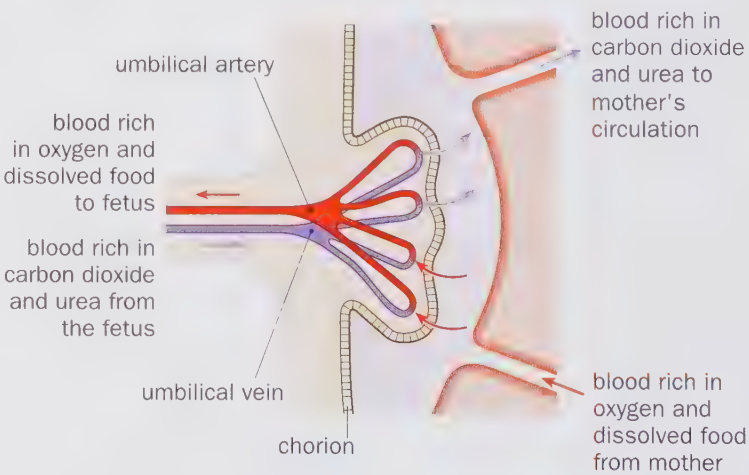
The placenta is a plate-like structure formed from the tissues of both the mother and the fetus.
The chorionic villi project into blood-filled spaces within the endometrium of the mother's uterus.
The blood of the mother comes into close association with that of the fetus without the two actually mixing.
This is important since the fetus is genetically different from the mother; an immune response could be produced if the placental barrier was breached.
In addition, the relatively higher blood pressure of the mother's blood could damage the delicate blood vessels of the fetus.



Section of the uterus to show fetus and placenta

However, this close blood contact does mean that rapid and efficient exchange of materials can take place down diffusion gradients between the two.
The functions of the placenta include:

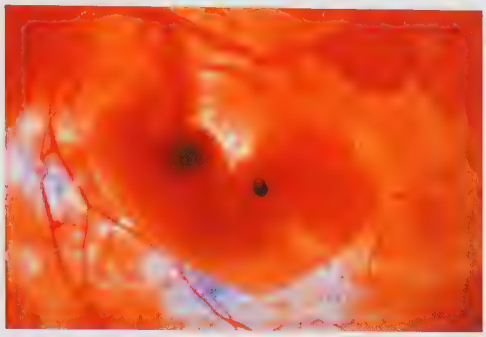
- The transfer of oxygen and soluble food, such as amino acids and glucose, from the mother to nourish the fetus.
- The transfer of carbon dioxide and nitrogenous waste products from the fetus to the mother so that they can be excreted by the mother.
- To act as a molecular filter to prevent toxins and disease-causing microbes getting into the fetal blood. Unfortunately viruses such as *Rubella* and HIV are able to cross the placenta and can damage the fetus. Toxic materials such as alcohol and nicotine are also potentially damaging.
- Some antibodies can pass from the mother to the fetus and provide immunity to certain diseases. This is called **passive natural immunity** since the antibodies are not actually produced by the fetus itself.
- The placenta must take over, from the corpus luteum, the role of secreting progesterone, which prevents ovulation and menstruation from occurring.



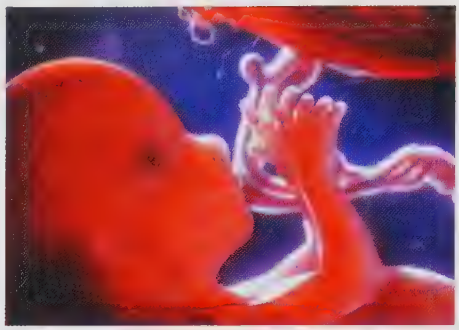
Diffusion pathways across the human placenta

Birth

In humans, birth (or **parturition**) takes place about 40 weeks after conception. During the later stages of this development (**gestation**), the fetus normally moves to a position facing downwards inside the uterus, with its head just above the cervix.



Human embryo at 7 weeks



Human fetus at 4 months

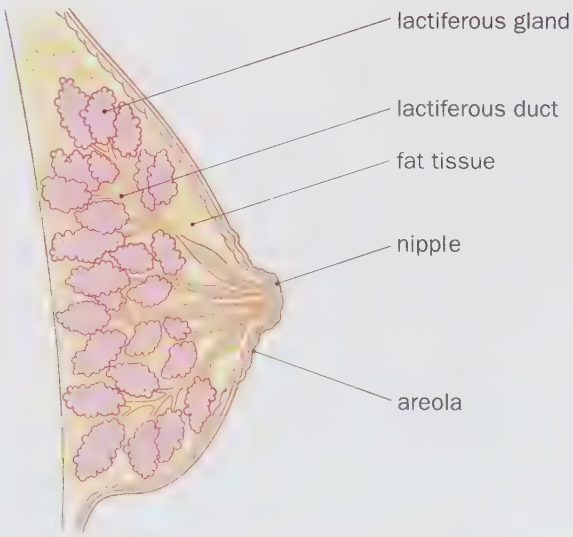
The placenta continues to produce progesterone and oestrogen during pregnancy.
The level of progesterone production rises steadily until just before birth, when it falls dramatically.
This makes the myometrium increasingly sensitive to **oxytocin**.
Oxytocin is a hormone released by the posterior pituitary gland.
Together with prostaglandins secreted by the placenta, oxytocin stimulates the smooth muscle of the myometrium to contract.
The pressure on the muscle in the uterine wall and on the cervix stimulates more oxytocin to be released (this is a rare example of **positive feedback**).
As a result, the contractions increase in force and frequency during labour.

The contractions eventually force the fetus through the cervix and out through the vagina.
Continued contractions result in the placenta becoming detached from the uterus wall and being expelled as the 'afterbirth', along with the umbilical cord.

Lactation

Lactation is the production of milk by the breasts.
The **mammary glands** develop during puberty under the influence of the sex hormones.
Each mammary gland is composed mainly of fat and contains a number of **lactiferous glands** that secrete milk.
Progesterone and oestrogen from the placenta stimulate the development of these glands during pregnancy.

At birth, the loss of the placenta stimulates an increase in the secretion of **prolactin** by the anterior pituitary.
Prolactin is a hormone that causes the lactiferous glands to secrete milk, which is then stored in the lactiferous ducts.
The baby sucking on the nipple initiates a reflex that results in the release of oxytocin, which stimulates the release of milk from the lactiferous ducts.
So the hormone prolactin stimulates the production of milk in the breasts and the hormone oxytocin stimulates the release of the milk in response to suckling.



Internal structure of a human mammary gland

Biology at work: Human sub-fertility

One in six couples in the UK seek specialist help during their reproductive lives because of difficulty with getting pregnant.

Infertility is the complete inability to conceive a child and is very rare.

Much research has focused on the causes of and treatments for **sub-fertility.** This is defined as difficulty with conceiving naturally for reasons affecting the man, woman, or both partners.

Many reproductive technology procedures are surrounded by considerable moral and ethical controversy.

Treatment centres in the UK are subject to regulation by the government 'watchdog', the Human Fertilisation and Embryology Authority (HFEA).

Causes of infertility

- **Ovulation failure** – the greatest cause of female infertility, usually associated with absence of, or irregular, menstrual periods. It can be caused by a failure in the feedback loop of the hormonal interactions between the hypothalamus, pituitary and ovaries.
- **Blockage of the Fallopian tubes (oviducts)** – any blockage in the passage of the egg from the ruptured follicle to the site of fertilisation in the Fallopian tubes will cause infertility.
- **Endometriosis** – a condition where small pieces of the endometrium are found outside the uterus attached to the ovaries. During menstruation these bleed, causing pain and infertility.
- **Mucus defects** – mucus that does not thin under the influence of oestrogen at ovulation may prevent sperm penetration or contain anti-sperm antibodies.
- **Polycystic ovary disease (PCOD)** is another common cause of ovulation failure caused by abnormal levels of male androgens.

Treatments

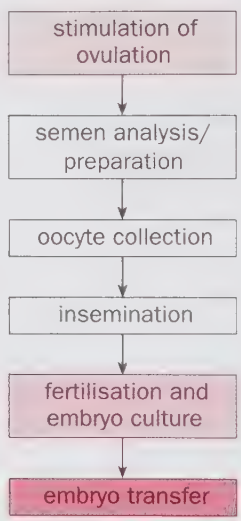
- **Ovulation failure** – 95% of cases are treatable with the use of the anti-oestrogen drug **clomiphene citrate.** This binds to oestrogen receptors in the brain more readily than oestrogen.
- **Blockage of the Fallopian tubes** – microsurgery is often used to remedy the problem.
- **In-vitro fertilisation (IVF)** – male and female gametes are mixed in a glass petri dish in such conditions that fertilisation can occur. It is a complex and demanding treatment made up of several stages. The success rate as measured by the number of live births per treatment cycle is about 13% and rising.
- **Gamete intra-Fallopian transfer (GIFT)** – involves oocyte collection and immediate replacement into the Fallopian tube, together with 2–300 000 sperm. It is less successful than IVF but simpler and cheaper.
- **Micromanipulation of individual sperm and oocytes** – the outer coat of the oocyte is difficult for sperm to penetrate, owing to their poor motility and structure. These outer cells can be easily dissected away by hand, allowing entry of the sperm.
- **Intracytoplasmic sperm injection (ICSI)** – involves the direct injection of the sperm into the oocyte cytoplasm. This technique has largely superseded **sub-zonal insemination (SUZI)** where a single sperm cell is injected between the zona pellucida and the oocyte surface membrane.

Relative frequency of the various causes of infertility

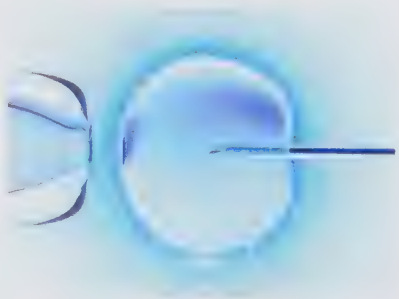
Cause	% Couples
ovulatory failure	21
tubal damage	14
endometriosis	6
mucus defect	3
sperm defect	24
other male factor	2
coital failure	6
unexplained	28

The total comes to more than 100% as some couples have more than a single cause.

Source: after Hull M.G.R. *et al* BMJ.291 (1985)



IVF treatment involves several stages



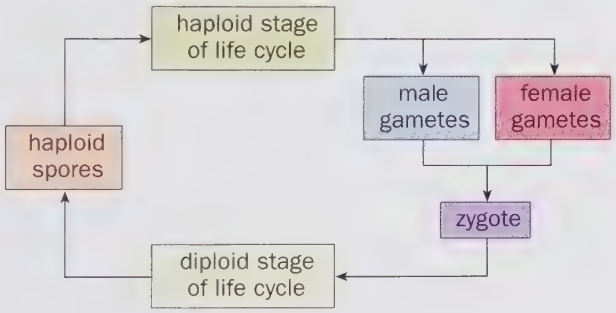
Sperm are injected directly into the oocyte cytoplasm during ICSI

Summary

- Sexual reproduction involves the production of haploid gametes, which fuse at fertilisation to produce a diploid zygote.
- Sexual reproduction results in offspring that show variation as a result of random segregation of chromosomes at metaphase I of meiosis, crossing over at prophase I of meiosis, random fertilisation of gametes, and random mating between individuals of the same species.
- The basic structure of a flower consists of the sepals, petals, stamens and carpels.
- Pollination is the transfer of pollen from the anther to the stigma of a plant of the same species.
- The two main methods of pollination are by insects and by the wind.
- Cross-pollination results in far greater genetic variation in a population than self-pollination.
- A double fertilisation occurs in flowering plants, since one male gamete fuses with the egg nucleus and the other fuses with the endosperm nucleus.
- The human testes produce spermatozoa and the male hormone testosterone.
- The human ovaries produce eggs and secrete the female sex hormones.
- Spermatogenesis is the production of sperm, and oogenesis is the production of eggs. Both of these processes involve meiosis to form haploid gametes.
- The menstrual cycle involves the production and release of eggs (ovulation), and the preparation of the uterus to receive the egg if it becomes fertilised (implantation).
- The placenta has a number of important functions involving the transfer of materials between the mother's blood and the fetus' blood.
- The pituitary secretes hormones that have an important role in the onset of birth and lactation.

Questions

1 The diagram shows the life cycle of a moss.



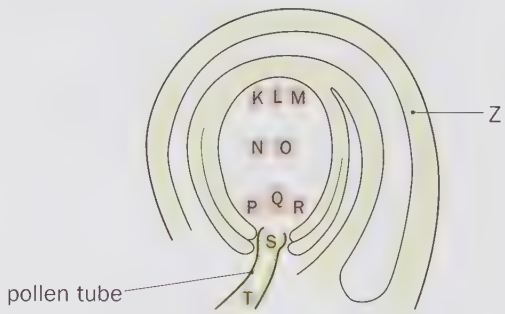
a) Copy the diagram and mark on it the place where meiosis occurs.

b) A spore from this moss contains 16 chromosomes. How many chromosomes would you expect there to be in:
   i) a female gamete,
   ii) a cell taken from the moss during the diploid stage of its life cycle?

c) Some DNA was extracted from cells during the haploid stage of the life cycle. It was found to contain 14% adenine.
   i) What percentage of thymine would you expect the sample to contain?
   ii) What percentage of cytosine would you expect the sample to contain?

d) Suggest two ways in which the male gametes of this organism are likely to differ from the female gametes.

2 The diagram shows the nuclei K–T found in the ovary of a pollinated flower with a diploid number of 12.

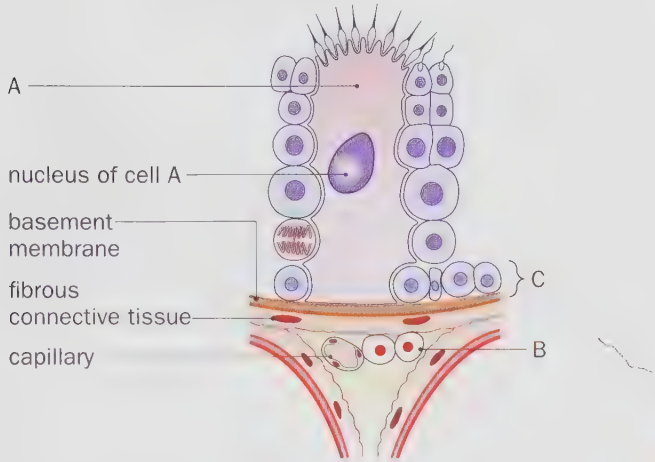


a) Copy and complete the table to show which of the nuclei K–R will fuse with S and T at fertilisation, the function of the fusion product, and the number of chromosomes that it will contain.

Nucleus with which it will fuse (K–R)	Function of fusion product	Number of chromosomes
S		
T		

b) After fertilisation the structure labelled Z swells.
   i) Name the structure that is produced.
   ii) Describe fully how this structure might help to ensure the survival of the species.

3 The diagram shows a section through part of one seminiferous tubule in the testis.



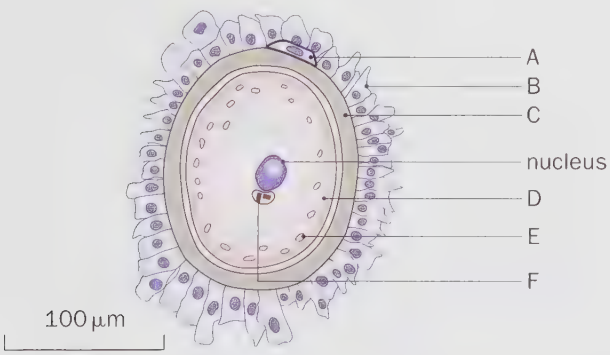
a) Name:
   i) the cells labelled A and B in the diagram,
   ii) the layer of cells labelled C.

b) Write a detailed account describing the way in which sperm are produced, and the part played by A, B and C in their production.

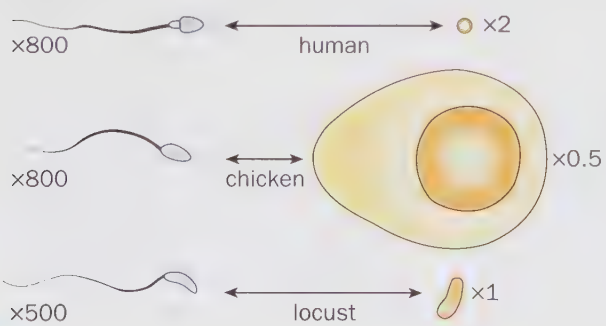
4 The diagram below shows a section of a human secondary oocyte.

a) Name the structures labelled A, B, C, D, E and F.

b) i) How does the nucleus of a secondary oocyte differ from that of a primary oocyte?
   ii) Describe how a secondary oocyte reaches the site where fertilisation may occur from the site in the ovary where it was produced.



5 The drawings show the sperm and eggs of three different animals.



a) i) How many times longer is the chicken egg than the chicken sperm?
   ii) Calculate the actual length of the chicken sperm in millimetres, showing your working.

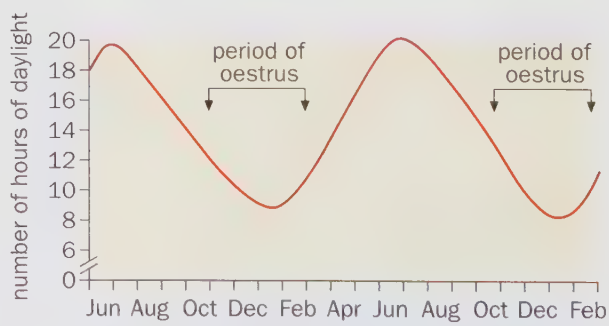
b) What is the main advantage to the locust of producing eggs that are much larger than sperm?

c) Explain why human eggs are smaller than chicken eggs, even though adult humans are much larger than adult chickens.

6 Copy and complete the table to show the correct function(s) of luteinising hormone, oestrogen and progesterone by marking the appropriate box or boxes with a tick (✓). Some hormones may have more than one function.

Function	Luteinising hormone	Oestrogen	Progesterone
immediate cause of ovulation			
immediate cause of repair of the uterine lining after menstruation			
inhibits production of follicle stimulating hormone			
maintains the uterus for implantation			
stimulates formation of a structure that produces progesterone			

7 The graph shows the effect of the number of hours of daylight on the timing of oestrus in sheep. The graph refers to daylight conditions in northern Europe over a period of 21 months.



a) Comment on the relationship between hours of daylight and the periods of oestrus shown in the graph.

b) Why is it an advantage that the periods of oestrus in sheep occur at the times shown on the graph?

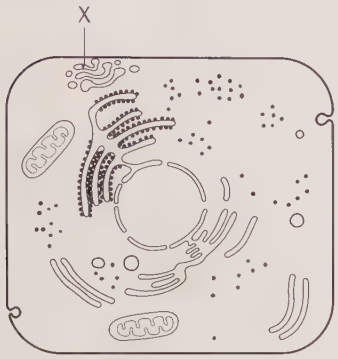
Cell structure

1 a) Magnification and resolution are both higher with an electron microscope than with an optical (light) microscope.

i) Explain the difference between magnification and resolution. [2]

ii) Explain how an electron microscope has a higher resolution than an optical microscope. [1]

b) The diagram below shows the structure of an animal cell as it would appear when seen with an electron microscope.



i) Give *one* structure that is present in this cell but would *not* be in a prokaryotic cell. [1]

ii) Give *one* structure that is *not* present in this cell but may be present in a prokaryotic cell. [1]

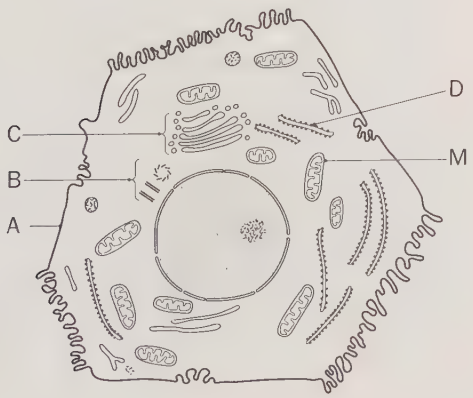
c) Describe *one* function of the organelle labelled X. [1]

AQA (formerly AEB) **[6]**

2 The diagram below shows the structure of a liver cell as seen using an electron microscope.

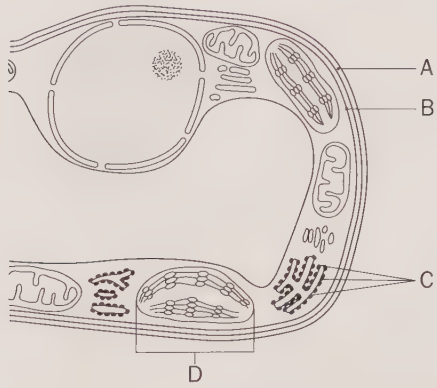
a) Name the parts labelled A, B, C and D. [4]

b) The magnification of this diagram is ×12 000. Calculate the actual length of the mitochondrion labelled M, giving your answer in μm. Show your working. [2]



Edexcel **[6]**

3 The diagram below shows part of a cell as seen under an electron microscope.

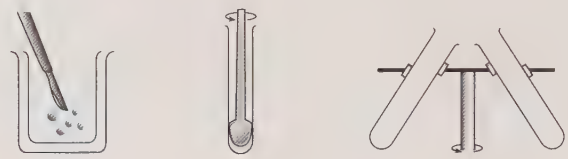


a) Give the names of the structures labelled A, B, C and D. [2]

b) Some of the detail in this diagram would not be seen using a light microscope. Explain why. [4]

AQA (formerly NEAB) **[6]**

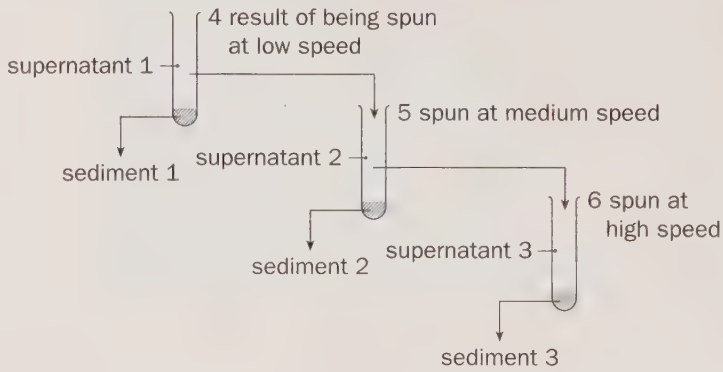
4 Cell fractionation is a technique used to isolate organelles. Students wanted to isolate mitochondria from liver cells. The diagram below shows the method they used.



1 tissue is cut up and kept in a cold, isotonic buffered solution

2 cut-up tissue further broken up in a homogeniser

3 homogenised tissue is spun in an ultracentrifuge at a low speed for 10 minutes



a) Explain each of the following steps in the method

i) the liver was homogenised [1]

ii) the solution was kept ice cold [1]

iii) the solution was isotonic [2]

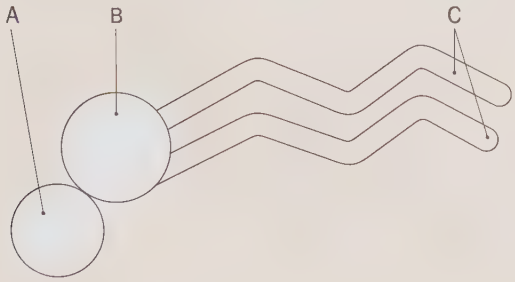
iv) a buffer was added to the solution. [2]

b) Which sediment 1, 2 or 3 will contain the mitochondria? [1]

[7]

Cell membranes and transport

5 The diagram below shows a phospholipid molecule.



a) i) Name the parts of the molecule labelled A, B and C. [1]

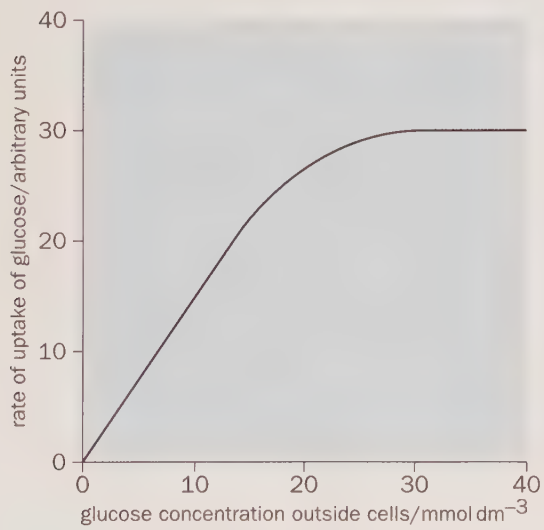
ii) Explain how the phospholipid molecules form a double layer in a cell membrane. [2]

b) Cell membranes also contain protein molecules. Give *two* functions of these protein molecules. [2]

AQA (formerly NEAB) **[5]**

a) Give *two* differences between facilitated diffusion and active transport. [2]

Some biologists put red blood cells in a buffer solution containing glucose. They investigated the effect of changing the concentration of glucose in the buffer solution. The graph below shows their results.



b) i) Use evidence from the graph to suggest how glucose enters the red blood cells. [2]

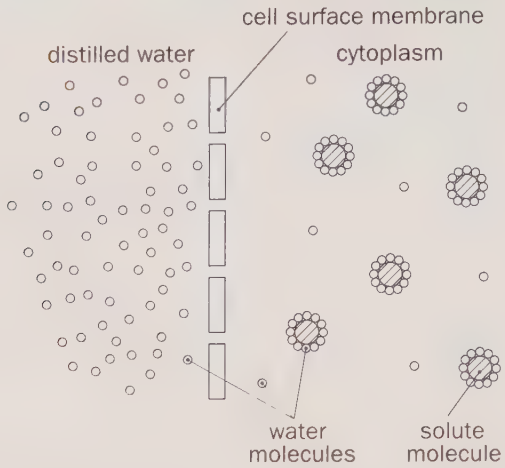
ii) Explain the shape of the curve above glucose concentration of 30 mmol dm^{-3}. [2]

c) i) A buffer solution was used in this investigation. Explain why. [2]

ii) Suggest the pH value of the buffer used. [1]

AQA **[9]**

7 The diagram below represents part of an animal cell which has been put in distilled water.



a) Use the diagram to:

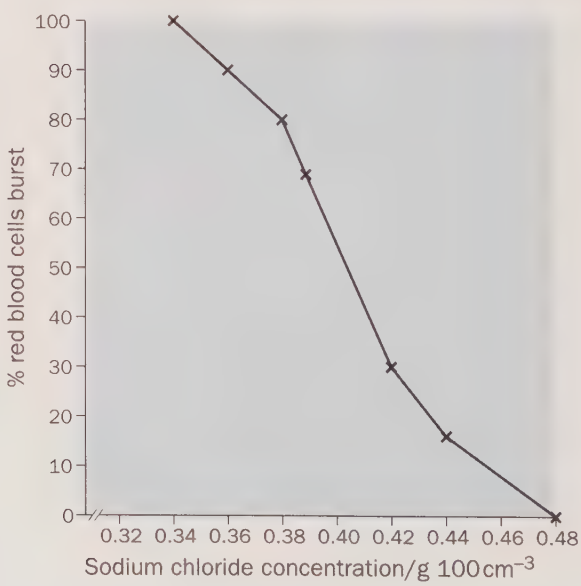
i) explain why the water potential of the distilled water is higher than the water potential of the cytoplasm of the cell; [2]

ii) describe the property of the cell-surface membrane which allows osmosis to take place. [1]

b) Osmosis has been described as a special case of diffusion. Describe two ways in which you would expect the movement of water into a cell by osmosis to be similar to the diffusion of oxygen into a cell. [2]

AQA (formerly AEB) **[5]**

A scientist placed samples of red blood cells in a series of sodium chloride solutions of different concentrations. After 3 hours, the samples were examined to find the percentage of the cells which had burst. The results are given in the graph below.



a) The scientist produced the solutions by diluting a stock solution of sodium chloride of concentration $1.0\,g\,100\,cm^{-3}$ with distilled water. Describe how the scientist produced $20\,cm^3$ of sodium chloride solution of concentration $0.40\,g\,100\,cm^{-3}$. [2]

b) i) All the red blood cells burst when placed in a sodium chloride solution of concentration $0.34\,g\,100cm^{-3}$. Explain why. [3]

ii) The red blood cells burst over a range of sodium chloride concentrations. Suggest a reason for this. [1]

c) When cells from an onion were placed in the same range of sodium chloride solutions, none of the cells burst. Explain why. [1]

AQA (formerly NEAB) **[7]**

Cell division

9 Which of the following is the correct sequence of stages in the cell cycle?

A G1, G2, S, nuclear division, cell division

B G1, S, G2, nuclear division, cell division

C S, G1, G2, nuclear division, cell division

D G1, S, G2, cell division, nuclear division

[1]

10 The table below refers to the processes of mitosis and meiosis. Copy the table.
If the statement is true, put a tick (✓) in the appropriate box. If the statement is false, put a cross (✗) in the box.

Statement	Mitosis	Meiosis
Crossing over occurs		
Reduction in chromosome number from diploid to haploid		
Genetic uniformity of daughter cells		
Homologous chromosomes associate in pairs		

AQA (formerly AEB) **[4]**

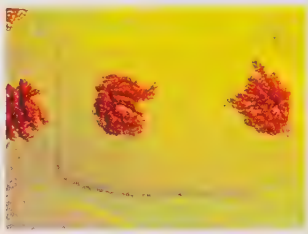
11 Students stained and squashed cells from the tip of an onion root. They then observed the cells under the microscope and counted the number of cells in each stage of the cell cycle.

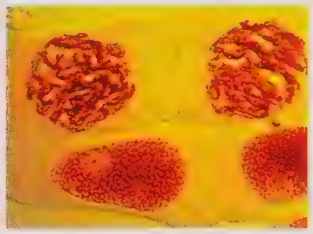
a) Students used the root tip for this investigation. Explain why. [1]

b) i) The students stained the cells. Explain why. [1]

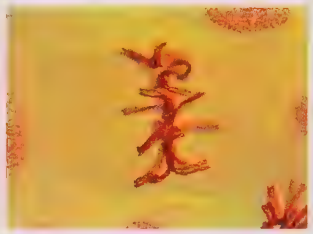
ii) The students squashed the cells. Explain why. [1]

c) The photographs below show cells in different stages of mitosis.

A 

B 

C 

D 

Complete the table with the correct letter for each stage. [2]

Prophase	Metaphase	Anaphase	Telophase

d) The following table shows the results of the students' investigation. The figures represent the number of cells in the different stages of the cell cycle.

Stage of cell cycle	Number of cells
interphase	138
prophase	56
metaphase	6
anaphase	2
telophase	7
Total	**209**

If one whole cycle takes 24 hours, calculate how long the cell will spend in stages where the chromosomes are visible as chromatids attached to each other at the centromere. Give your answer in minutes. Show your working. [2]

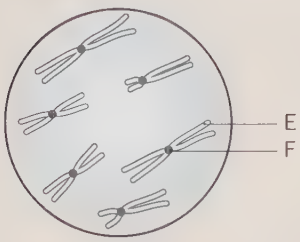
[7]

12 When a diploid cell divides by meiosis, haploid cells are produced.

a) Reduction of the chromosome number when a cell divides by meiosis is biologically important. Explain why. [2]

Figure 1 shows a cell in the early stages of cell division.

Figure 1



b) i) Give the name of the parts labelled E and F. [2]

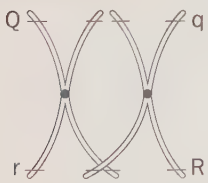
ii) Identify one pair of homologous chromosomes by shading them on the diagram. [1]

iii) Give the diploid number of this cell. [1]

iv) Calculate the number of different types of gametes that could be produced from the cell as a result of the different combinations of maternal and paternal chromosomes. Assume no crossing over occurs. [1]

Figure 2 below shows a pair of homologous chromosomes in the process of crossing over during the early stages of meiosis. The letters represent the alleles of two different genes.

Figure 2



c) i) What is an allele? [1]

ii) Complete the labelling of the alleles by putting the correct letter in the spaces on the diagram. [2]

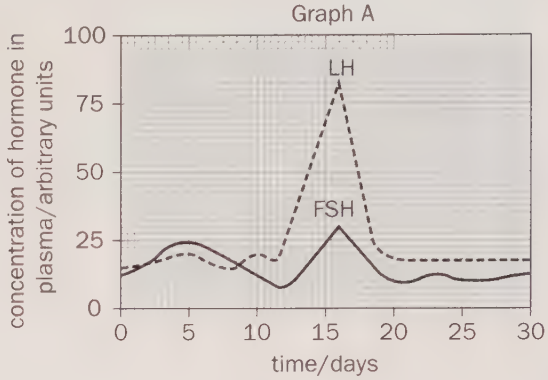
iii) The cell containing this homologous pair divided by meiosis to form four gamete cells. Sketch the distribution of chromosomes from this homologous pair in four of the gametes in the circles below. Each circle represents a gamete cell. [3]

[13]

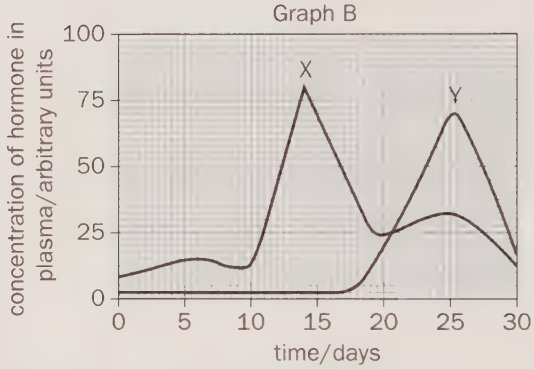
Reproduction

13 Graph A below shows the concentration of FSH and LH in plasma during a woman's menstrual cycle. Graph B shows the concentration of two hormones, X and Y, produced in the ovary during the same menstrual cycle.

Graph A



Graph B



a) Where are FSH and LH produced? [1]

b) Name hormones X and Y. [2]

c) On which day did ovulation occur in this woman's cycle? Explain the evidence for your answer. [3]

d) Describe an example of negative feedback involving two of these hormones. [2]

AQA (formerly NEAB) **[8]**

8 Gas exchange

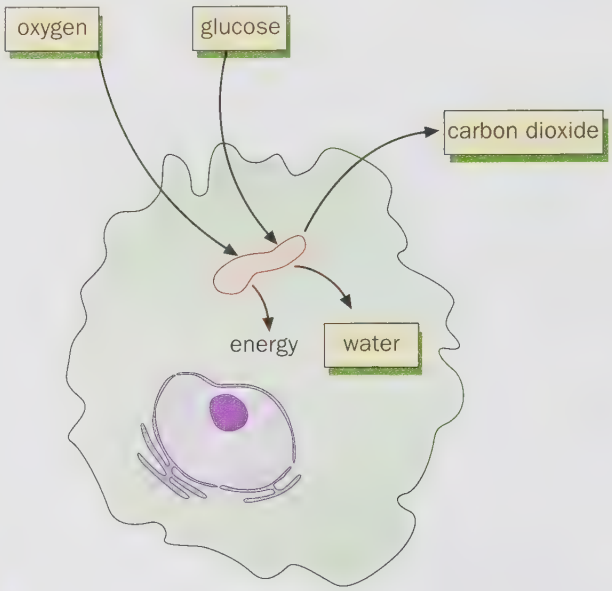
How long can you hold your breath underwater?
Not as long as a seal can, that's for sure!
Seals can remain underwater for up to 48 minutes.

Divers continue to breathe underwater using air tanks.
The deeper they go, the greater the water pressure on their bodies.
The air in their lungs gets compressed and this forces nitrogen, as well as oxygen, through the membranes of the lungs into the blood.
If a diver resurfaces too quickly, bubbles of nitrogen can form in the tissues, causing blocked blood vessels, pain and even death.
This is known as the 'bends'.
A seal does not hold its breath at all when it dives. It actually breathes as much air out of its lungs as it can.
Not only does this make the seal less buoyant, it also avoids the bends.

Many diving mammals, like seals, are able to store oxygen during a dive.
Their muscles contain a lot of myoglobin, which combines with oxygen even more readily than haemoglobin.
Also, oxygenated blood is redirected away from regions such as the skin, to critical areas such as the brain and the heart.

Gas exchange and respiration

If cells are to stay active, grow and divide, they need a source of energy.
This energy comes from the oxidation of organic molecules such as glucose in **respiration**.
As respiration involves oxidation reactions, all cells must have a constant supply of oxygen.
As well as releasing energy, respiration also produces the waste products carbon dioxide and water.

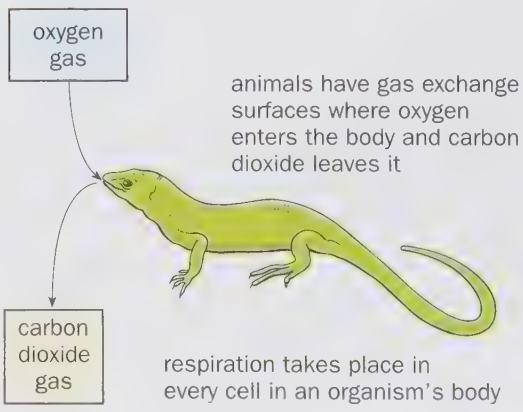


Respiration is a series of oxidation reactions taking place in all living cells. It results in the release of energy from organic compounds such as glucose.

$$\underset{C_6H_{12}O_6}{\text{glucose}} + \underset{6O_2}{\text{oxygen}} \longrightarrow \underset{6CO_2}{\text{carbon dioxide}} + \underset{6H_2O}{\text{water}} + \underset{2880\,kJ}{\text{energy}}$$

Gas exchange is not the same as respiration. It is the process by which oxygen gets to the cells and carbon dioxide is removed.
Respiration is the release of energy in cells.
Respiration creates a constant demand for oxygen and a constant release of toxic carbon dioxide, which must be removed.

Animals and plants have evolved special **gas exchange surfaces**, which allow the exchange of oxygen and carbon dioxide with the environment.



Gas exchange involves the diffusion of gases into and out of cells allowing respiration to take place.

What is the difference between gas exchange and respiration?
How would you describe the function of a gas exchange surface such as the gills of a fish?

Gas exchange surfaces

What do the gills of a fish, the alveoli (air sacs) in the lungs of a mammal and the spongy mesophyll cells in the leaves of a plant have in common?

They are all excellent gas exchange surfaces.
They allow quick and efficient gas exchange between the cells of the organism and its environment.

What features do these gas exchange surfaces have in common?

- They have a large surface area relative to the volume of the organism, over which gas exchange can take place rapidly.
- They are thin, so there is a short diffusion pathway over which gases can diffuse rapidly.
- They have a moist surface where gases can dissolve first before they diffuse in or out.
- They are able to maintain a concentration gradient down which gases can diffuse.

Humans and other mammals have a high metabolic rate.
This means that they have high oxygen requirements.
Gas exchange is even more efficient where there is:

- a blood transport system with red blood cells containing the pigment haemoglobin,
- a means of ventilation to get gases to and from the gas exchange surface.

The external gills of this axolotl allow for efficient gas exchange

This flatworm's body has a large surface area to volume ratio

Gas exchange in unicellular organisms

In unicellular organisms, the gas exchange surface is the cell-surface membrane.
These unicells have a high surface area to volume ratio.

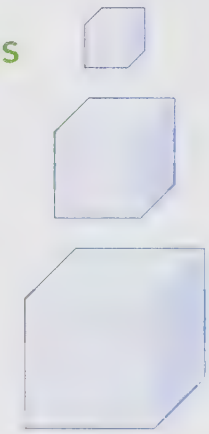
Look back to page 88 to see how the surface area to volume ratio decreases as the volume of a cell increases.

The smaller the organism, the greater its surface area to volume ratio and the greater the efficiency of diffusion of gases through the membrane.

What happens when a unicellular organism uses up oxygen in respiration?

Oxygen diffuses down a concentration gradient into the cell as the oxygen inside the cell is quickly used up in respiration.
The build-up of carbon dioxide inside the cell sets up another concentration gradient and this gas diffuses out of the cell.

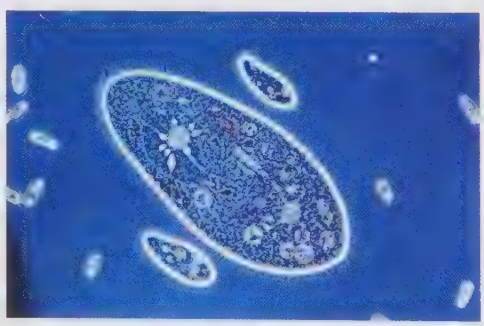
The cell-surface membrane of a unicellular organism is moist, allowing gases to dissolve, and thin to allow rapid gas exchange.



1 cm^3 'cell'
surface area to volume ratio = 6:1

8 cm^3 'cell'
surface area to volume ratio = 3:1

27 cm^3 'cell'
surface area to volume ratio = 2:1



Gases diffuse across the cell-surface membrane of this Paramecium

Gas exchange in animals

Worms

What is the gas exchange surface of a worm?
Oxygen and carbon dioxide diffuse across the skin surface.
Worms do not have any special gas exchange organs.
But they have evolved a tubular shape.
How do you think this is an advantage?

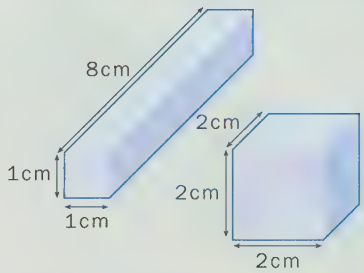
The tubular shape of the earthworm and other **annelids** is efficient for the exchange of gases.
The skin is moist for gases to dissolve, and thin so there is only a short distance between the air and the blood capillaries beneath the skin surface.
The blood system maintains a diffusion gradient by constantly removing oxygen and taking it to the cells and bringing carbon dioxide back.
Some types of worm have evolved a flattened shape.
How do you think this is an advantage?

Gas exchange occurs across the earthworm's skin

Maths skills

Look at the 'tubular animal' and the 'cube-shaped animal'.
Calculate the surface area and volume for each 'animal'.
Then work out the surface area to volume ratio for each.

Can you see that they have the ***same*** volume ($8\,cm^3$)?
But the surface area of the elongated animal is $34\,cm^2$ and that of the cube-shaped animal is $24\,cm^2$.
The elongated animal has a greater surface area to volume ratio than the cube-shaped animal (4.25 compared with 3.0).

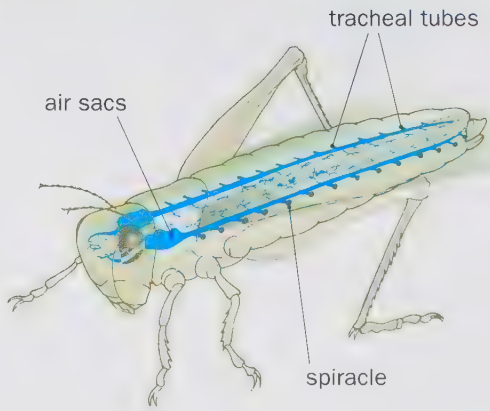


Insects

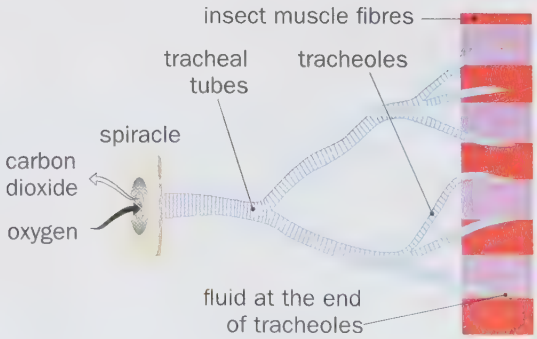
Insects have evolved a system of **tracheal tubes** throughout their bodies.
Openings at the side of the body called **spiracles** open into a branching system of tubes that supply the tissues with air.
The spiracles can open and close like valves, allowing gas exchange but also reducing water loss.

The smallest of the branching tubes are called **tracheoles** and are in contact with the tissues.
The end of each tracheole contains a small amount of fluid in which the gases dissolve.
When tissues such as muscle are active, the fluid is drawn into the tissue, supplying oxygen.
The fluid is released back into the tracheole when the muscle is at rest, so removing the waste carbon dioxide.

- The tracheal system provides the insect with a large surface area for exchange of gases.
- Small insects can rely on diffusion through the tracheal system alone to get gases in and out of the tissues.
- Larger or more active insects ventilate their tracheal system by rhythmical body movements that pump air in and out.



The gas exchange system of an insect



Insect tracheal system

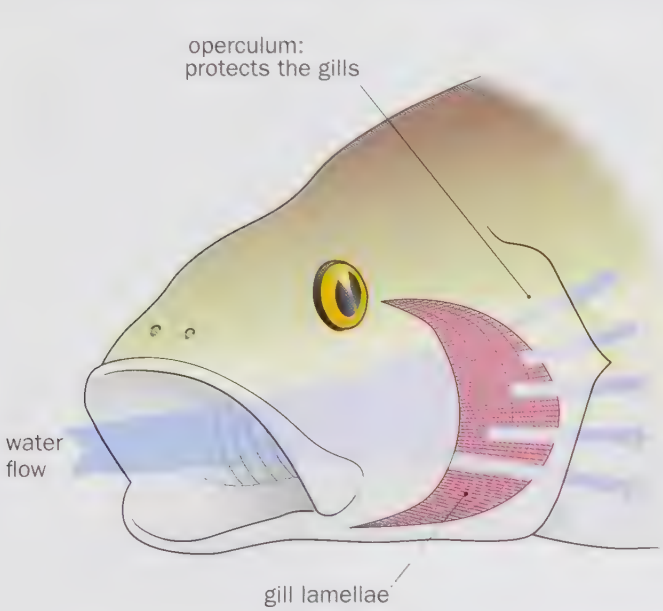
Gas exchange in fish

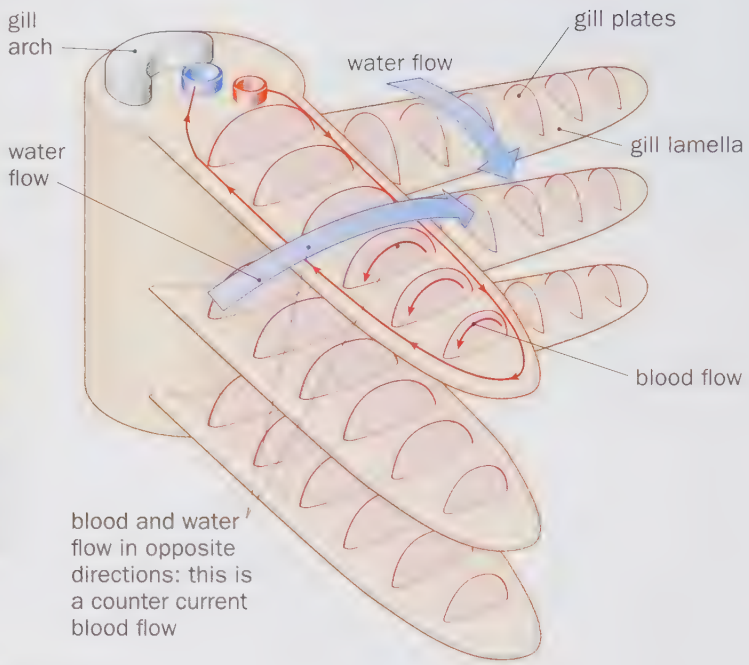
Fish use water as a gas exchange medium instead of air.
This brings problems, as you can see from the table.

	Water	Air
oxygen content (%)	0.7	20
oxygen diffusion rate	low	high
density (kg per litre)	1.0	0.0013
viscosity	1.0	0.02

Water contains far less oxygen than air and the rate of diffusion of gases in water is slower.
Water is denser and more viscous, so it does not flow as freely as air.

Specialised gas exchange organs have evolved: the **gills**.
Gills are made up of numerous folds, providing a huge surface area over which water can flow and gases can be exchanged.





In bony fish there are four pairs of gills in the **pharynx** (throat).
Each gill is supported by a bony **gill arch**.
Along each gill arch is a double row of **gill lamellae**.
These thin flaps lie on top of each other like the pages in a book.
When surrounded by water they give a large surface area for gas exchange.
Out of water, the lamellae stick together and the gill collapses, so the fish suffocates.

Can you see from the diagram that each gill lamella has **gill plates** along each of its sides?
The gill plates are the gas exchange surfaces.

Blood vessels bring deoxygenated blood to the gill lamellae.
The blood then passes through tiny capillaries present in each of the gill plates.
Oxygen passes through the gill plates into the capillaries and carbon dioxide passes out into the water.
Blood vessels carry oxygenated blood away.

Ventilation of the gills

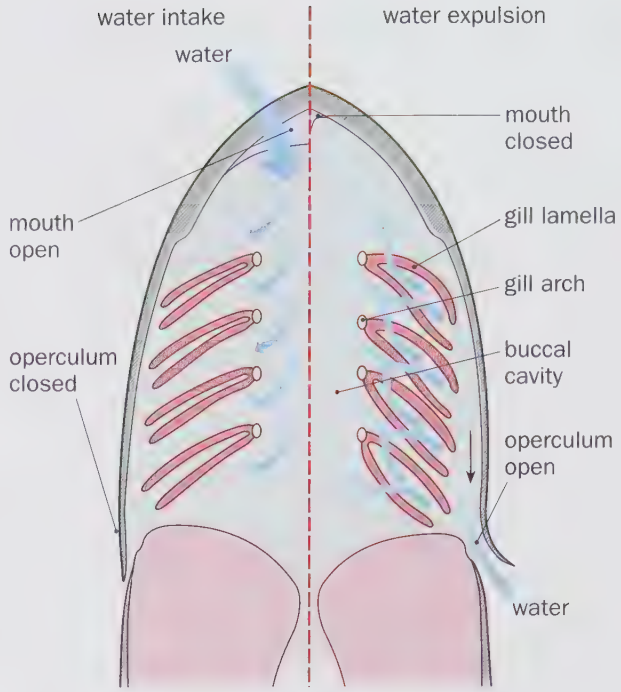
Bony fish have evolved a ventilation mechanism that allows water to pass across the gill more or less continuously.

To take in water:

- The mouth opens.
- The **operculum** (gill cover) closes the opening at the back of the pharynx.
- The floor of the **buccal cavity** (mouth cavity) is lowered.
- The volume inside the buccal cavity increases and so the pressure inside the cavity falls.
- This allows water to rush in through the mouth.

For water to pass out:

- The mouth closes.
- The floor of the buccal cavity is raised.
- The volume inside the buccal cavity decreases and so the pressure inside the cavity rises, forcing water back over the gills.
- The operculum opens and water flows out.



Ventilation of fish gills

A counter current system

Bony fish have gills that use a **counter current principle**. In the lamellae, the blood in the capillaries flows in the ***opposite*** direction to the water flowing over the surface.

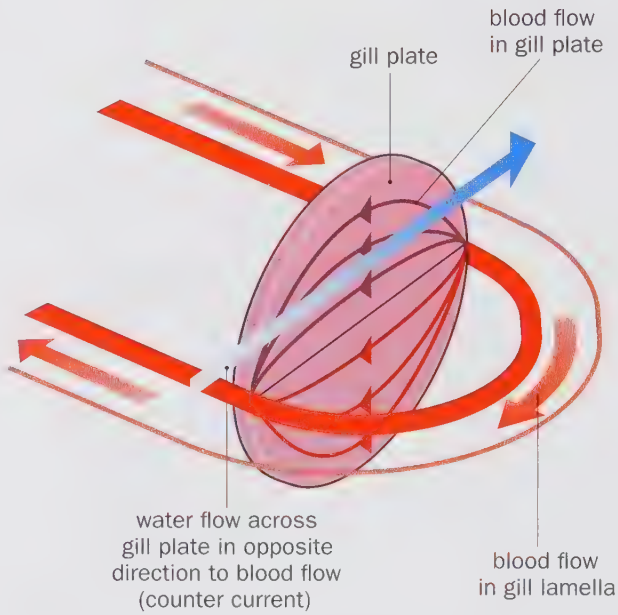
Can you see from the diagram that the blood is flowing forward in the capillaries of the gill plate?
At the same time water passes over the gill plate in the opposite direction.

This means that a diffusion gradient is maintained between the blood and the water right across the gill plate.

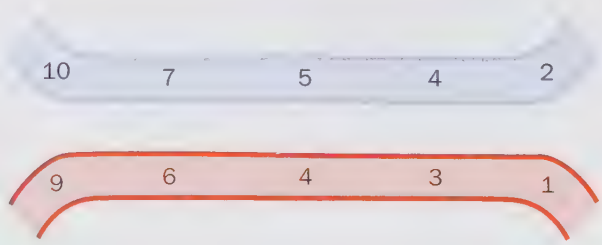
Can you see in this diagram that:

- blood with a relatively high oxygen concentration meets water with a lot of oxygen in it?
  So there is still a diffusion gradient for oxygen ***into*** the blood.
- blood with a relatively low oxygen concentration meets water which has had most of its oxygen removed?

The counter current system allows the gills of a bony fish to achieve an 80% extraction of oxygen from water.
That's three times the rate of extraction of human lungs from air!
This helps to overcome the problem of there being less oxygen in water.



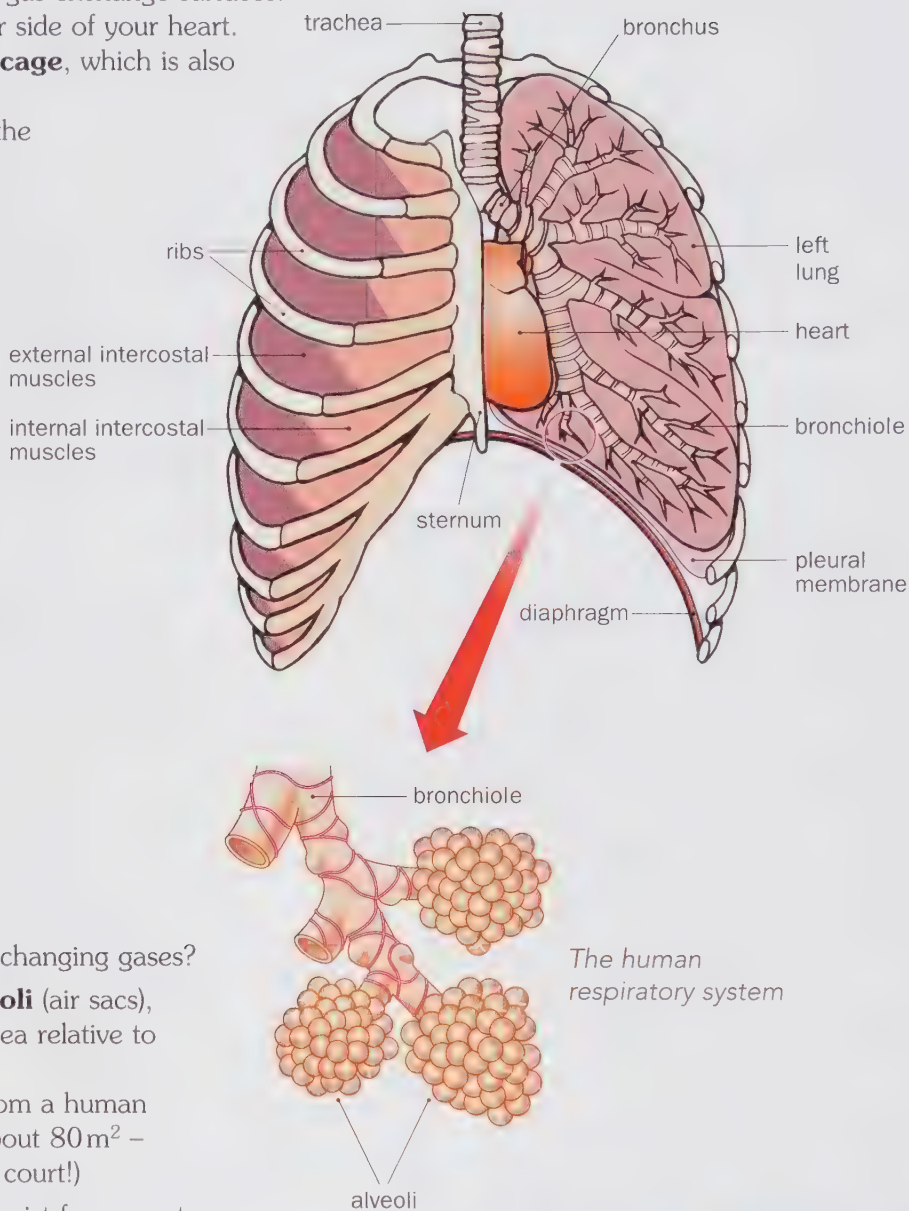
the counter current system maintains a diffusion gradient along the whole length of the gill plate



(numbers represent relative oxygen concentrations)

Gas exchange in humans

Do you remember learning about the lungs?
The lungs are specialised ***internal*** gas exchange surfaces.
They are found in your chest, either side of your heart.
The lungs are protected by the **rib cage**, which is also involved in breathing.
A muscular **diaphragm** separates the lungs from the organs below.



The human respiratory system

Why are the lungs so efficient at exchanging gases?

- The exchange surfaces are **alveoli** (air sacs), which provide a large surface area relative to the volume of the body.
  (If you unfolded all the alveoli from a human lung, it would give an area of about $80\,m^2$ – that's nearly the size of a tennis court!)
- The surfaces of the alveoli are moist for gases to dissolve.
- The alveoli are very thin, which helps diffusion by providing a very short diffusion pathway.
- Each alveolus is surrounded by a capillary network. This good supply of blood helps to maintain concentration gradients, because the blood is always taking oxygen away from the alveolus and returning with carbon dioxide.
- Ventilation of the lungs ensures that the air in the air passages is changed.
  This again helps to maintain the gas concentration gradients between the air in the alveoli and that in the blood.

Where ***exactly*** in the lungs does gas exchange occur?

A resin cast of the human lungs

Breathing

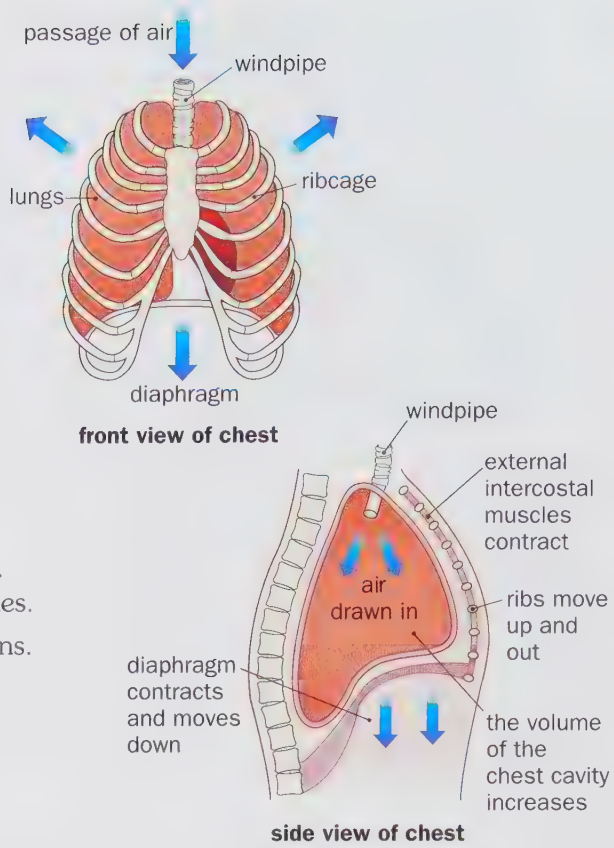
Your lungs contain elastic tissue – they have no muscle. So how are you able to breathe in and out?

Air is drawn into the lungs by reducing the pressure inside them to below atmospheric pressure.
Air is blown out of the lungs by increasing the pressure inside them to above atmospheric pressure.
Movements of the ribcage and diaphragm produce these pressure changes.

Inspiration (breathing in)

If air is to enter the lungs, then the pressure inside them must be lower than atmospheric pressure.

- The external intercostal muscles ***contract*** and the internal intercostal muscles ***relax***, raising the ribs upwards and outwards. There is antagonistic interaction between these two sets of muscles.
- At the same time, the muscular diaphragm ***contracts*** and flattens.
- Both these actions ***increase*** the volume inside the thorax, causing the pressure inside the thorax to ***decrease***.
- Since atmospheric pressure is greater, air rushes ***into*** the lungs and they inflate.



Inspiration

Expiration (breathing out)

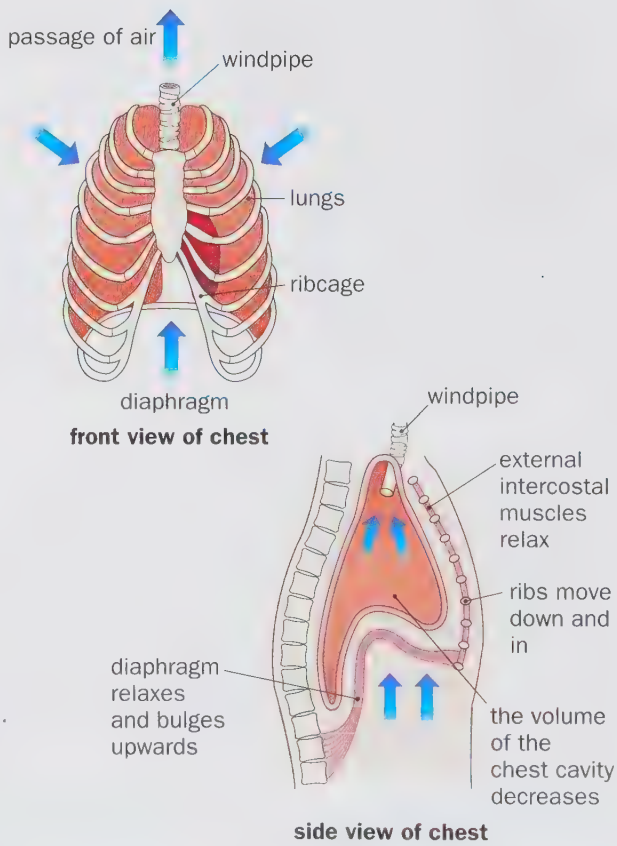
Breathing in is an active process but breathing out is usually passive. Inspiration stretches the elastic tissue of the alveoli.
When the inspiratory muscles relax, this tissue recoils, pushing the air out.

- The internal intercostal muscles ***contract*** and the external intercostal muscles ***relax*** (antagonistic interaction again). This lowers the ribs downwards and inwards.
- The diaphragm ***relaxes*** and bulges upwards due to pressure from the organs below, for example the liver.
- Both these actions ***decrease*** the volume inside the thorax, causing the pressure inside the thorax to ***increase***.
- Air is forced ***out*** of the lungs as the elastic tissue of the alveoli recoils.

Around each lung and lining the thorax are the **pleural membranes**. Between these two membranes is a space called the **pleural cavity**, which contains **pleural fluid**.
During breathing the pleural fluid acts as a lubricant. This allows friction-free movement of the lungs against the inner wall of the thorax.

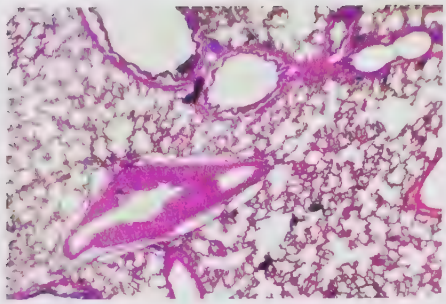
The alveoli do not collapse when we breathe out because they have an anti-sticking chemical called a **surfactant** covering their surfaces. This chemical acts by reducing the surface tension and so keeps the alveoli open.

See Chapter 15 for ventilation rate, vital capacity and tidal volume measurements.



Expiration

Gas exchange at the alveolus



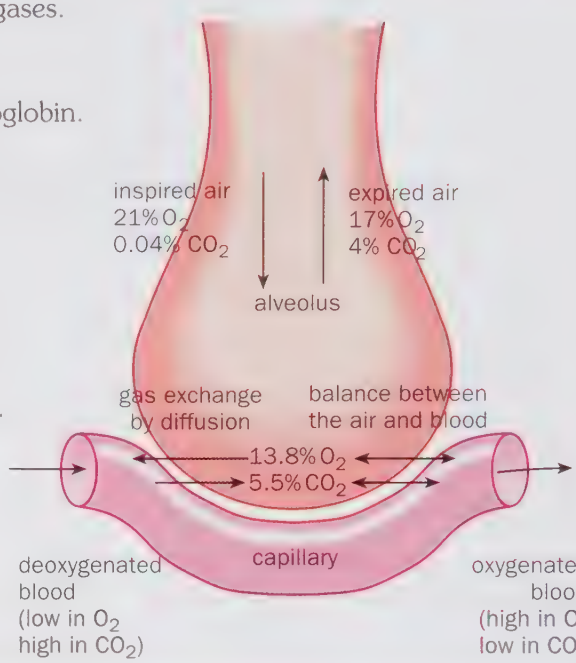
A photomicrograph of lung tissue

Gas exchange takes place in the lungs at the tiny air sacs or alveoli. As you have seen, these provide a huge surface area for the respiratory gases to pass across.

Ventilation ensures that air is moved into and out of the air passages of the lungs regularly.
This helps to maintain the necessary concentration gradients of oxygen and carbon dioxide.

The capillaries around the alveoli provide efficient blood transport of gases. This prevents a build-up of oxygen and carbon dioxide and maintains gas concentration diffusion gradients.
In addition, the red blood cells contain the respiratory pigment haemoglobin. This has a high affinity for oxygen, making the removal of oxygen from the alveoli even more efficient.

Look at the diagram.
Deoxygenated blood enters the capillaries around the alveolus.
This blood has less oxygen and more carbon dioxide than the air inside the alveolus.
So oxygen diffuses out of the alveolus into the blood in the capillary.
Carbon dioxide diffuses out of the capillary into the air in the alveolus.



Can you see that by the time the blood leaves the alveolus, the concentration of oxygen and carbon dioxide in the alveolus and in the blood are balanced?

The lining of the alveolus is moist and gases diffuse in solution.
The walls of the alveolus and blood capillary are each only one cell thick, making it easy for diffusion to take place.

Look at the table.
Why is the percentage of oxygen in the alveolus ***less*** than that in inspired air?
The answer is because the inspired air mixes with air already in the lungs (residual air), which has a lower percentage of oxygen.

Explain the differences in carbon dioxide concentration between inspired and expired air.
Why does the percentage of nitrogen hardly change?
Why is expired air saturated with water?

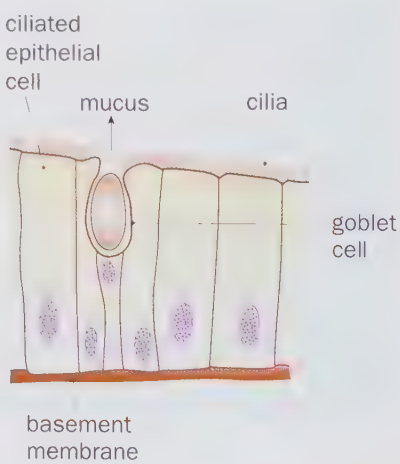
Percentage composition of inspired, alveolar and expired air

Gas	Inspired	Alveolar	Expired
oxygen (O_2)	20.95	13.80	16.40
carbon dioxide (CO_2)	0.04	5.50	4.00
nitrogen (N_2)	79.01	80.70	79.60
water (H_2O)	variable	saturated	saturated

Air conditioning

The first part of the respiratory system treats the air that you breathe in.
As you breathe in through your nose, the air is warmed, moistened and filtered.

The trachea, bronchi and bronchioles are lined with ciliated epithelial cells.
There are also **goblet cells**, which secrete slimy mucus.
The mucus traps dust particles and some pathogens that you breathe in.
Then the cilia beat to move a stream of mucus up to your throat.
This removes the dust and pathogens.



Tobacco smoke temporarily anaesthetises the cilia and stops them beating.
What effect does this have on the accumulation of mucus in the lungs?

See Chapter 15 for diseases of the lungs and data linking smoking to lung disease.

Control of breathing

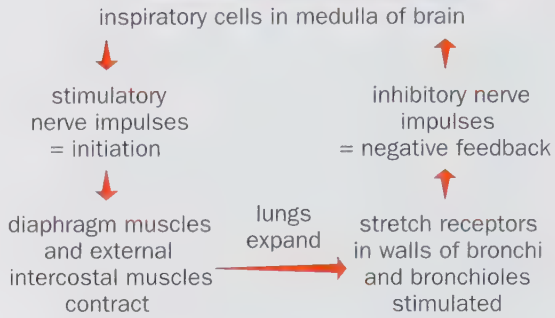
You can exercise voluntary control over your breathing muscles.
You do this when you shout, sing, sigh, or play the saxophone.
But you do not usually think about breathing – it's automatic.

Regular breathing

The basic rhythm of breathing is controlled by part of the brain called the **medulla**.
There is a group of nerve cells in the medulla that make up the **respiratory centre**.

During quiet breathing, nerve impulses from the respiratory centre travel along nerves to the external intercostal muscles and the diaphragm.
The muscles then contract and you breathe in.

As air enters the lungs, **stretch receptors** in the walls of the bronchi and bronchioles send impulses back to the medulla.
When the lungs are sufficiently inflated, the increased feedback from the stretch receptors causes the respiratory centre to stop sending out impulses for about 3 seconds.
The external intercostal muscles and the diaphragm relax and expiration takes place.

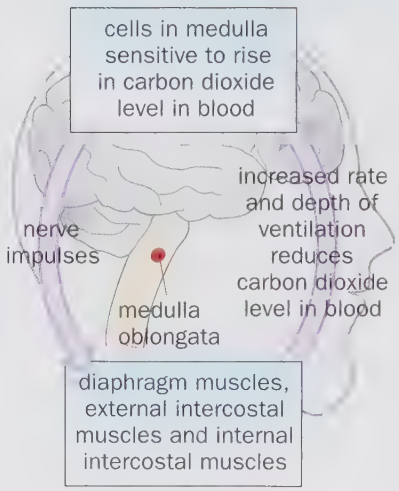


Varying your breathing

Your breathing rate alters when you exercise.
Greater muscular action means you need more energy from respiration.
More oxygen is needed in the muscles and extra carbon dioxide must be removed quickly. So,

- the ***depth*** of breathing must be increased,
- the ***rate*** of breathing must be increased.

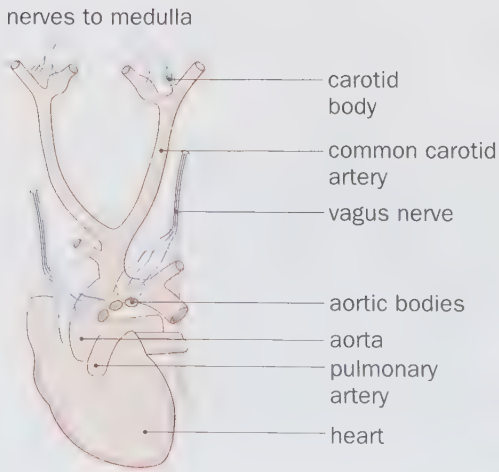
During exercise the rate of respiration increases in your cells.
So more oxygen is used up and more carbon dioxide is produced.
It is the ***rise in carbon dioxide*** that triggers the changes in your breathing.



Chemoreceptors are cells that are sensitive to changes in the presence and concentration of specific chemicals in the blood.
Some are able to detect very small changes in pH.
The most sensitive chemoreceptors are located in the medulla itself.
A rise in carbon dioxide concentration results in more carbonic acid.
This dissociates, increasing the hydrogen ion concentration and producing a more acidic pH.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

There are chemoreceptors in areas lining the carotid arteries and aorta.
These areas are called the **carotid bodies** and **aortic bodies**, respectively.
These are sensitive to changes in carbon dioxide and pH, and can also detect changes in oxygen.



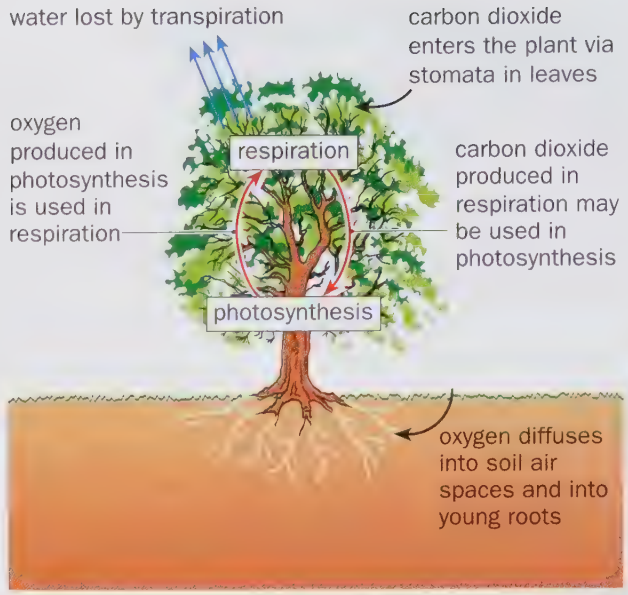
When the chemoreceptors detect any of these changes, they send nerve impulses to the respiratory centre in the brain.
The respiratory centre sends more frequent nerve impulses to the external intercostal muscles and the diaphragm.
As a result, breathing becomes faster and deeper until carbon dioxide levels return to normal.

Gas exchange in plants

All plant cells need a supply of oxygen because they carry out respiration all the time.
Some plant cells also carry out photosynthesis, so they need a supply of carbon dioxide.
Carbon dioxide produced during plant respiration may be used by photosynthesising cells.
And oxygen produced during photosynthesis can be used by cells for respiration.

Oxygen enters root tissue by diffusion from air spaces between soil particles.
Respiration keeps the oxygen concentration in the cells below that in the soil, so maintaining a diffusion gradient.
The root system is extensive, giving a large surface area, and the thin root hairs provide little barrier to diffusion.

However, most gas exchange in plants occurs in the leaves.
Leaves are thin and have a large surface area to volume ratio.
They also have an extensive internal system of air spaces.
Gases are able to diffuse in and out of these air spaces through tiny pores called **stomata**.

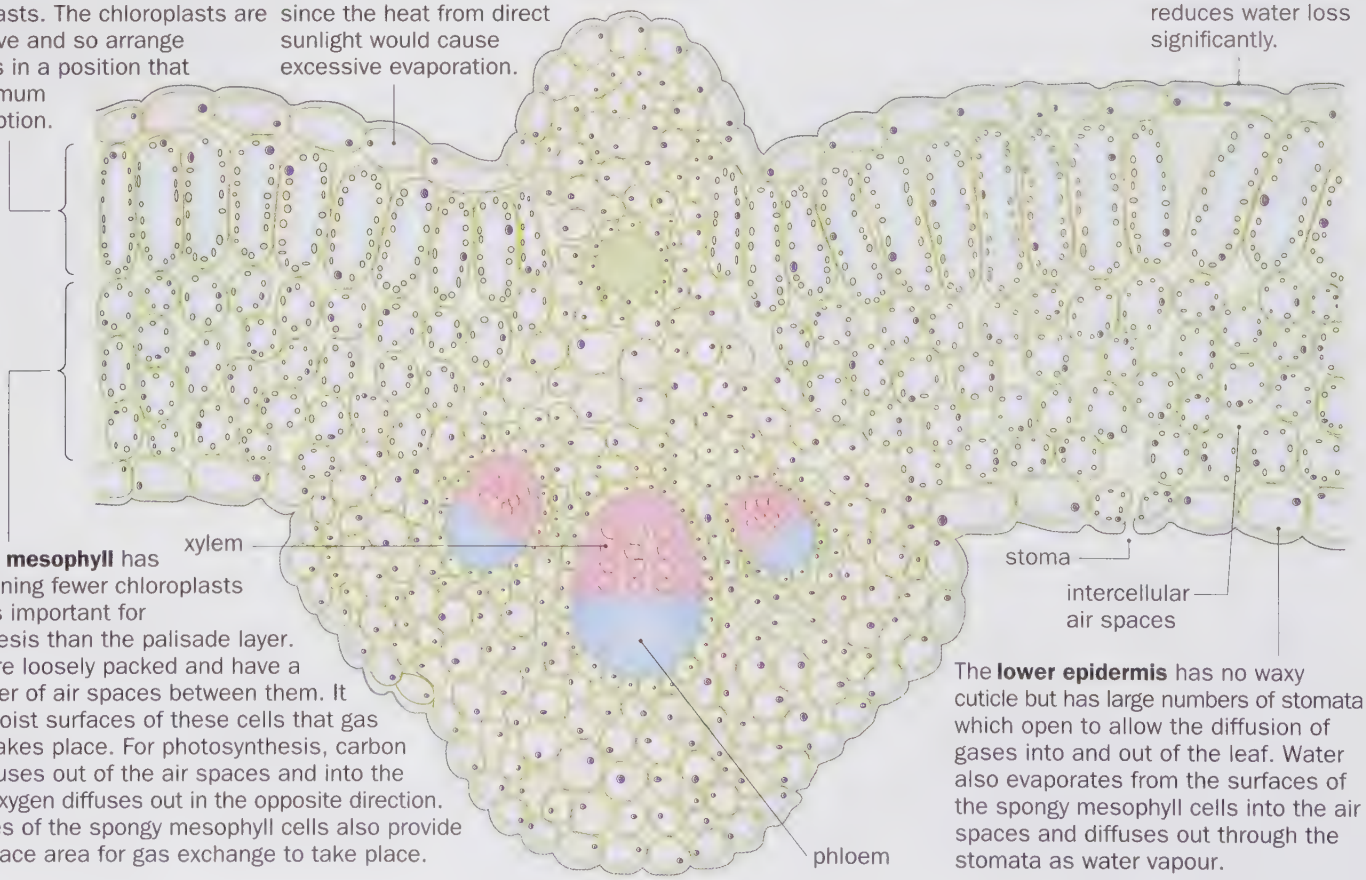


Leaf structure

The **palisade mesophyll** is the main photosynthetic tissue. The cells are deep and packed full of chloroplasts. The chloroplasts are able to move and so arrange themselves in a position that gives maximum light absorption.

The **upper epidermis** is a single layer of cells. It is transparent since no chloroplasts are present and this means that light can pass straight through to the tissues below. There are few stomata in the upper epidermis (if any at all) since the heat from direct sunlight would cause excessive evaporation.

The leaf's upper surface is covered by a waxy **cuticle**. This reduces water loss significantly.



The **spongy mesophyll** has cells containing fewer chloroplasts so it is less important for photosynthesis than the palisade layer. The cells are loosely packed and have a large number of air spaces between them. It is on the moist surfaces of these cells that gas exchange takes place. For photosynthesis, carbon dioxide diffuses out of the air spaces and into the cells, and oxygen diffuses out in the opposite direction. The surfaces of the spongy mesophyll cells also provide a large surface area for gas exchange to take place.

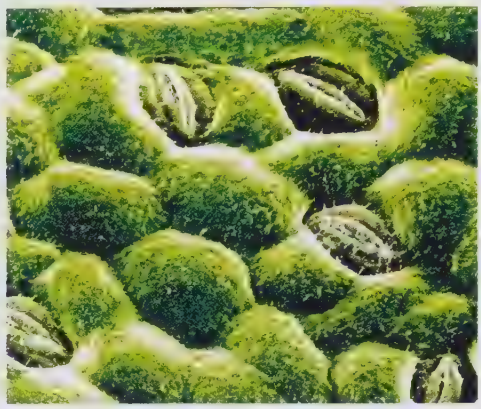
The **lower epidermis** has no waxy cuticle but has large numbers of stomata which open to allow the diffusion of gases into and out of the leaf. Water also evaporates from the surfaces of the spongy mesophyll cells into the air spaces and diffuses out through the stomata as water vapour.

Stomata

Stomata are the tiny pores found on the underside of a leaf.
Each stoma is bordered by two **guard cells**.
These guard cells have chloroplasts, unlike other epidermal cells.
The stomata allow exchange of gases to occur between the air and the internal tissues of the leaf.
About 90% of water evaporation from a plant takes place through the stomata.

The opening of the stomata depends upon environmental conditions.
The guard cells have thickened inner walls.
When the guard cells take up water by osmosis, they swell and become turgid.
The thickened inner walls of the guard cells become more curved and the stoma pore opens.
When the guard cells lose water they become flaccid.
Their thickened inner walls spring back and close the stoma.
By opening and closing, the stomata control the amounts of gases diffusing into and out of the leaf.
They also control the amount of water vapour evaporating.
(The mechanism of stomatal opening and closing is covered in Chapter 11.)

SEM of a stoma with two guard cells



SEM of lower leaf epidermis showing stomata

Opening and closing

The most important environmental factors affecting the opening and closing of stomata are light, carbon dioxide and water.

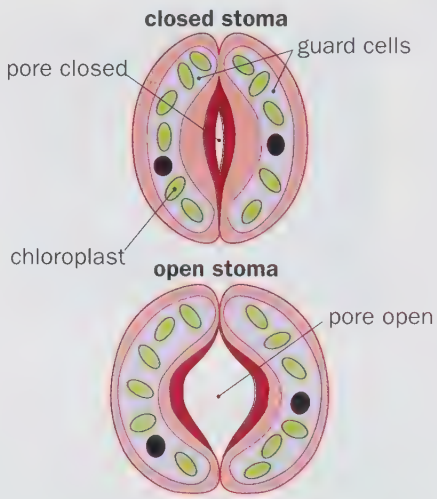
The stomata tend to open during the day and close at night.
Increased light intensity results in more photosynthesis, which reduces the carbon dioxide concentration in the cells.
Low carbon dioxide stimulates the stomata to open.

Low light intensity reduces photosynthesis.
Carbon dioxide accumulates in the cells since it is not being used up.
High carbon dioxide stimulates the stomata to close.

Lack of water also causes the stomata to close because the guard cells lose their turgidity and become flaccid.

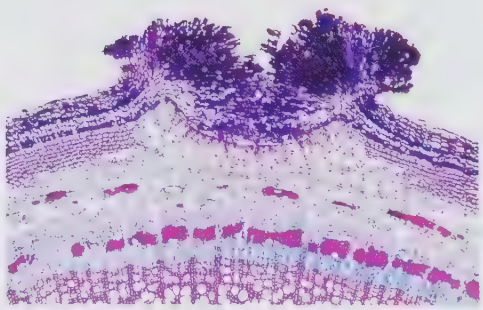
Water stress can also cause an increase in **abscissic acid (ABA)** in the leaves, which stimulates the stomata to close.

Why do floating water plants only have stomata on the upper surface of their leaves?



Lenticels

The protective layer of bark in woody plants prevents gas exchange.
But underneath the bark are respiring cells, so how can they get oxygen?
A **lenticel** is a small area of bark where the cells are loose.
The lenticels allow oxygen to diffuse into the living stem tissue and carbon dioxide to diffuse out.



Photomicrograph of a section of a lenticel

Different gas exchange systems

Look at the photographs of the organisms on this page.
Each has evolved a method of gas exchange that adapts it for life in a particular environment.
What special gas exchange surface does each organism have?
What features of this surface assist in the diffusion of gases?

A sea anemone

A nematode worm

A flatworm

A lugworm (Arenicola marina) showing gills

A vine leaf (Vitis sp.)

A tadpole with external gills

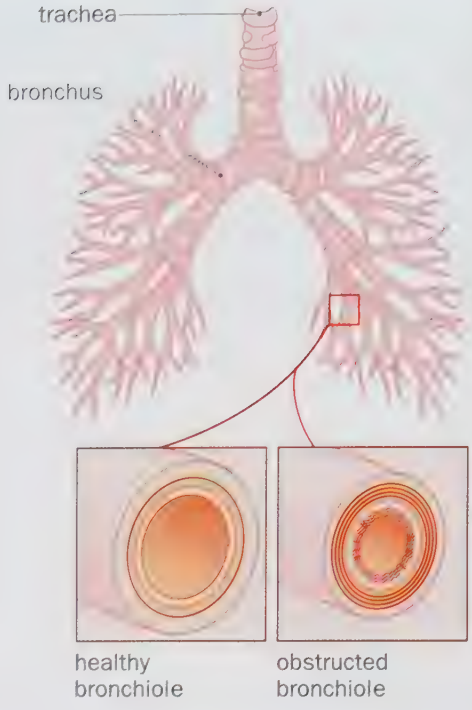
Copy and complete the table using your observations of each organism.

Name of organism	Gas exchange surface	Ways in which it makes gas exchange efficient

Biology at work: Asthma

Asthma is a common long-term ailment that affects about 1 in 12 adults and 1 in 11 children. In 2012 there were about 5.4 million people in the UK receiving treatment for asthma. The symptoms include attacks of breathlessness, which can vary from quite mild to very severe.
More than half the children with asthma grow out of it by the time they reach 21.

Asthmatic attacks occur due to inflammation of the bronchioles. These are the air passages in the lungs that lead to the alveoli, and their obstruction means that more effort is required to deliver sufficient air to the lungs.
The obstruction is made worse by increased secretion of phlegm due to the inflammation, and sufferers develop a dry cough in an attempt to clear the airways.



The effect of asthma on the lungs

What causes asthma?

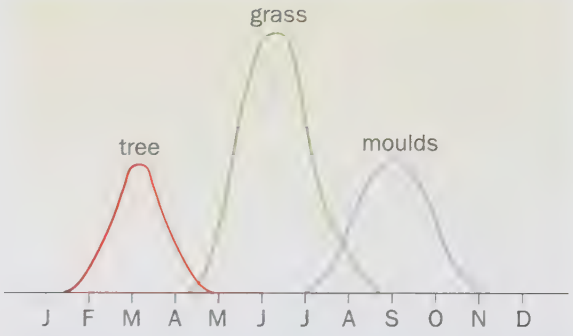
The causes of asthma are not fully understood but it is known to run in families.
There are many common triggers of asthmatic attacks, such as air pollution, respiratory infections, exercise, cold air and allergies. The most common allergens are pollen, house dust, dust mites, fur and feathers.

Where asthma occurs for only a few months of the year, the cause is likely to be pollen or spores, and this is known as seasonal asthma.

Treatment for asthma

There is no cure for asthma, and treatment and prevention involves a combination of medicines, lifestyle advice, and then avoiding potential asthma triggers. The key aims of treatment are to relieve symptoms and prevent future attacks from occurring.

Bronchiodilators (which are often related to adrenaline) give quick relief by causing the smooth muscle which lines the bronchioles to relax – so widening the airways.
Steroids may be taken to reduce the amount of inflammation of the bronchioles.



Seasonal variation in pollen and spore counts

Both these drugs can be taken in aerosol form using an inhaler. When used correctly, these devices deliver the correct dose directly to the inflamed tissue, so allowing a speedy response.


A sportswoman using an inhaler

Asthma should not be confused with emphysema, which has some common symptoms, such as breathlessness and a shortage of oxygen.
Emphysema is one of a group of lung diseases that are known as **chronic obstructive pulmonary disease (COPD)**. COPD in most cases is caused by cigarette smoking and is due to permanent lung damage. The alveoli burst and blend together to form a greatly reduced surface area for gas exchange, combined with a loss of elasticity. It is thought that in the UK there are over 3 million people with COPD, but many are undiagnosed because they dismiss their symptoms as a 'smokers cough'.

Biology at work: Everest without oxygen

On 8th May 1978 Reinhold Messner and Peter Habeler were the first to climb Mount Everest (8848 m) without an additional supply of oxygen.
At the time this was commonly believed to be an impossible feat.
Their success was made possible by a careful and slow process of **acclimatisation**.

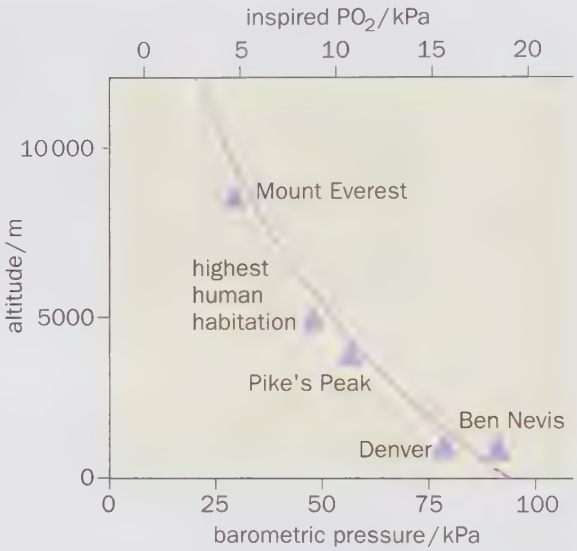
Reinhold Messner and Peter Habeler in 1978

This involved a gradual exposure to an atmosphere where the air contained less than a third the amount of oxygen present in air at sea level.
This reduction in oxygen has severe consequences on the body.

A reduction in blood oxygen levels causes a condition known as **hypoxia**.
If allowed to continue, hypoxia may lead to acute mountain sickness or more life-threatening conditions.
Such conditions include high altitude cerebral oedema, which is swelling of the brain and high altitude pulmonary oedema, leading to excess fluid in the lungs.
Death can result from these conditions unless the climber descends rapidly.

The body's response to hypoxia depends on a number of factors, including the speed and severity of exposure to low oxygen levels.
In general, the following responses occur.

- Rate and depth of breathing increases to increase oxygen uptake and carbon dioxide removal.
- Heart rate increases to transport more oxygen-laden red blood cells from the lungs to the tissues.
- Concentration of the blood increases owing to water reabsorption into the tissues.
- Production of red blood cells from bone marrow increases after the first week, due to an increased secretion of a hormone called **erythropoietin**, which is made in the kidneys.
- Growth of more blood capillaries to the tissues, allowing a quicker diffusion of oxygen to the cells.
- The number of mitochondria per cell increases, so oxygen is used up quickly and a steep diffusion gradient is maintained.



The relationship between altitude and inspired oxygen pressure (PO_2)

There is a limit to how much oxygen can be extracted from the air at such altitudes because of the above responses.
For instance, the heart can only beat so fast before it has an inadequate amount of time to refill between contractions.
A few hundred metres higher and Everest would be impossible to climb without supplemental oxygen.
It is for this reason that the area above 8000 m on the world's highest mountains is referred to as the 'Death Zone'!

Everest base camp where climbers spend time acclimatising before attempting the summit

Biology at work: Training at altitude

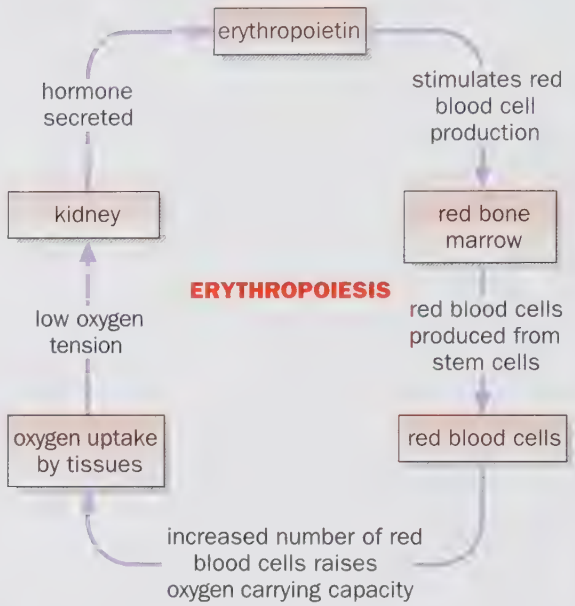
In the 1968 Olympics, athletes who were used to training and competing near sea level experienced a significant reduction in performance.
This was because the Games were held in Mexico City, which is at an altitude of 2242 m.
The athletes who normally lived at a high altitude did well and won many of the endurance races, such as the 10 000 m.

In the 1968 Mexico Olympics, the 10 000 m race was won by Naftali Temu, who lived and trained at altitude

The body's response to altitude

The lower oxygen levels at altitude mean that less oxygen is delivered to the muscles.
The body's immediate response is to release red blood cells stored in the spleen.
Within 12 hours there is also an increase in the rate at which red blood cells are formed.
The hormone erythropoietin is responsible for this increase.
These changes lead to a total increase in circulating haemoglobin by as much as 50–90%.
Consequently, the capacity of the blood to carry oxygen is also increased.

Other changes include an increase in phosphate substances inside the red blood cells.
These combine with the haemoglobin to reduce its affinity for oxygen.
This means the haemoglobin will give up its oxygen more easily when it reaches the muscle tissue.
At altitudes of 2000–2500 m, it takes about 2 weeks for the body to acclimatise and bring about these changes.
Haemoglobin reversal occurs between 3 and 8 days after return to sea level. Any respiratory changes are reversed immediately, and cellular changes reverse within 1 to 2 weeks.



Benefits of training at altitude

Recent developments have tried to reproduce the benefits of altitude training but at sea level.
These range from crude and uncomfortable breathing apparatus worn by athletes, to complex hypoxic training rooms.

An obvious benefit of this is a reduction in travel cost.
Others benefits include:

- no air pressure change,
- no exposure to higher levels of ozone and UV light,
- no tissue enlargement,
- no accelerated dehydration,
- better oxygen supply to peripheral tissues,
- longer maintenance of improvements such as drop in heart rate.

A hypoxic training room

Summary

- Gas exchange is needed for respiration to take place. Oxygen has to be taken up and carbon dioxide released.
- Efficient gas exchange surfaces:
  i) have a high surface area to volume ratio,
  ii) are thin and permeable,
  iii) are moist so that gases can diffuse in solution.
- In unicellular organisms the cell-surface membrane has enough surface area relative to the volume of the cell to act as a gas exchange surface.
- In larger organisms, structures such as gills, tracheal tubes and alveoli have evolved enabling efficient gas exchange to take place.
- Many fish use a counter current system to increase the rate of gas exchange across the gills.
- Insects have evolved a system of air tubes called tracheoles that carry air directly to the tissues.
- Mammals have internal lungs and a method of ventilation to change the air in them.
- Some animals have an efficient respiratory pigment, such as haemoglobin.
- The spongy mesophyll cells of a leaf provide the main gas exchange surfaces in a plant.
- Gases pass into and out of plant tissues through stomata and lenticels.
- Both animals and plants have developed gas exchange systems that enable them to overcome their reduced surface area to volume ratio.

Questions

1 a) Unicellular organisms such as *Amoeba* have no special organs for gas exchange. Large, multicellular organisms such as mammals have complex internal systems for gas exchange. Explain why a mammal needs such a system when a single-celled organism does not.

b) How does a molecule of carbon dioxide in the atmosphere reach the palisade mesophyll cells of a leaf?

2 The rate of diffusion of a molecule across a membrane depends on the relative concentration of the molecule on either side of the membrane, the membrane's thickness and its surface area.

$$\text{Rate of diffusion} = \frac{\text{surface area} \times \text{difference in concentration}}{\text{thickness of the membrane}}$$

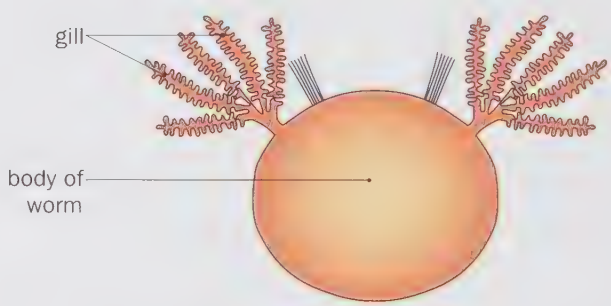
a) For maximum diffusion to take place, which factor should:
   i) be as large as possible,
   ii) be as small as possible?

b) Use the equation to explain how the following are adapted for efficient gas exchange:
   i) a single-celled *Amoeba*,
   ii) the human lungs.

3 *Arenicola* is a marine worm that lives in a burrow in the mid-shore.
It has gills along its body that extract oxygen from the seawater that it pumps through its burrow.

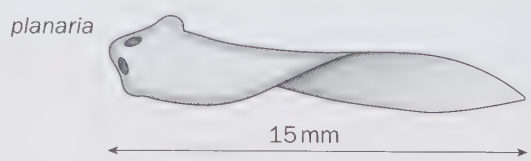
a) The diagram shows a cross-section through the body and gills of *Arenicola*. How do you think that the structure of the gill makes it efficient for gas exchange?

b) What is the advantage to the worm of pumping seawater through its burrow?



Cross-section of Arenicola

4 a) What are the essential features of a good respiratory surface?



b) This aquatic animal is able to carry out gas exchange by diffusion.
Explain how this occurs taking into account size and shape of the animal, its environment, metabolic activity and maintenance of diffusion gradients.

c) Outline how a continuous supply of oxygen reaches all tissues of an earthworm.

5 The data in the table shows that the carbon dioxide concentration in the alveoli of the lungs affects the amount of air taken in by breathing movements.

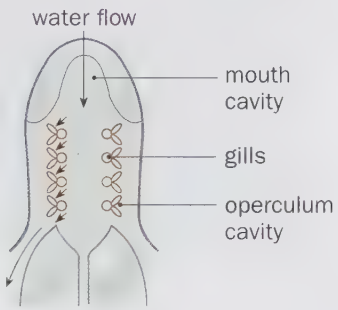
Carbon dioxide concentration in the alveoli (arbitrary units)	40	42	44	46	48	50	52
Air taken in ($dm^3\ min^{-1}$)	8	15	22	29	36	43	50

a) If the lowest carbon dioxide concentration is increased by 5%, calculate (showing your working)
   i) the new carbon dioxide concentration,
   ii) the percentage increase in ventilation rate.

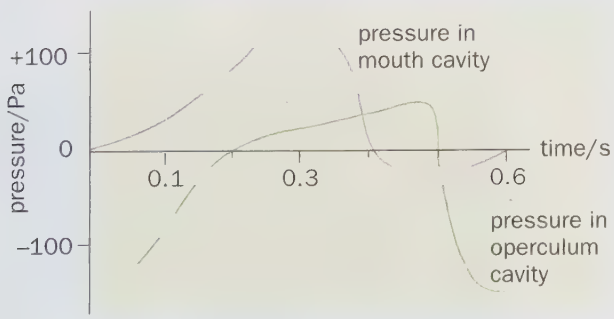
b) In what two ways do the respiratory movements change to bring about this effect?

6 Describe the pathways and mechanisms in the movement of oxygen in a mammal from:
a) the air to the alveolus,
b) the alveolus to the lung capillary,
c) the lung capillary to the tissues.

7 The diagram shows the way in which water flows over the gills of a bony fish.



The graph shows the changes in the mouth cavity and operculum cavity during ventilation.

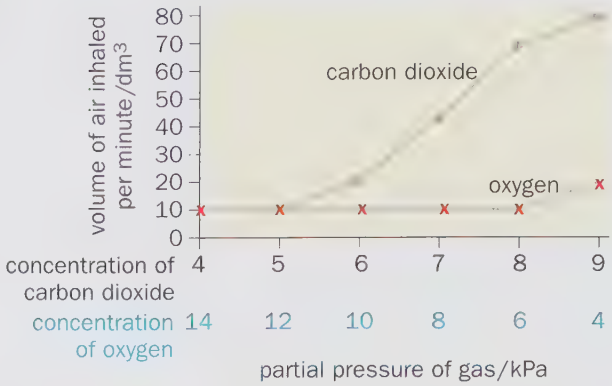


a) Use the graph to calculate the ventilation rate in cycles per minute.

b) During ventilation, water flows over the gills in one direction.
What evidence is there in the graph that supports this statement?

c) How does the counter current principle make the diffusion of oxygen across the gills more efficient?

8



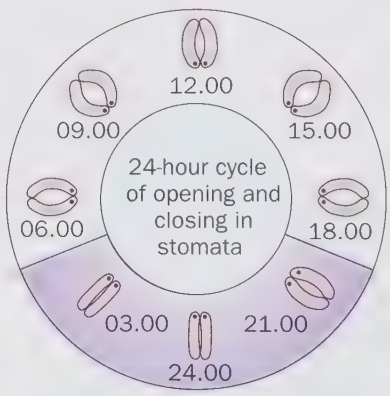
The graph shows the effects of changing the concentration of oxygen and carbon dioxide in the air on the volume of air inhaled in 1 minute by a person.

a) Calculate the percentage increase in the volume of air inhaled per minute when the partial pressure of carbon dioxide rises from 5 kPa to 8 kPa.

b) Describe the effect of changing oxygen concentration on the volume of air breathed in per minute.

c) The volume of air breathed in per minute is regulated by the respiratory centre. Where in the brain is the respiratory centre located?

9 The drawing shows the 24-hour cycle of stomatal opening and closing for a plant.
Explain how this cycle of opening and closing is advantageous to the plant.



9 Digestion

The prehistoric supershark

Dozens of teeth marks on the fossil remains of a whale show that it was the victim of a massive predatory shark.
Scientists have used a formula relating tooth size of sharks to their body length.
They calculate that the shark, *Carcharodon megalodon*, could have grown to a length of 17 m.
A scaled-up version of the great white shark of *Jaws* fame, megalodon preyed on whales up to 9 m long.
Megalodon was probably the largest carnivorous fish that ever lived on the Earth.
Weighing an estimated 65 tonnes, it would have made the great white shark of today look like a kitten.

Heterotrophic nutrition

Autotrophs such as green plants, algae and some bacteria are able to use light energy or energy from chemical reactions to make their own food.

Heterotrophs are unable to do this and have to feed on other organisms.
Heterotrophs include all animals, fungi, some protoctists and some bacteria.
There are a number of different types of heterotrophic nutrition:

- **Saprophytes** (sometimes called saprobionts) feed on dead or decaying material using **extracellular digestion**.
  Saprophytes include fungi and bacteria.
  They feed by secreting digestive enzymes onto their food externally.
  The food is then digested and the soluble products are absorbed.
  Microscopic saprophytes are called **decomposers**.
- **Parasites** feed on other living organisms, their **hosts**.
  They digest the cells of the host and absorb the products.
  The relationship between the parasite and the host is beneficial to the parasite but harmful to the host.
  Parasites may be animals, plants, fungi, protoctists or bacteria.
  Examples of parasites are tapeworms, liver flukes and potato blight.
  Some parasites cause human disease, such as the malarial parasite.
- **Holozoic feeders** include virtually all animals.
  They take their food into their bodies and digest it.
  Many do this inside a specialised digestive system.
  Holozoic feeders include **herbivores** (plant eaters), **carnivores** that feed on other animals, and **detritivores**, animals that feed on dead and decaying material, digesting it internally.
- **Mutualism** is a form of nutrition where there is a close association between two species where both partners benefit.
  Lichens are made up of fungal cells and algal cells.
  The fungal hyphae store water for the algal cells and in return get sugars from the photosynthetic alga.

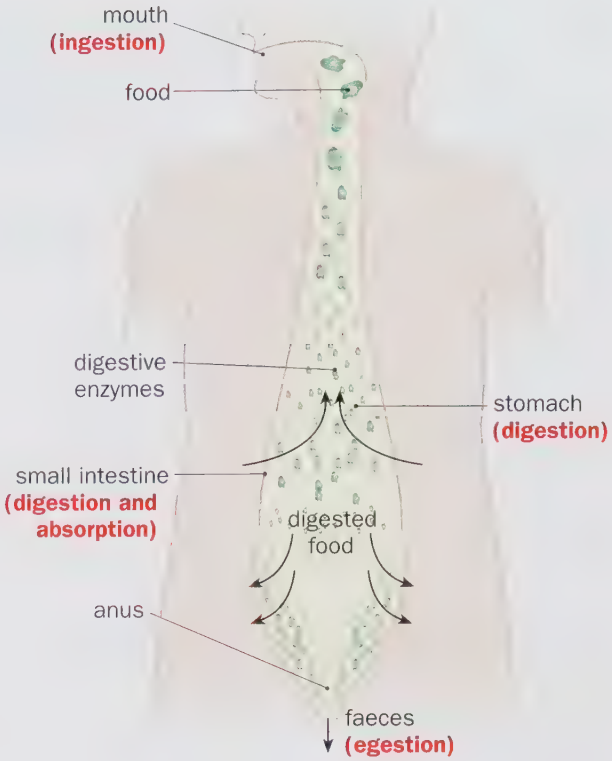
Fleas are ectoparasites

Brown bear feeding on salmon in Alaska

The human digestive system

The components of the human diet are covered in Chapter 14. Here we will look at the human digestive system and its five main functions.

- **Ingestion**: taking food into the body.
  Other animals employ a variety of different feeding mechanisms depending upon the nature of their food.
- **Peristalsis**: propelling food along the alimentary canal by muscular contractions of the gut wall.
- **Digestion**: the breakdown of large, insoluble food molecules into simple, soluble molecules.
  Digestion can be **mechanical**, involving the physical breaking up of food by our teeth when biting or chewing.
  Digestion can also be **chemical**, involving the hydrolysis of complex food molecules by digestive enzymes (as we have seen earlier in Chapter 2).
- **Absorption**: the passage of digested food through the gut wall into the bloodstream.
- **Egestion**: the elimination of undigestible food from the body, mainly plant cell wall material such as cellulose.



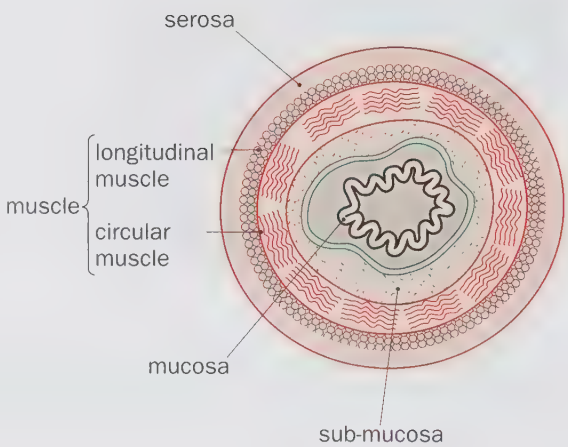
The structure of the gut wall

The alimentary canal is a muscular tube that runs from mouth to anus. Throughout its length the gut wall consists of four different tissue layers:

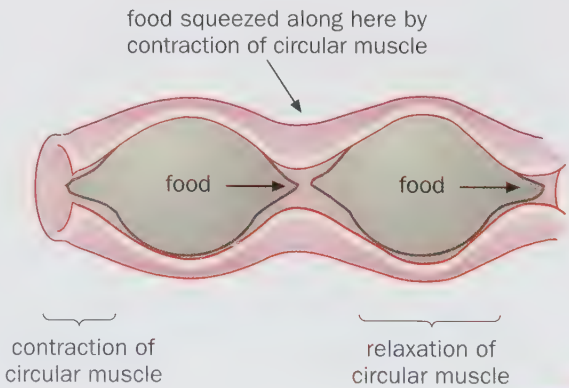
- The **inner mucosa** lines the gut wall.
  It secretes mucus, which lubricates the passage of food and helps to protect the gut from damage.
  The cells of the mucosa are layered (stratified) for protection.
  In some regions of the gut, the mucosa secretes digestive juices, whereas in others it absorbs digested food.
- The **sub-mucosa** contains blood and lymph vessels, which take away the absorbed food products.
  It also contains a rich network of nerve fibres that coordinate the muscular contractions involved in peristalsis.
- The **muscle layer** consists of two layers of muscle running in different directions.
  The inner circular muscle has fibres arranged in rings.
  The outer longitudinal muscle has fibres running lengthways.
  Both layers are made up of smooth involuntary muscle and are responsible for the waves of muscular contraction that move food along the gut. This is called **peristalsis**.

Behind the ball of food, the circular muscle contracts and the longitudinal muscle relaxes. This helps move the food along. In front of the ball of food, the longitudinal muscle contracts and the circular muscle relaxes. This causes the gut to widen and shorten, so that it can receive the food as it is pushed forward.

- The outer **serosa** is a layer of tough connective tissue which protects the gut wall from friction from other organs in the abdomen.



General structure of the gut wall in section

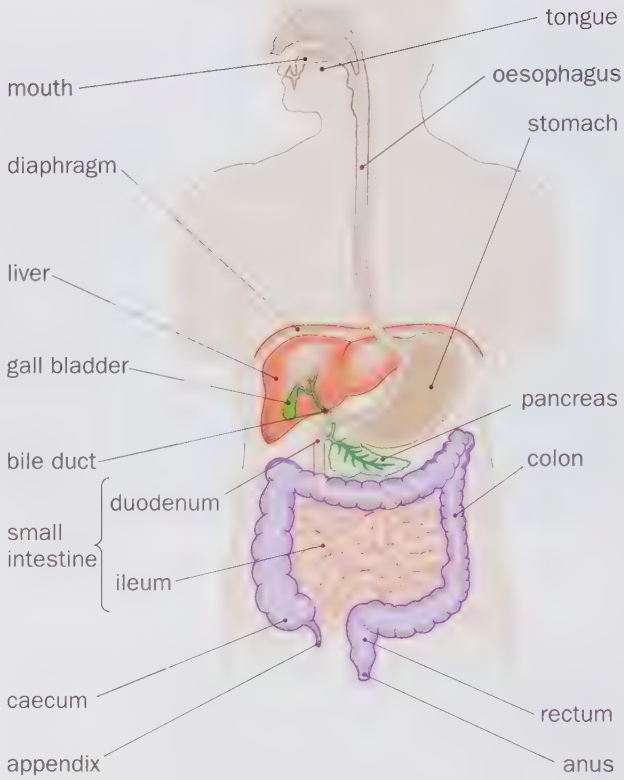


The gut: an overview

The human digestive system consists of the alimentary canal and its associated organs, such as the **salivary glands**, **liver** and **pancreas**.

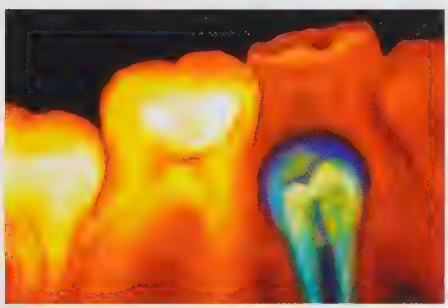
In adults, the alimentary canal is about 10 m long.
It is divided up into distinct parts, which have been adapted to carry out different functions.

Food passes from the mouth to the stomach down a tube called the **oesophagus**.
Food leaves the stomach and enters the **duodenum**, the first part of the **small intestine**.
The second and longer part of the small intestine is called the **ileum**.
The rest of the alimentary canal is made up of the **large intestine**, which can be sub-divided into the **caecum**, the **appendix**, the **colon**, and the **rectum** ending at the **anus**.



Mechanical digestion

Teeth are important in the mechanical digestion of food.
Mastication is the chewing of ingested food.
This makes the food easier to swallow and also increases the surface area for enzyme action.

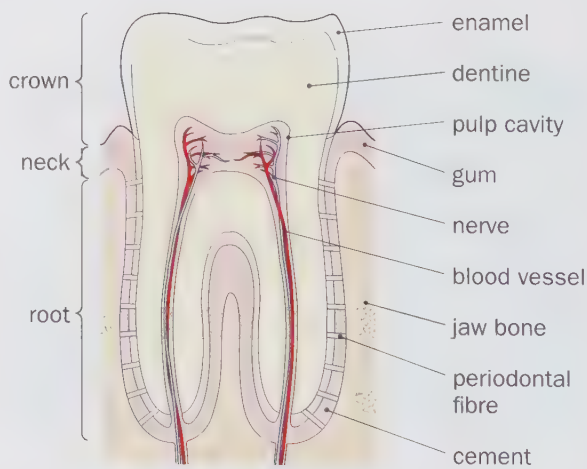


Permanent tooth (green) erupting under a child's milk teeth

Humans have two sets of teeth: the **milk teeth** and the **permanent teeth**.
We have different types of teeth, which perform different roles:

- **Incisors** are chisel-shaped for biting and cutting.
- **Canines** are pointed in many carnivores for piercing and tearing. In humans their role is more like that of incisors.
- **Premolars** have uneven 'cusps' for grinding and chewing.
- **Molars** are like premolars and are used for chewing. The lack of specialisation of our teeth reflects the mixed plant and animal nature of our diet: we are **omnivores**.

A tooth is made up of the **root**, which is embedded in the jaw, and the visible **crown**.
The hard outer layer of the crown is made up of **enamel**.
This covers the bone-like layer of **dentine**, which makes up the bulk of the tooth and surrounds the **pulp cavity**.
Inside the pulp cavity are nerves and blood vessels.
The dentine of the root is kept firmly in place in the jaw by a layer of **cement** and by **periodontal fibres**.



Section of a molar

Chemical digestion

As you saw in Chapter 2, some enzymes are involved in the **hydrolysis** of complex food molecules to smaller ones. The digestive enzymes are classified as **hydrolases**.

Usually more than one enzyme is needed for the complete digestion of a particular food molecule.
For instance, **amylase** will hydrolyse starch to the disaccharide maltose, but a second enzyme, **maltase**, is then needed to digest the maltose into the monosaccharide glucose.

Proteins, as you know, are large, complex molecules.
They may require several different enzymes to digest them.
Peptidases is the name given to protein-digesting enzymes.

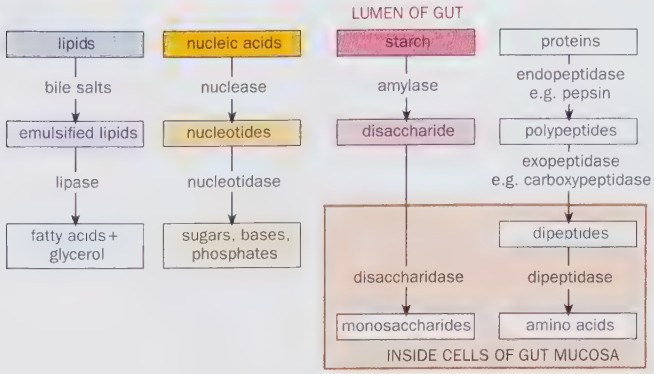
Endopeptidases hydrolyse peptide bonds within the protein molecule. This essentially slices the protein up into shorter lengths of amino acids.
The peptide bonds at the ends of these short lengths are then hydrolysed by **exopeptidases**.

Why do you think that endopeptidases act on proteins before exopeptidases?

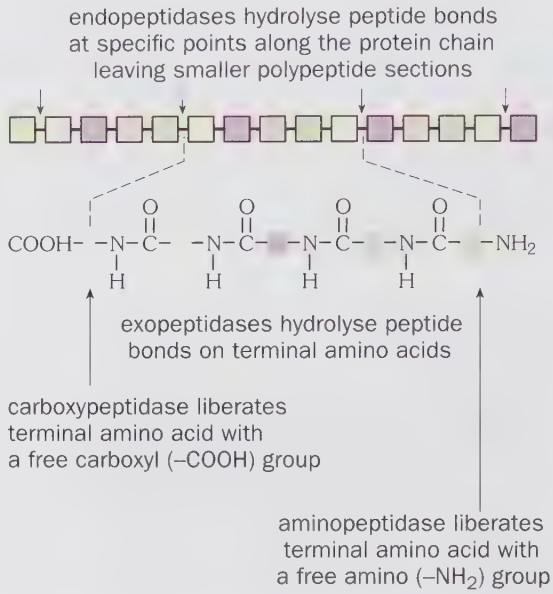
Well, if exopeptidases were to act first, they would only have two 'ends' of the molecule to work on.
By using endopeptidases first, the protein is split up and far more 'ends' become available for exopeptidases to work upon.

Compared with proteins, fats are much smaller molecules.
They can be hydrolysed to fatty acids and glycerol by just one enzyme, **lipase**.

Different parts of the alimentary canal have different pHs.
As you know, different enzymes have different optimum pHs.
For instance, pepsin in the stomach has an optimum pH of 2.0, whereas lactase in the ileum has a pH optimum of 8.5.



Note that disaccharidases and dipeptidases work inside the cells of the gut mucosa. For this reason, they are referred to as 'membrane-bound'



Summary of the digestive enzymes in the human gut

Region of gut	Secretion	Site of production	pH	Enzymes produced	Substrate	Products
mouth	saliva	salivary glands	6.5–7.5	amylase	starch	maltose
stomach	gastric juice	gastric glands	2.0	pepsin	protein	polypeptides
				rennin	milk protein	
duodenum	pancreatic juice	pancreas	7.0	amylase	starch	maltose
				trypsin	protein	polypeptides
				chymotrypsin	protein	polypeptides
				carboxypeptidase	polypeptides	amino acids
				lipase	fats	fatty acids + glycerol
				nuclease	nucleic acids	nucleotides
ileum	intestinal juice	ileum mucosa	8.5	maltase	maltose	glucose
				sucrase	sucrose	glucose + fructose
				lactase	lactose	glucose + galactose
				peptidases	polypeptides	amino acids
				lipase	fats	fatty acids + glycerol
				nucleotidase	nucleotides	sugar, base, phosphate

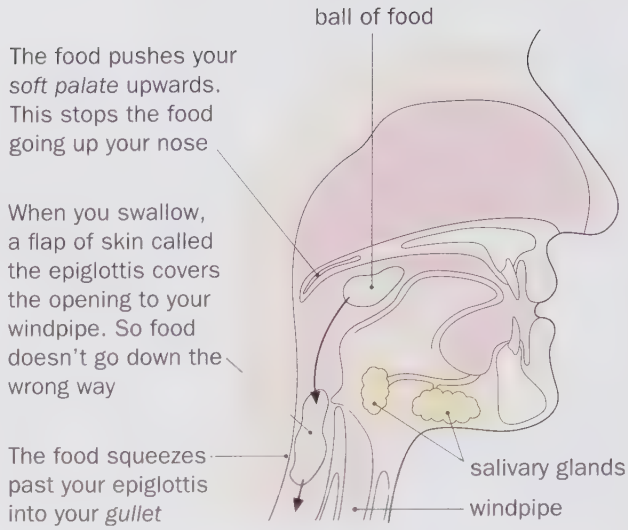
Digestion in the mouth

In the mouth food is chewed by the teeth and mixed with saliva.
Saliva is secreted by three pairs of salivary glands.
Saliva is a solution containing the following dissolved substances:

- **Salivary amylase**, which hydrolyses starch to maltose.
- **Mineral salts**, mainly sodium hydrogen carbonate, which helps to keep the pH in the mouth at about 6.5–7.0 (the optimum for amylase). Chloride ions in saliva activate salivary amylase.
- **Mucin**, a slimy glycoprotein which helps to bind particles of food together and lubricates its passage down the oesophagus.

After chewing, the food is rolled into a ball or **bolus** by the tongue and pushed to the back of the mouth to be swallowed.

Swallowing involves a number of reflexes.
Look at the diagram.



What prevents the food from going

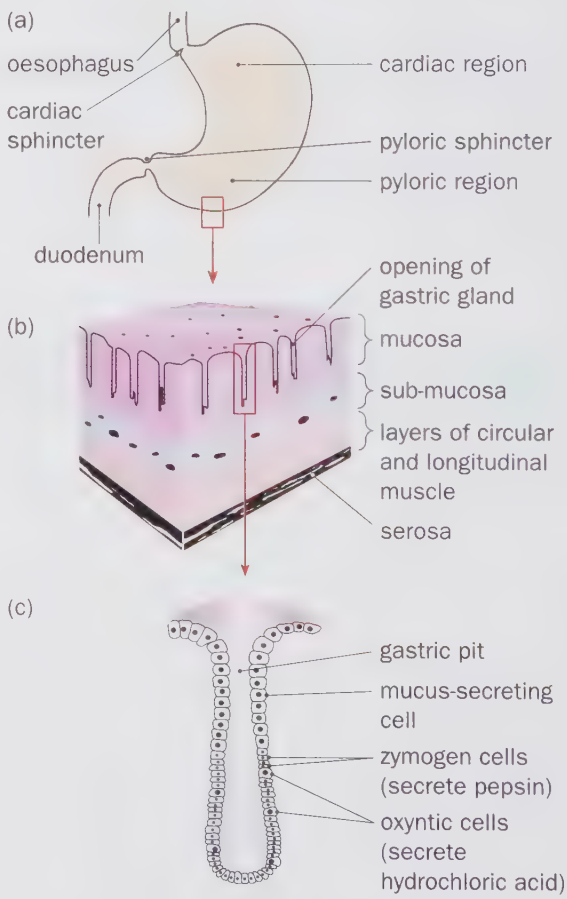
a) into your nasal passage,
b) down your windpipe?

Once inside the oesophagus, the bolus is forced down to the stomach by a wave of muscular contraction known as peristalsis.

Digestion in the stomach

The stomach is a large, muscular bag that can hold up to 2 dm^3 of food.
Food is kept in the stomach by contraction of the **cardiac sphincter** and **pyloric sphincter**, two rings of smooth muscle.
Food may stay in the stomach for up to 4 hours depending upon what has been eaten.
During this time the contractions of the muscular stomach wall churn up and mix the food with **gastric juice**, secreted by cells in **gastric pits** in the mucosa of the stomach lining.
Gastric juice is a solution containing the following dissolved substances:

- **Hydrochloric acid**, secreted by **oxyntic cells**, gives the stomach contents a pH of 2.0.
  This activates the stomach enzymes and provides the optimum pH for their action.
  It also kills most bacteria in the food and inactivates salivary amylase.
- **Pepsin** is secreted in inactive form by **zymogen cells**.
  It becomes activated by stomach acid.
  Pepsin is an endopeptidase, breaking specific peptide bonds within the protein molecule, so hydrolysing it to polypeptides.
- **Rennin** is also secreted by zymogen cells. This is activated by acid and converts soluble caseinogen in milk into insoluble **casein**.
  The milk curdles and so it stays in the stomach longer for digestion.
  Why do you think this is particularly important in young mammals?
- **Mucus** is secreted by **goblet cells**, and is important in protecting the stomach wall from the digestive actions of pepsin and hydrochloric acid. A peptic ulcer can form if too much acid is secreted as a result of irregular eating, stress or smoking.



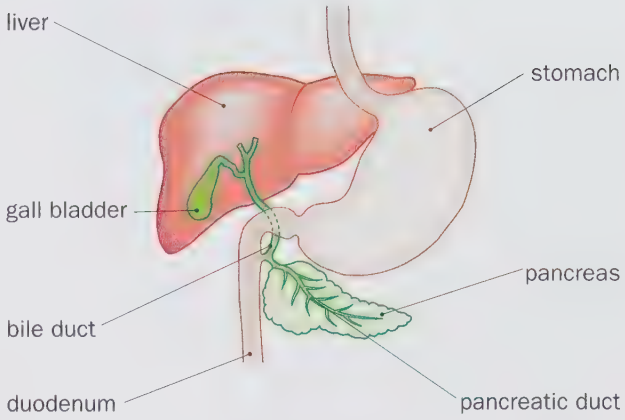
(a) section through stomach (b) part of stomach wall (c) gastric gland

Digestion in the small intestine

After a few hours in the stomach, the food becomes a creamy fluid called **chyme**.
Periodic relaxation of the pyloric sphincter releases the chyme into the duodenum a little at a time.

Most of the digestive activities of the small intestine take place in the duodenum. The role of the ileum is mainly absorption.

There are three main secretions concerned with digestion that are released into the duodenum: **bile**, **pancreatic juice** and **intestinal juice**.



Bile

Bile is made in the liver and stored in the **gall bladder**.
It enters the duodenum along the **bile duct**.
Bile contains no digestive enzymes, but it does contain substances that have an important role in digestion:

- **Sodium hydrogencarbonate** neutralises the acid chyme as it enters the duodenum from the stomach to produce a neutral pH (pH 7) at which the enzymes in the small intestine work best.
- **Bile salts** (sodium glycocholate and sodium taurocholate) **emulsify** fats (break them down into minute droplets) and give them a much larger surface area over which lipase can act.

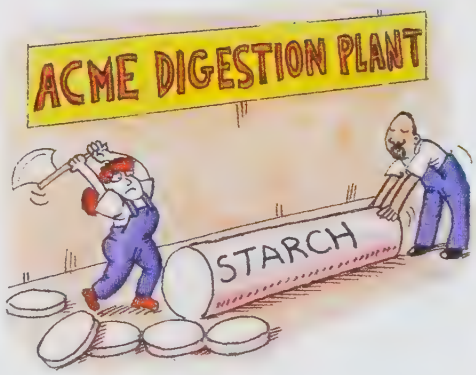
Pancreatic juice

Pancreatic juice is made in the pancreas by secretory cells.
It is released into the pancreatic duct in response to the presence of acid food in the duodenum.
It is alkaline and performs a similar role to bile in neutralising stomach acid.
Pancreatic juice contains a lot of water, as is the case with other digestive juices.
This water must eventually be reabsorbed into the blood in the large intestine, otherwise we would dehydrate.

Pancreatic juice contains the following enzymes:

- **Trypsin**, an endopeptidase.
  Its role is to hydrolyse proteins to polypeptides.
- **Chymotrypsin**, another endopeptidase.
  When activated in the presence of trypsin, it hydrolyses proteins to peptides.
- **Carboxypeptidase**, also activated by trypsin.
  This enzyme is an exopeptidase that hydrolyses polypeptides to smaller peptides and some amino acids.
  Trypsin, chymotrypsin and carboxypeptidase together continue the digestion of proteins.
- **Pancreatic amylase** completes the hydrolysis of starch to maltose, which was started by salivary amylase in the mouth.
- Lipase hydrolyses fats to fatty acids and glycerol.
- **Nuclease** breaks down nucleic acids into their constituent nucleotides.

Secretory cells in the pancreas



Splitting starch the hard way!

Intestinal juice

There are deep folds in the wall of the duodenum called the **crypts of Lieberkühn**.
Here, **Brunner's glands** secrete intestinal juice, which contains water, mucus and sodium hydrogencarbonate.
Intestinal juice does not contain any enzymes but it protects the mucosa of the duodenum from the effects of stomach acid.

Enzymes released by the secretory cells at the tips of the villi complete digestion.
Digestive enzymes are also present in the cell-surface membrane and cytoplasm of these cells. These are membrane-bound disaccharidases and dipeptidases.

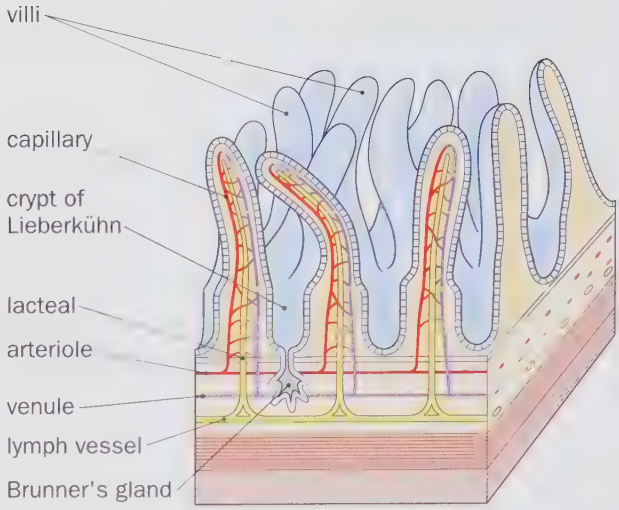
- **Aminopeptidase** is an exopeptidase that hydrolyses peptides to smaller peptides and amino acids.
- **Dipeptidase** breaks down dipeptides to amino acids.
- **Nucleotidase** hydrolyses nucleotides into their constituent sugars, phosphates and bases.
- **Maltase** breaks down maltose to glucose.
- **Lactase** converts lactose to glucose and galactose.
- **Sucrase** hydrolyses sucrose to glucose and fructose.

Absorption in the small intestine

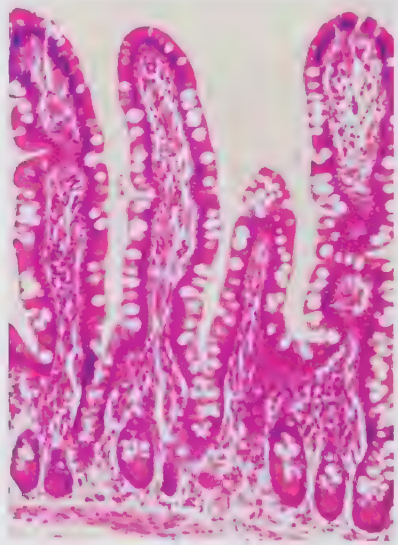
As a result of digestion, there is a high concentration of small, simple molecules inside the lumen of the ileum.
These have to be absorbed across the wall of the ileum so that they can be transported around the body in the bloodstream.

Although there is a concentration gradient established, simple diffusion would be too slow to supply the body's needs for some of these molecules.
So the following methods of transport occur:

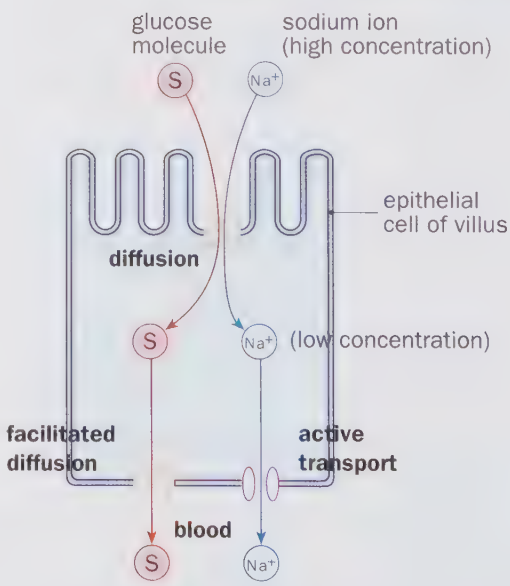
- **Diffusion**
  Fatty acids, glycerol and most vitamins diffuse easily into the epithelial cells.
  The fatty acids and glycerol recombine inside the cells to form triglycerides. These combine with protein to form **chylomicrons** (so that the triglycerides do not stick together).
  They leave the epithelial cells and enter a **lacteal**, to be circulated by the **lymph system** (see page 176).
- **Facilitated diffusion**
  As you saw in Chapter 5, some molecules, such as fructose, cross the cell-surface membrane of the epithelial cell via carrier proteins.
  This form of transport does not involve energy from ATP.
- **Active transport**
  As you saw in Chapter 5, the epithelial cells of the small intestine use energy from ATP for the active uptake of glucose, galactose, amino acids, dipeptides and some salts.
  Dipeptides are then digested ***intracellularly*** (within the epithelial cells) into simple amino acids.



Three-dimensional diagram of a section of the duodenum



Section of the ileum to show villi

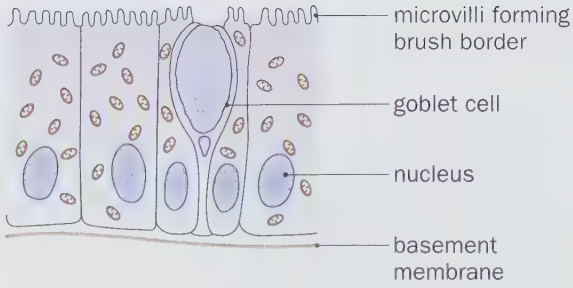


Co-transport of glucose with sodium (Na^+) into and out of an epithelial cell of a villus

The structure of the small intestine

The ileum is well adapted for absorption.
It has a number of structural features that significantly increase the surface area over which absorption can take place.

- The ileum is very long – about 6 m in adult humans.
- The internal lining is thrown into folds, giving a much larger surface area than a simple, smooth tube would.
- On the folds are numerous finger-like projections called **villi**.
- The epithelial cells lining the villi have microscopic projections called **microvilli**. These vastly increase the absorptive surface of the cell-surface membrane of the epithelial cells.

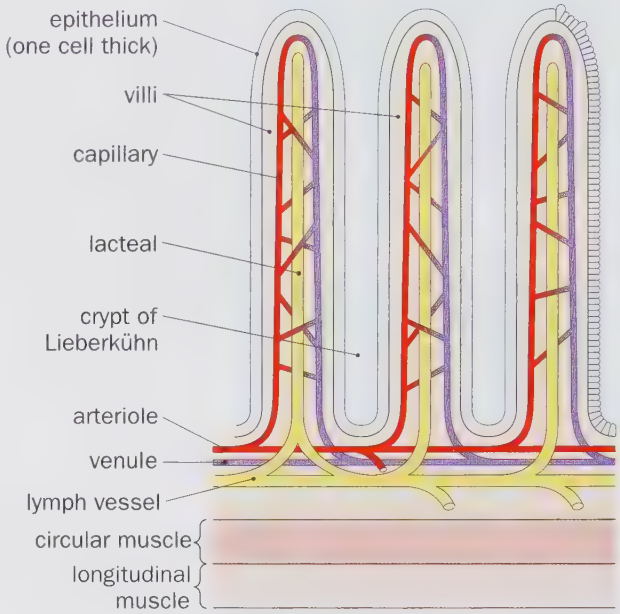


Detailed structure of the epithelial cells lining the small intestine

Simple sugars, amino acids, salts and vitamins that have been absorbed pass out of the epithelial cells into a blood capillary inside the villus.
From here, the blood travels along the **hepatic portal vein** to the liver, where the levels of absorbed food are monitored and regulated, before being delivered to all the cells of the body.

Any excess glucose is converted to the polysaccharide glycogen and stored in the liver.
Excess amino acids are broken down by a process called **deamination** to make **urea**, which is excreted by the kidneys.

As we shall see in Chapter 10, triglycerides form chylomicrons and are transported by the lymph system, which joins to the blood system near the heart.
In the blood, they eventually break down into fatty acids and glycerol and are transported to cells to be used to make lipids.



Detailed section of the ileum

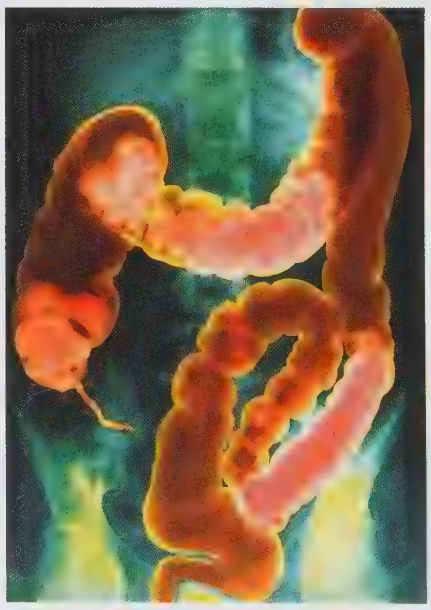
The large intestine

The large intestine is about 1.5 m long and can be divided up into the caecum, the appendix, the colon and the rectum.

Any undigestible food not absorbed by the ileum enters the caecum.
In humans this is little more than a short connection between the ileum and the colon.
At the base of the caecum is a small tube called the appendix.
In humans neither of these two parts of the large intestine are important in digestion, but they have a major function in the alimentary canals of herbivores.

About 10 dm^3 of digestive fluids may be secreted into the human gut every day. The vast majority of this is water, which has to be reabsorbed into the blood, otherwise we would dehydrate. This is one of the major roles of the colon.
As reabsorption takes place, the consistency of the undigestible food changes from liquid to semi-solid.
The semi-solid **faeces** consists of undigested food, particularly fibre, dead cells lost from the gut lining, bacteria and waste material from bile. These are stored and compacted in the rectum before **defaecation**.

Populations of microbes such as *Escherichia coli* (*E. coli*) present in the colon are responsible for the manufacture of vitamin K and folic acid.



X-ray photograph of the large intestine after a barium meal

Control of digestive secretions

Have you ever thought what causes you to produce saliva? The sight, smell, taste and even the thought of food are enough to cause you to salivate.

The release of digestive juices in the gut is under the sophisticated control of both the **nervous system** and the **hormonal system**.

Hormones are chemical messengers that are carried around the body in the bloodstream to affect **target organs**. We will look more closely at the hormonal and nervous systems in Chapters 19 and 20.

Control of saliva production

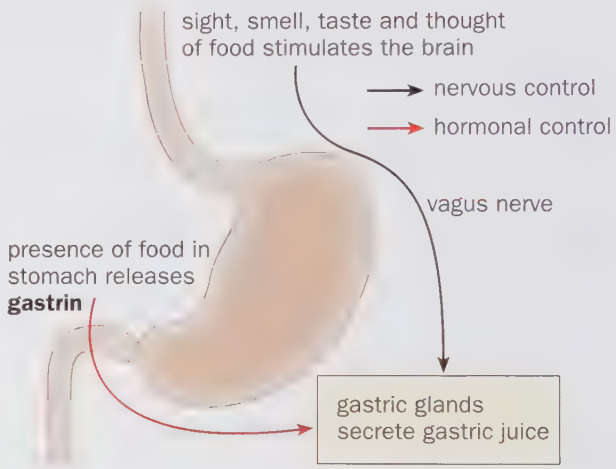
The release of saliva by the salivary glands is entirely under nervous control.
The presence of food in your mouth triggers a **simple reflex** to the brain, which sends out nervous impulses along the **vagus nerve** instructing the glands to secrete saliva.

But the mere thought of food can cause you to salivate due to a **conditioned reflex** action involving the brain.
In other words, your brain learns to associate the thought of food with its presence in the mouth.

Control of gastric juice production

At the same time, the brain stimulates the stomach lining to secrete gastric juice by nerve impulses passing along the vagus nerve.

The presence of food in the stomach stimulates the release of the hormone **gastrin** from **G cells** in the stomach wall. This further stimulates the secretion of gastric juice for up to 4 hours.

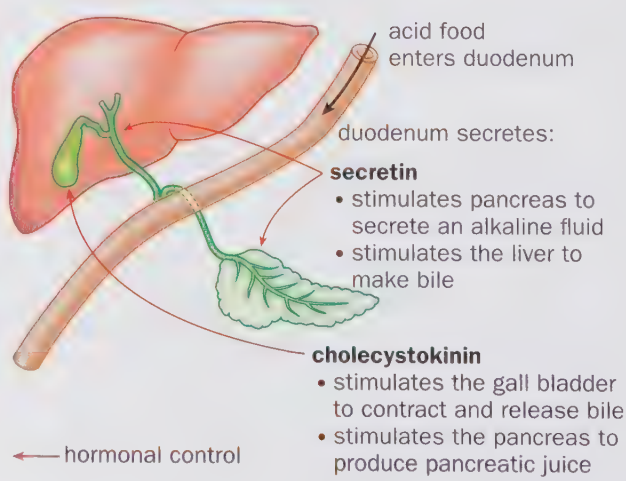


Control of bile and pancreatic juice production

When food leaves the stomach, it enters the duodenum.
The presence of acid food in the duodenum stimulates the production of ***two*** hormones from the duodenal wall:

- **Secretin** travels to the liver in the bloodstream, where it stimulates the production of bile.
  Secretin also passes to the pancreas, where it stimulates the secretion of an alkaline fluid.
- **Cholecystokinin** (CCK) causes the gall bladder to contract, so releasing bile into the duodenum.
  CCK also stimulates the pancreas to produce pancreatic juice.

The control of the release of digestive secretions in the gut highlights the differences between nervous and hormonal control. One relies upon the transmission of nerve impulses from the brain along the vagus nerve.
The other involves the transport of chemical messengers to target organs via the bloodstream.



Other types of heterotrophic nutrition

Saprophytism

Saprophytes (also called saprobionts) feed on dead organisms and waste organic matter.
Most saprophytes are fungi or bacteria.
They secrete powerful digestive enzymes to the outside of their cells. This is called extracellular digestion.
The digestive enzymes include proteases, amylases, lipases, cellulases and lignases.
Can you suggest which substrate each of these enzymes hydrolyses?

The digestive enzymes break down organic material into smaller, soluble molecules, which can then be absorbed across cell membranes.
The bread mould *Rhizopus* is a saprophytic fungus.

As we shall see in Chapter 22, many saprophytic fungi and bacteria are important in the large-scale production of chemicals, such as antibiotics, as well as the commercial production of foods, such as yoghurt, cheese and bread.

SEM of the bread mould Rhizopus

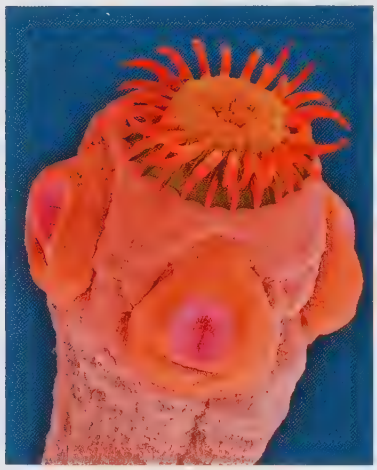
Parasitism

A parasite feeds on another living organism, its host.
It is a one-sided relationship because the parasite gains food and harms the host.

Ectoparasites, such as fleas, feed on the outside of their hosts.

Endoparasites, such as the tapeworm *Taenia*, live inside the host.
The tapeworm embeds its head, or **scolex**, into the lining of the human small intestine by means of a crown of hooks and four suckers.
A long chain of segments, or **proglottids**, grows from the scolex.

The tapeworm lies in the gut surrounded by predigested food.
It absorbs this predigested food across its body wall by a combination of diffusion and active transport. (The tapeworm has no mouth or gut.)
The tapeworm's body wall is resistant to digestive enzymes and to the immune response of the host.



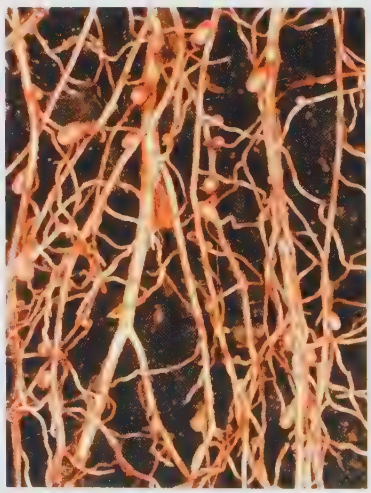
The scolex of the tapeworm Taenia

Mutualism

Mutualism is an association between two organisms in which both benefit in some way, often nutritionally.

As you will see in Chapter 23, different types of bacteria are vital to the nitrogen cycle. **Nitrogen-fixing bacteria** are able to convert atmospheric nitrogen into ammonia, which can be used to make organic molecules such as amino acids and proteins.
Rhizobium is a nitrogen-fixing bacterium that lives in the roots of plants of the Papilionacae, the family to which peas, beans and clover belong.
The bacteria cause swellings on the plant roots called **root nodules**.

The association is a good example of mutualism.
On the one hand, the bacterium provides the plant with a source of nitrogen to make amino acids and proteins.
In return, the plant provides the bacterium with sugars.
Nitrogen-fixing bacteria are very important in maintaining soil fertility.

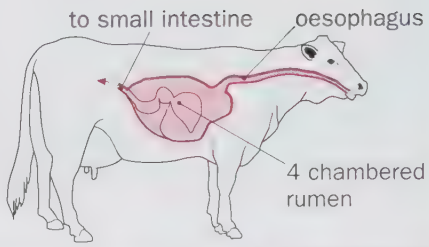


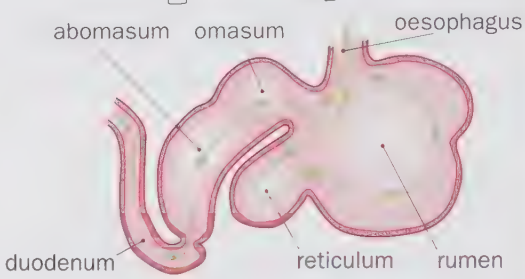
Root nodules contain nitrogen-fixing bacteria

Biology at work: Protein producers

Most of the meat and milk consumed by humans comes from cows and sheep. These animals have the ability to eat grass and other vegetation.

Like other herbivorous mammals, cows and sheep do not secrete **cellulase** enzymes.
Instead they have mutualistic bacteria that produce the enzymes for them. In return, the bacteria gain other digestive products and suitable conditions for growth.
The bacteria live in a specific part of the specialised stomach of cows and sheep, called the **rumen**. Such animals are called **ruminants**.
The 'stomach' has four chambers, with three chambers deriving from the lower part of the oesophagus and one chamber which is the true stomach.





Cellulose digestion takes place in six stages:

1 ***Mouth*** – grass is cropped by teeth, ground with saliva into a 'cud' and swallowed.
2 ***Rumen*** – the cud is mixed with cellulose-digesting bacteria to produce glucose, which is then fermented to organic acids. These are absorbed into the blood and provide a major source of energy. Carbon dioxide and methane are belched out. Rumen bacteria also form protein from inorganic nitrogen.
3 ***Reticulum*** – the cud is formed into balls and regurgitated into the mouth for further chewing before being re-swallowed and passing into the omasum.
4 ***Omasum*** – here much water is reabsorbed from the cud. The firmed up remainder then passes into the abomasum.
5 ***Abomasum*** – here normal gastric secretions begin to digest proteins from the grass and bacteria.
6 ***Duodenum*** – chyme passes into here and then into the small intestine where the products of digestion are absorbed.

Protein sources

Ruminant animals provide about 70% of all the animal protein consumed worldwide.
Much of this comes from cows and sheep, although goat meat is commonly eaten in many countries and makes up about 63% of the red meat eaten around the world.
Ruminants feed on grass, forage and other fibrous vegetation that humans cannot digest.

Fish is the main source of animal protein in many developing countries, and provides about 20% of the animal protein consumed around the world.

Only about 35% of the protein eaten worldwide comes from animal sources, and people in some countries eat little or no meat, gaining their protein from vegetable sources such as grains, nuts and seeds.

Harvesting alfalfa, an important forage crop

Summary

- The human digestive system has five basic functions: ingestion, peristalsis, digestion, absorption and egestion.
- The human alimentary canal is composed of four different layers of tissue: the inner mucosa, the sub-mucosa, the muscle layer and the outer serosa.
- Mechanical digestion involves mastication by the teeth and churning by the muscular stomach wall.
- Chemical digestion involves enzymes called hydrolases, which hydrolyse food substrates.
- Salivary amylase starts the digestion of starch in the mouth before the food is swallowed.
- The stomach secretes gastric juice containing hydrochloric acid, pepsin, rennin and mucus.
- Bile is released into the duodenum from the gall bladder. It emulsifies fats and neutralises the acid chyme entering the duodenum from the stomach.
- Pancreatic juice containing a number of enzymes enters the duodenum from the pancreas.
- Digested food molecules are absorbed in the ileum by diffusion, facilitated diffusion or by active transport.
- The ileum is well adapted for absorption because it has a large surface area: it is very long, and it has a folded inner lining with villi and epithelial cells with microvilli.
- Saprophytes feed on dead organisms and waste organic matter.
- Parasites feed on other living organisms (the host).
- Mutualism is an association between two organisms in which both benefit, often nutritionally.

Questions

1 a) Describe the digestion of carbohydrate in the human alimentary canal.
b) How is the structure of the wall of the ileum (small intestine) adapted to its function in the absorption of the products of digestion?

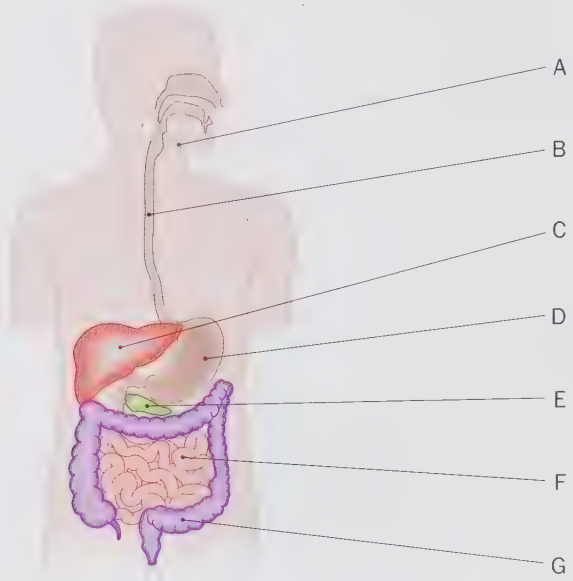
2 Copy and complete the table showing some of the enzymes in the human gut.

Name of enzyme	Site of production	Substrate	Product(s)
trypsin	duodenal mucosa		
	pancreas	fat	fatty acids, glycerol
lactase		lactose	

3 a) Explain exactly what the following types of enzymes do:
i) endopeptidases
ii) exopeptidases
iii) nucleases
b) Why are endopeptidases secreted into the gut ***before*** exopeptidases?

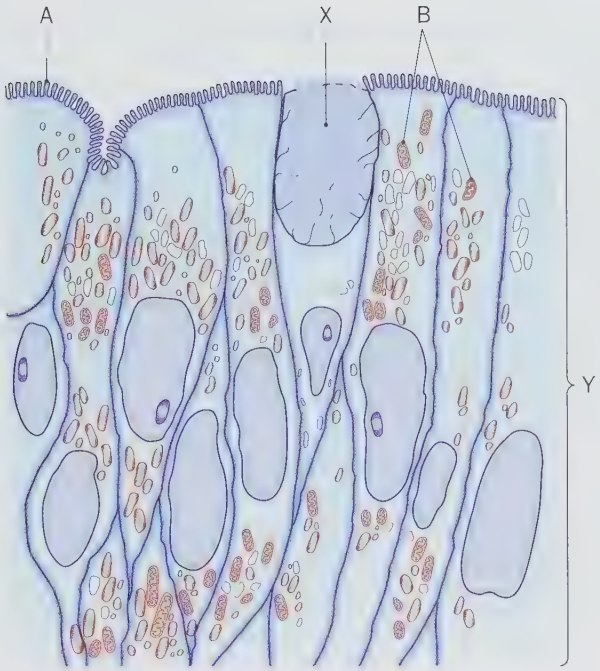
4 a) Describe one function of the muscle layers in the stomach wall.
b) i) Name the type of protein-digesting enzyme secreted by the gastric glands.
ii) Describe the function of this type of enzyme.
c) Explain briefly why a different enzyme is required for the digestion of each type of food substance.

5 The drawing shows the human digestive system.



a) Give the letter of the organ where each of the following is produced:
i) endopeptidase
ii) maltase
b) Name the compounds produced when triglycerides are digested.
c) Describe two roles of bile in the digestion of triglycerides.

6 The diagram on the following page shows some of the cells that form the lining of the small intestine of a mammal.

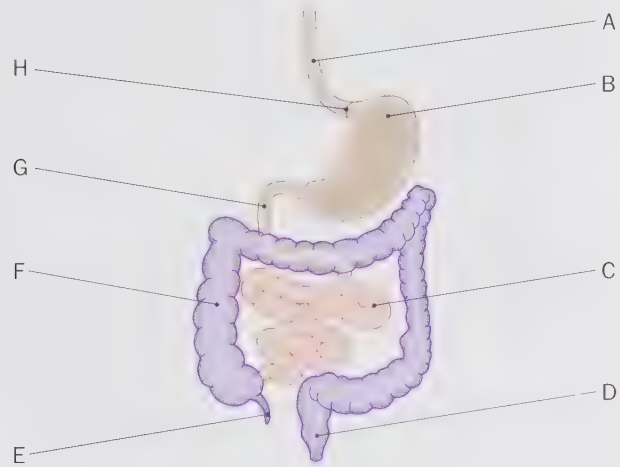


a) What is the general name given to tissue such as that labelled Y?

b) i) Name the features labelled A and B on the diagram.
ii) Explain fully how A and B function in this tissue.

c) i) Name the secretion labelled X.
ii) State two functions of this secretion.

7 The diagram shows the human digestive system.



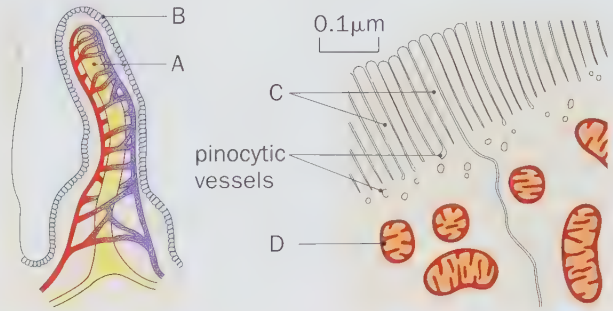
a) Name the parts labelled A to H.

b) Different regions of the gut have different pH values.
i) Why is it important to have a particular pH in a particular region?
ii) Name a region where the gut has a low pH and one where it has a high pH.

c) Use one of the letters A to H to indicate the main region where absorption of each of the following occurs:
i) digested proteins
ii) glucose
iii) water

8 a) What are the main features of
i) autotrophic nutrition
ii) heterotrophic nutrition?

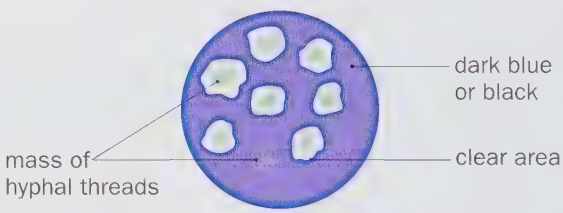
The diagram shows part of a transverse section of the ileum.



b) Name the parts labelled A to D.

c) Briefly describe how three features shown in the diagram enable the ileum to carry out its function of absorption.

9 A number of fungal spores were scattered on the surface of a sterile growth medium containing starch. All the spores germinated and each formed a typical fungal growth consisting of a mass of hyphal threads. At this time iodine solution was poured over the surface of the growth medium.
The result is shown in the diagram.



a) Explain the clear area around most of the fungal growths.

b) Despite the fact that all these spores were produced asexually from the same fungus, one of them did not have a clear area around it. Suggest an explanation for this.

10 Copy and complete the table about the method of feeding of each organism. Tick (✓) if the statement is true or cross (✗) if the statement is not true.

Statement	Saprophytic fungus	Tapeworm	Human
takes undigested food into its body			
carries out extracellular digestion			
carbohydrates absorbed as monomers, such as glucose			

10 Transport in animals

Marathon runners have great endurance.
They are able to maintain intense exercise for long periods, up to 26 miles!
But they have to train to achieve this level of fitness.
Training improves the efficiency of both the respiratory system and the cardiovascular system.

The cardiovascular system is made up of the heart, the blood vessels and the blood.
Endurance training can bring about the following changes.

- The heart size increases, particularly the left ventricle. Its muscular wall becomes thicker and the space inside becomes larger.
- This means that the volume of blood pumped with each heartbeat increases (this is called the **stroke volume**).
- So the resting heartbeat rate decreases.
- The volume of blood in the body increases.
- Recovery of the breathing and heartbeat rates is quicker after exercise.

If we look more closely at our transport system, you will be able to see why these changes improve an athlete's performance.

Mass flow transport

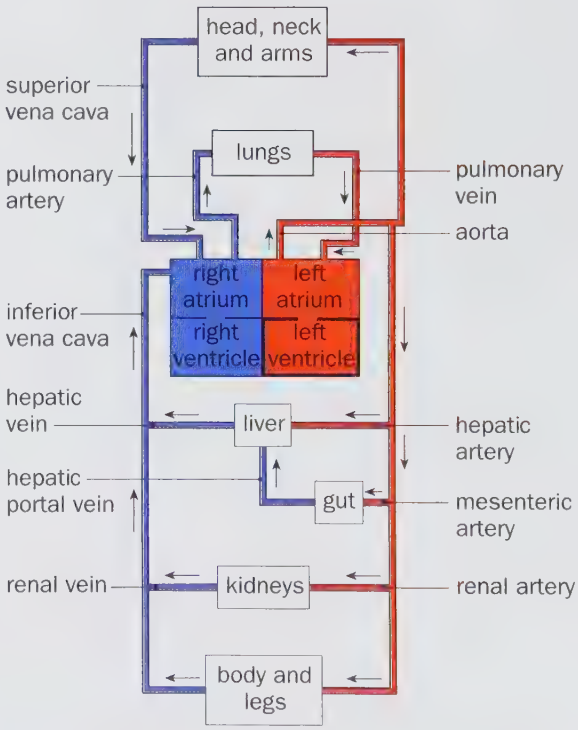
Animal cells need a constant supply of oxygen and nutrients. They also need to get rid of waste products such as carbon dioxide.

Simple, small animals, such as sea anemones, flatworms and nematodes, can do this by ***diffusion*** across their moist body surfaces.
But in larger animals, diffusion is too slow to supply all the body cells efficiently.
They need a **transport system** to carry oxygen, nutrients, carbon dioxide, waste products and hormones to and from the special exchange surfaces.

Our own circulatory system transports large volumes of fluid to all parts of our bodies.
It is an example of a **mass flow system**.

Our circulatory system consists of:

- **blood** – the fluid that is transported through the system,
- **blood vessels** – the tubes that carry the blood,
- a **heart** – to pump the blood through the network of blood vessels.



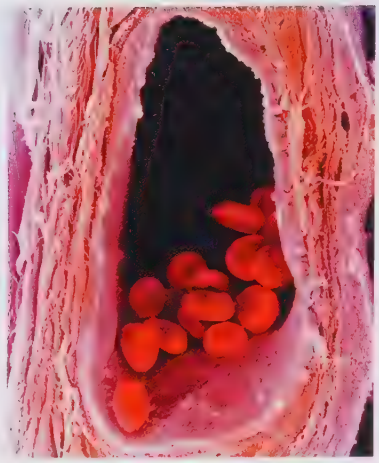
The human circulatory system

Closed or open?

Animals such as snails and insects have **open blood systems**.
The blood does not flow through blood vessels.
It is pumped out of the heart into large spaces in the body cavity.
The blood comes into close contact with the tissues and exchange of materials takes place.
The blood then returns to the heart.

The blood of an insect does ***not*** transport oxygen.
Can you remember how oxygen gets to the tissues of an insect?

In a **closed circulation system**, the blood flows through vessels.



SEM of a vein (×1400)

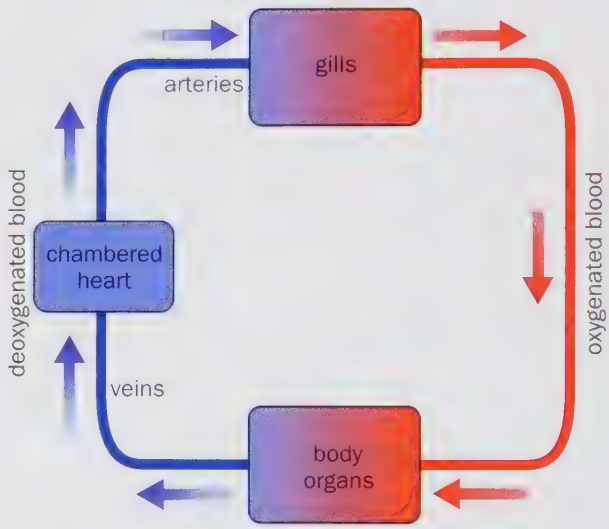
Single or double?

Fish have a **single circulation system**.

The heart pumps deoxygenated blood to the gills.
Oxygenated blood is then carried to the tissues.
Deoxygenated blood returns to the heart.

So how many times does the blood pass through the heart of a fish in ***one*** circuit of the body?

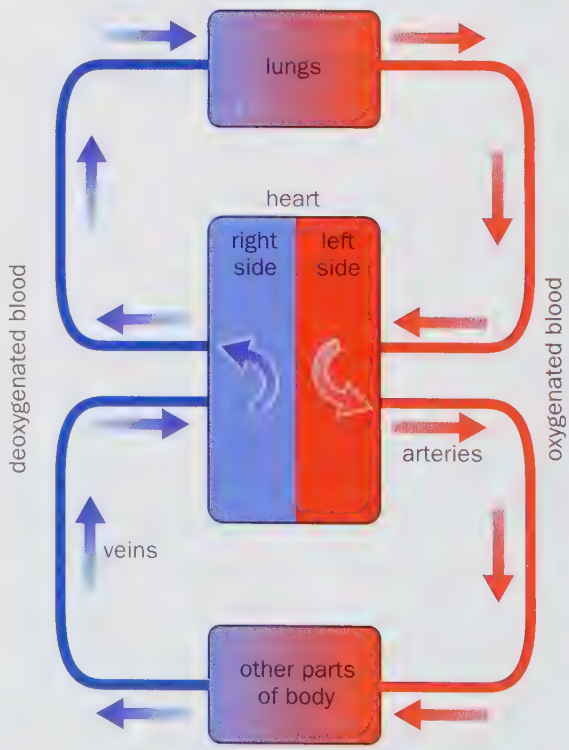
You can see from the diagram that the blood passes through the heart only ***once*** in one circuit of the body.
That's why it is called a ***single circulation***.



We have a **double circulation system**.

The right side of the heart pumps deoxygenated blood to the lungs.
Oxygenated blood then returns to the left side of the heart.
The left side of the heart pumps the oxygenated blood to the tissues.
Deoxygenated blood then returns to the right side of the heart.

So blood passes through the heart ***twice*** in each circuit of the body: once through the right side and once through the left side.
This is called a ***double circulation***.



In mammals, the flow of blood is maintained by:

- a muscular heart, which pumps blood out through the arteries to the capillaries,
- rhythmical contractions of the muscle in the thick-walled arteries, which can be felt as a pulse,
- contraction of the body muscles during normal movements, which help to squeeze blood along the thin-walled veins. Veins also have **semi-lunar valves** to prevent the blood from flowing backwards.
- breathing in creates a negative pressure inside the thorax, which helps to draw blood towards the heart.

Blood vessels

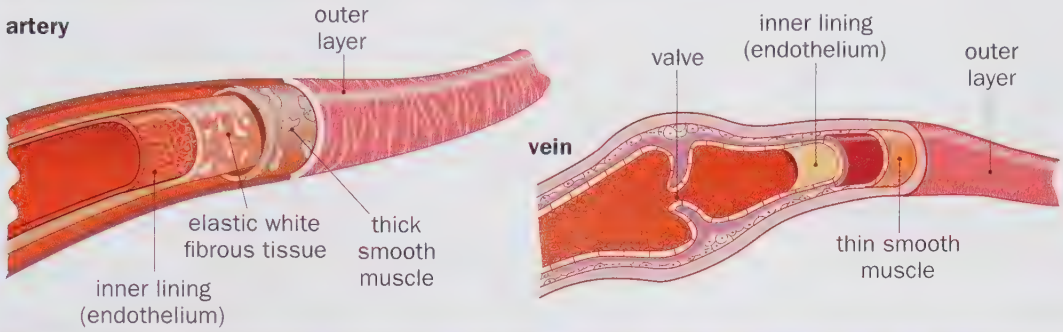
There are three main types of blood vessels:
arteries, **veins** and **capillaries**.

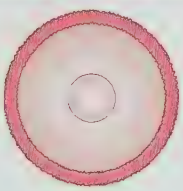
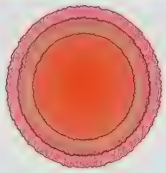
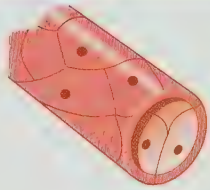
Arteries carry blood ***away*** from the heart. They branch to form smaller **arterioles**. Arterioles sub-divide into tiny, thin-walled capillaries. Capillaries form a branching network through a tissue. They allow rapid diffusion of materials between the blood and the cells. Capillaries join up to form **venules**. Venules then join up to form larger veins.

ARTERIES
↓
ARTERIOLES
↓
CAPILLARIES
↓
VENULES
↓
VEINS

The walls of arteries and veins are made up of three main layers:

- a thin inner lining of epithelial cells,
- a middle layer made up of elastic tissue and smooth muscle,
- an outer layer of collagen fibres and elastic tissue.

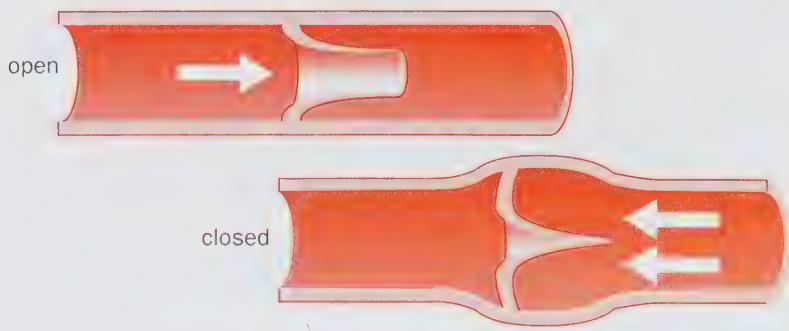


Arteries	Veins	Capillaries
a) 	b) 	c) 
carry blood away from the heart	carry blood back to the heart	link up arteries and veins in the tissues
thick muscular walls	thin muscular walls	no muscle: wall made up of one cell thick **endothelium**
lots of elastic tissue in wall	little elastic tissue in wall	no elastic tissue present
relatively small lumen	relatively large lumen	small lumen – just large enough for a red blood cell to squeeze through
blood under high pressure	blood under low pressure	pressure falls as blood passes along capillary
blood flow is rapid	blood flow is slow	blood flow is slowing down
blood flows in pulses	no pulse	no pulse
no valves	valves prevent backflow of blood	no valves

The elastic tissue in the artery wall allows the vessel to 'give' as blood surges through. So the artery wall first stretches as a result of the high blood pressure, before an elastic recoil of the wall pushes the blood on its way. This swelling can be felt as a pulse where arteries travel near the surface of the skin.

How the semi-lunar valves work in a vein:

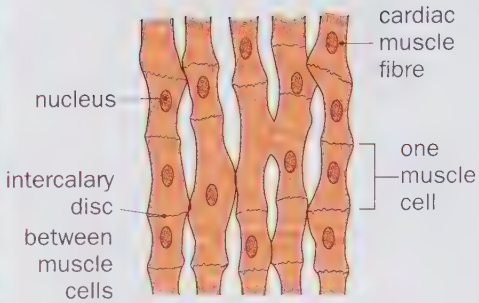
- The blood has enough pressure to force the valves open as it flows towards the heart.
- Backflow of blood causes the valves to close.



The action of semi-lunar valves

Heart structure

Your heart is made up of mainly **cardiac muscle**.
Cardiac muscle tissue is made up of muscle fibres.
Each muscle fibre is made of interconnecting muscle cells.
Each muscle cell is joined to the next by **intercalary discs**.
These allow the rapid spread of impulses through the tissue from cell to cell.
The heart is often described as being 'myogenic'.
This means that it can rhythmically contract and relax of its own accord throughout a person's life.



The structure of cardiac muscle

The heart is really two pumps side by side.
The right side of the heart pumps deoxygenated blood to the lungs.
The left side of the heart pumps oxygenated blood to the rest of the body.
Each side of the heart is kept completely separate, and so the deoxygenated blood and the oxygenated blood do not mix.

On each side of the heart there are two chambers.
The upper chambers are called **atria** (singular **atrium**).
The ***right atrium*** receives blood from the **vena cava**.
The ***left atrium*** receives blood from the **pulmonary veins**.

When the atria contract, blood passes into the lower chambers, called **ventricles**.
When the ***right ventricle*** contracts, it pumps blood out into the **pulmonary arteries**.
When the ***left ventricle*** contracts, it pumps blood out into the **aorta**.

The thickness of the walls of each chamber of the heart is related to the distance that it has to pump blood.

Why do you think that the atria have less muscular walls than the ventricles?

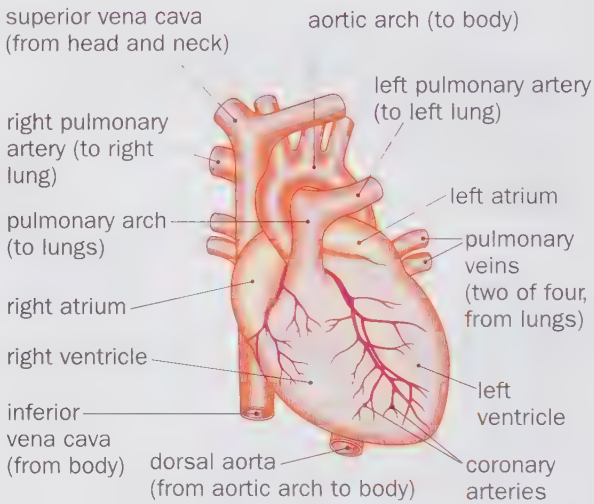
The two atria only have to pump blood a short distance down to the ventricles.
The ventricles have much thicker walls because they have to develop enough pressure to force the blood further.

Why do you think the left ventricle has a more muscular wall than the right ventricle?

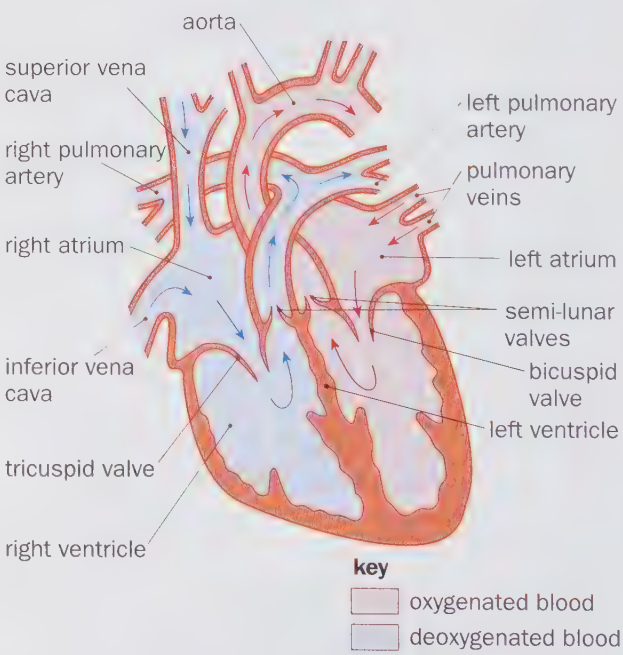
The right ventricle has only to force blood to the lungs, which are either side of the heart.
The left ventricle has to pump blood all round the body.

Two sets of valves are present in the heart to keep the blood flowing in one direction.

- The **atrio-ventricular valves** are found on each side, between the atria and the ventricles.
  They prevent the backflow of blood into the atria when the ventricles contract.
  On the right side, the **tricuspid valve** has three flaps.
  On the left side, the **bicuspid valve** has two flaps.
- The semi-lunar valves are found at the base of the pulmonary artery and the aorta.
  These valves close to prevent the backflow of blood into the ventricles when they relax.



External structure of the heart



Internal structure of the heart

The cardiac cycle

The **cardiac cycle** describes the sequence of events in one heartbeat. It is described in terms of alternate contractions (**systole**) and relaxations (**diastole**).

How long would each cardiac cycle last for a heart beating 75 times a minute?
Each cardiac cycle would last $\frac{60}{75} = 0.8$ seconds.

The valves in the heart respond to pressure changes during a cardiac cycle.
The noise of the blood when the sets of valves close makes the sound of your heartbeat (lub-dub).

There are ***three*** main stages to the cardiac cycle.

Atrial systole

- The heart is full of blood and the ventricles are relaxed.
- Both the atria contract and the blood passes down to the ventricles.
- The atrio-ventricular valves open due to the pressure of blood against them.
- 70% of the blood flows ***passively*** down to the ventricles so the atria do not have to contract a great amount.

Ventricular systole

- The atria relax.
- The thick muscular walls of the ventricles contract, forcing blood out of the heart into the pulmonary artery and the aorta.
- The pressure of blood against the atrio-ventricular valves causes them to shut, preventing blood going back into the atria. This produces the first part of the heart sound 'lub'.
- The pressure of blood against the semi-lunar valves opens them.
- The pulmonary artery then carries deoxygenated blood to the lungs and the aorta carries oxygenated blood to the different parts of the body.

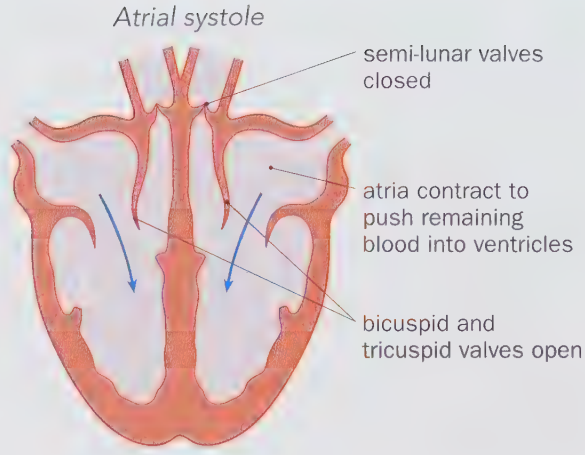
Diastole

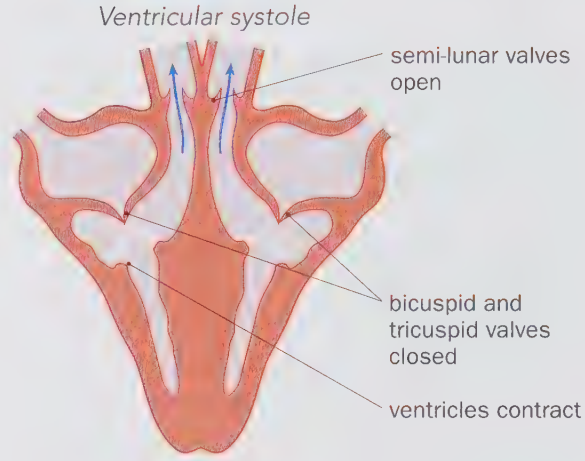
- The ventricles relax.
- The pressure inside the ventricles drops below that in the arteries.
- Blood under high pressure in the arteries causes the semi-lunar valves to shut, preventing blood from going back into the ventricles. This produces the second part of the heart sound 'dub'.
- During diastole, all the muscle in the heart relaxes.
- Blood from the vena cava and pulmonary veins enters the atria. The whole cycle starts again.

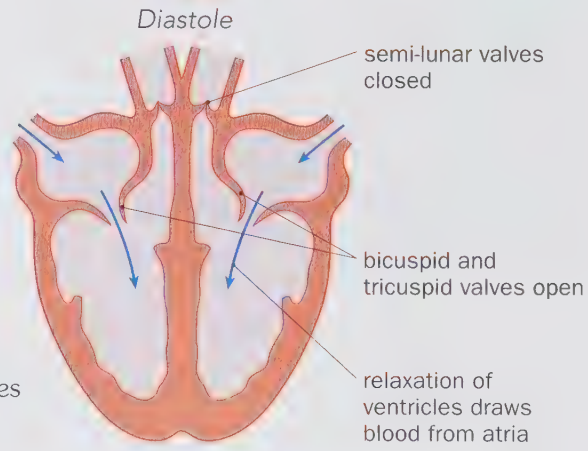
Look at the graph showing the pressure changes in the left atrium, the left ventricle and the aorta during a cardiac cycle.

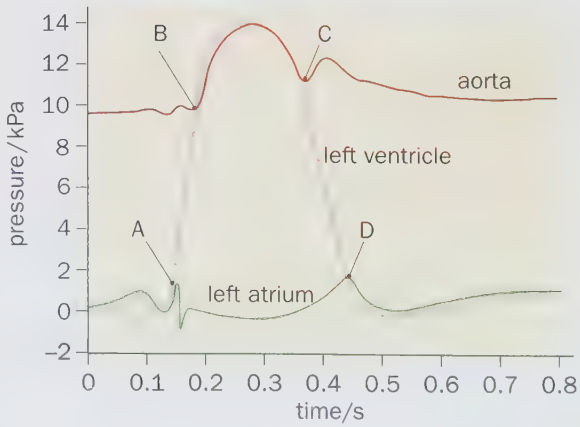
a) Calculate how many complete cardiac cycles there would be per minute.

b) Explain why the letters on the graph match the following events:
   i) semi-lunar valves open (B),
   ii) atrio-ventricular valves close (A),
   iii) semi-lunar valves close (C),
   iv) atrio-ventricular valves open (D).









Control of the heartbeat

As you know, the heart is myogenic: that is, it can contract and relax without having to receive impulses from the nervous system.

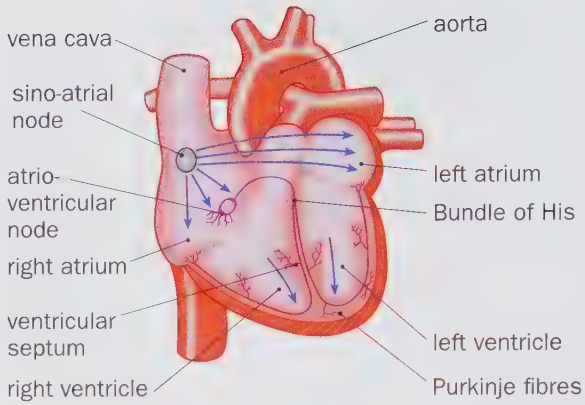
The cardiac cycle is started by specialised cardiac muscle tissue in the wall of the right atrium called the **sino-atrial node** (**SAN**). You may have heard it called the '**pacemaker**'.
The cells of the SAN set the rhythm at which all the other cardiac muscle cells beat, and so can control the speed of the cycle.

The SAN sends out electrical impulses to the rest of the atria. These impulses spread out in a wave of electrical activity (depolarisation) over the atrial walls. The cardiac muscle in the walls of both atria contract in rhythm with the impulses from the SAN. So both right and left atria contract at the same time.

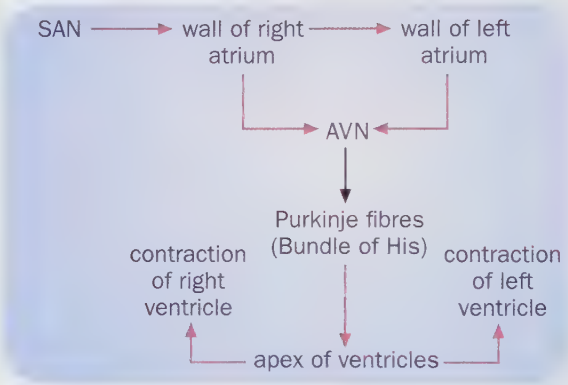
The electrical impulses do not pass down to the ventricles.
It is important that the muscles of the ventricles do not start to contract until the muscles of the atria have finished contracting.
Collagen fibres prevent the electrical impulses from passing through the heart wall from atria to ventricles.
The delay ensures that the ventricles do not start to contract before they fill with blood.

So what causes the ventricles to contract?

A second node, the **atrio-ventricular node** (**AVN**), picks up the impulses that have passed through the atrial muscle.
The AVN responds by generating its own electrical impulses, which travel along specialised muscle fibres called **Purkinje fibres**.
These fibres were discovered by a man called Purkyne, so either spelling is correct.
The fibres in the right and left ventricle walls are together known as the **Bundle of His**.
The impulses are carried rapidly to the apex of the ventricles.
This causes the cardiac muscle in each ventricle to contract simultaneously, from the bottom up.
So blood is squeezed up and out through the arteries.



Controlling the heartbeat



An **electrocardiogram** (**ECG**) can be used to detect changes in the electrical activity of the cardiac cycle. Electrodes are taped to the patient's chest and connected to a monitor that produces the ECG trace (see page 177 for examples).

Bradycardia is when the resting heart rate is less than 60 beats per minute.
This in a healthy athletic person may be 'normal'. But a slow heartbeat may be abnormal in cases such as hypoglycaemia, brain injury or drug abuse.
Tachycardia is when the resting heart rate exceeds 100 beats per minute. Its causes may include stress, fright, illness or exercise. So it can be related to certain circumstances when a person experiences changes, for example shock.
An **ectopic heartbeat** happens when cells from a part of the heart, other than the SAN, create an electrical discharge. These changes lead to extra or skipped heartbeats. They are mostly harmless and the heartbeat is otherwise normal.

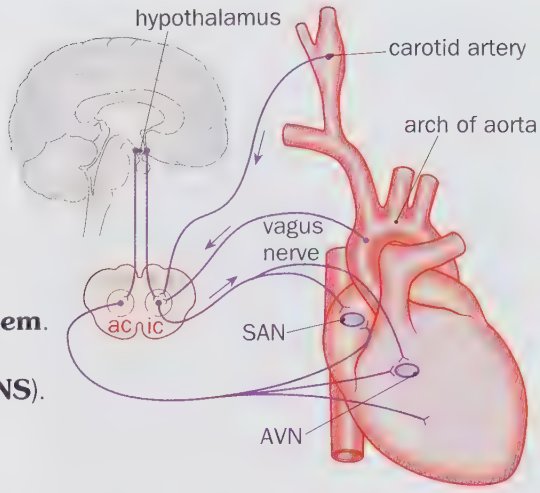

An electrocardiogram (ECG) producing an ECG trace

Modifying the heartbeat

You have seen that the heartbeat is started off and controlled by the heart itself. But what happens when you exercise?
Cardiac output can be adjusted to meet the varying needs of the body.

cardiac output = heartbeat rate × stroke volume

(Stroke volume is the volume of blood forced out of the heart by each muscular contraction.)
Changes in cardiac output are controlled by the **autonomic nervous system**.
There are two distinct parts to the autonomic nervous system:
the **sympathetic** (**SNS**) and the **parasympathetic nervous systems** (**PNS**).



Stimulation of the heart by the SNS ***increases*** cardiac output by:

- increasing the heartbeat rate,
- increasing the stroke volume.

Stimulation of the heart by the PNS ***decreases*** cardiac output by:

- decreasing the heartbeat rate,
- decreasing the stroke volume.

These two opposing systems work on a negative feedback principle, involving two centres in the medulla of the brain:

- The **cardiac acceleratory centre** is linked by the sympathetic nervous system to the SAN.
- The **cardiac inhibitory centre** is linked by the **vagus nerve** of the parasympathetic nervous system to the SAN, AVN and the Bundle of His.

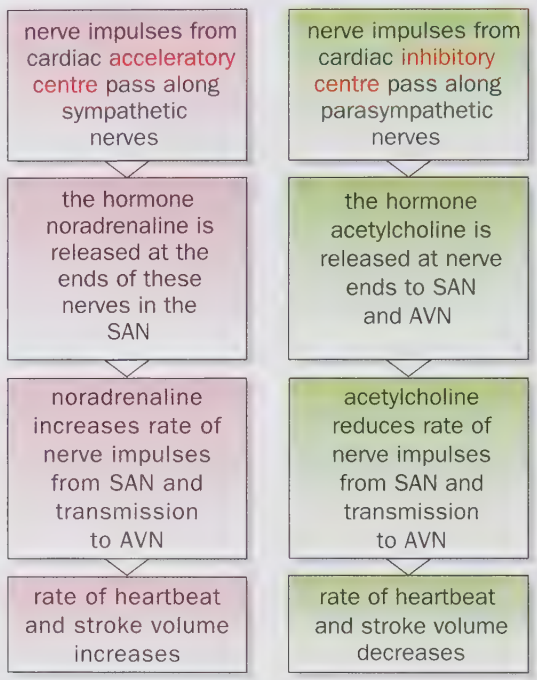
So what determines which of these cardiac centres is stimulated?
Well, carbon dioxide concentration and pH certainly have an effect.

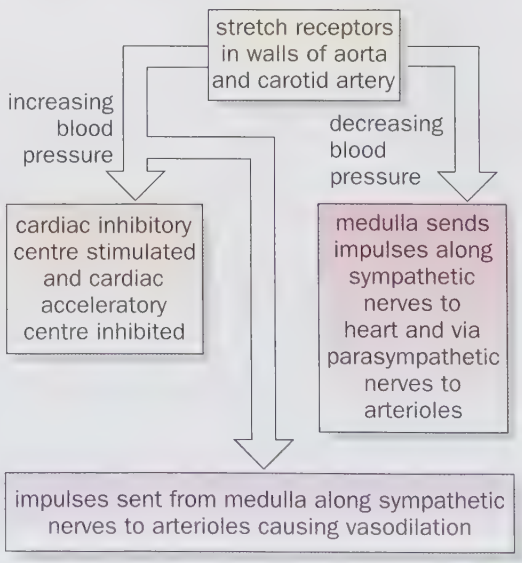
During vigorous exercise, the carbon dioxide level in the blood increases as a result of increased respiration.
This causes a lowering of blood pH (it becomes more acidic).

Sensory receptors in the **carotid body**, located on the carotid artery, detect this change.
They send nerve impulses to the cardiac acceleratory centre, resulting in an increase in cardiac output.
Blood flow to the lungs increases and so the extra carbon dioxide is removed.

If a fall in carbon dioxide level (a rise in pH) is detected by the carotid receptors, they send impulses to the cardiac inhibitory centre, resulting in a decrease in cardiac output.

Stretch receptors and **pressure receptors** in the walls of the aorta and carotid artery can detect changes in the blood flow to them, due to increased blood pressure.
If the blood pressure in the arteries is high, the stretch receptors are stimulated and send nerve impulses to the cardiac inhibitory centre.
This sends nerve impulses along the vagus nerve to the heart to reduce cardiac output.
If blood pressure is low, the stretch receptors trigger nerve impulses to the cardiac acceleratory centre, increasing cardiac output.

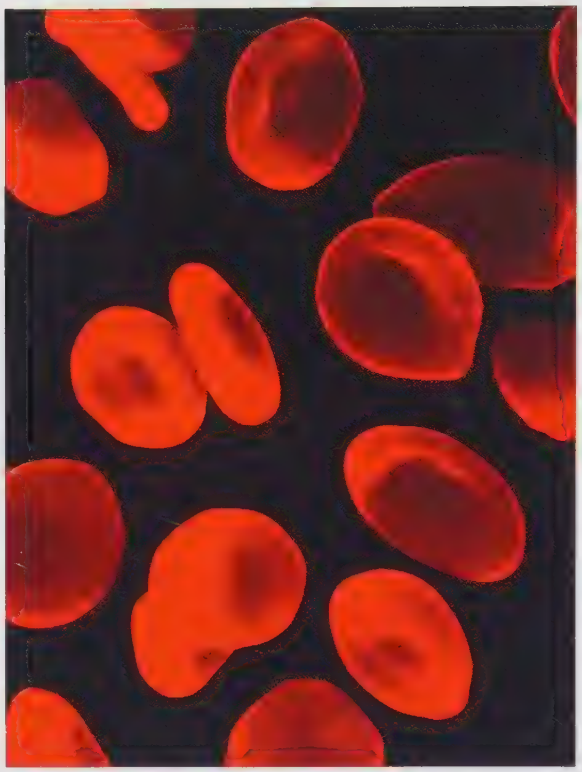




The blood

The blood is the main transport medium in your body. But it has several other functions as well.

- It defends the body against disease.
- It maintains diffusion gradients. For instance, it transports respiratory gases to and from the alveoli and it removes absorbed food from the villi of the small intestine.
- It acts as a buffer. Many of the blood proteins are able to neutralise excess acid or alkali and so keep the pH of the blood constant.
- It provides pressure for such processes as the formation of tissue fluid and filtration by the kidneys.
- It distributes heat around the body.



Blood plasma

About 55% of your blood is a liquid and about 45% is made up of cells.
The pale yellow liquid fraction is called **plasma**.
The composition of the plasma (its pH and salt concentration) is regulated by the kidneys.

Plasma contains:

- ***plasma proteins***, such as albumins (for the osmotic balance of the blood), antibodies (for immunity) and clotting factors such as fibrinogen,
- ***absorbed food molecules***, such as glucose, amino acids and fatty acids,
- ***excretory waste products***, such as carbon dioxide, urea and uric acid,
- ***hormones***, ***salts*** and ***heat***.

If you leave a sample of blood to stand the cells separate from the plasma

White blood cells

These are sometimes called **leucocytes** and there are at least five different types.
What they have in common is that they protect the body from disease-causing organisms as part of the immune system.

White blood cells are larger but much less abundant than red blood cells.
They all have a nucleus and they are spherical or irregular in shape.

The two best known types are **lymphocytes** (made in the lymph nodes and responsible for antibody production) and **neutrophils** (phagocytic cells made in the red bone marrow that are able to engulf microbes and cell debris).

White blood cells and their role in the immune system are dealt with in greater detail in Chapter 16.

SEM of human red and white blood cells and platelets

Red blood cells

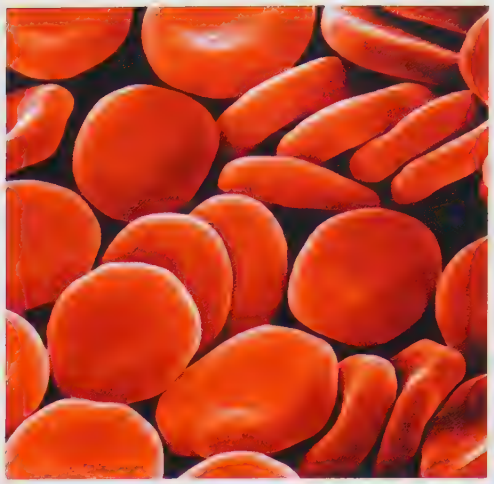
Red blood cells are also known as **erythrocytes**.
They transport oxygen from the lungs to the tissues.
Red blood cells contain the pigment **haemoglobin**, which combines with oxygen to give **oxyhaemoglobin**.

Packaging the haemoglobin inside red blood cells rather than dissolving it in the plasma means that the water potential of the blood is not affected.

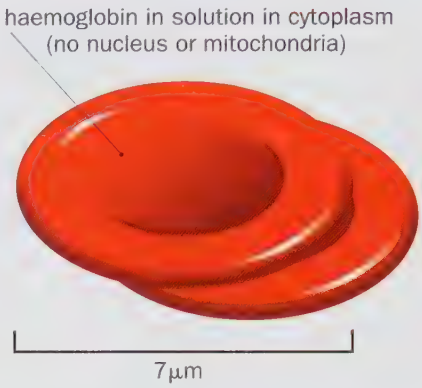
Unlike white blood cells, red blood cells do not have a nucleus.
This makes more room for the haemoglobin in the cell, and so increases the amount of oxygen that each red blood cell can carry.

Red blood cells are shaped like biconcave discs, a bit like doughnuts that have not had their centres taken out.
This disc shape gives a large surface area to volume ratio.
This means that there is a lot of membrane over which gas exchange can occur when loading and unloading oxygen.
Imagine a red blood cell with the same volume but in the shape of a sphere.
It would provide a far lower surface area to volume ratio.

Red blood cells are quite small (roughly half the size of white blood cells).
This means that no molecule of haemoglobin is far from the cell-surface membrane and its source of oxygen.
In fact, red blood cells can just about squeeze through a capillary.
This slows down the flow of red blood cells, making gas exchange in the capillaries more efficient because the red blood cells are there longer.
In the embryo, red blood cells are made by the liver, but this function is taken over by the red bone marrow soon after birth.
The average red blood cell only lasts for about 120 days, because it lacks a nucleus and other organelles needed to maintain the cell.



SEM of red blood cells

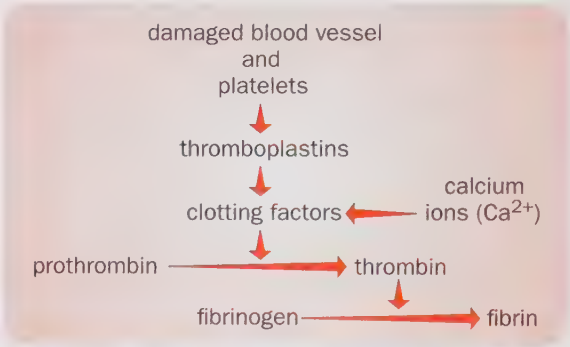


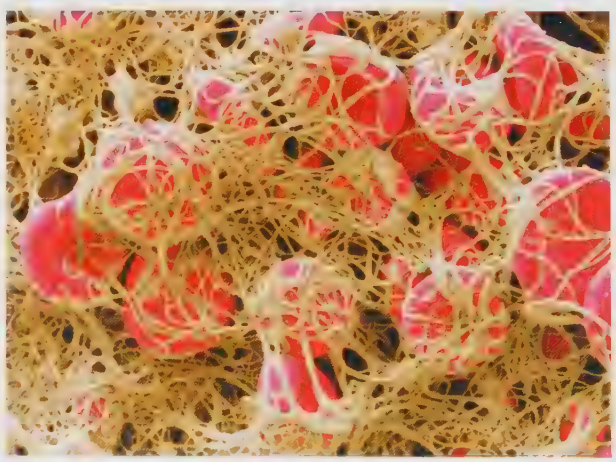
Platelets

Platelets are small cell fragments consisting of cytoplasm surrounded by the cell-surface membrane.
They do not have a nucleus and are only about 3 μm in diameter.
They are made in the bone marrow and last about 6 or 7 days.

The main role of platelets is in blood clotting.
Injury to the lining of a blood vessel exposes collagen fibres.
The platelets stick to these and swell up, releasing chemicals called **thromboplastins.**

The thromboplastins attract several plasma proteins called **clotting factors** to the site of the injury.
These set off a cascade effect in the presence of calcium ions.
The inactive plasma protein **prothrombin** changes to **thrombin**.
Thrombin converts another plasma protein, **fibrinogen**, to its insoluble form, **fibrin**.
Fibrin forms a mesh of threads, which trap the red blood cells, so helping to block the cut.
These dry to form a clot, preventing entry of bacteria and further loss of blood, and allowing the wound to heal.





Red blood cells caught up in a mesh of fibrin threads

Oxygen transport

Red blood cells contain the pigment haemoglobin.
You should remember that haemoglobin is a conjugated protein, because it is attached to a prosthetic group.
In this case the prosthetic group is called haem, and it contains iron
There are four haem groups in each haemoglobin molecule and each one can bind to an oxygen molecule.

$$\underset{\text{haemoglobin}}{Hb} + \underset{\text{oxygen}}{4O_2} \underset{\text{in tissues}}{\overset{\text{in lungs}}{\rightleftharpoons}} \underset{\text{oxyhaemoglobin}}{HbO_8}$$

The **partial pressure** of oxygen (pO_2) is a measure of the oxygen concentration.
The greater the concentration of dissolved oxygen, the higher its partial pressure.

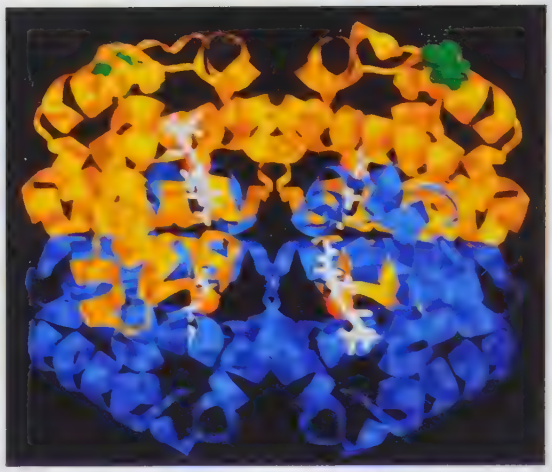
Red blood cells pick up oxygen in the dense network of capillaries that cover the alveoli of the lungs.
Here the partial pressure of oxygen is high and the haemoglobin becomes ***saturated*** with oxygen.
The cells carry the oxygen as oxyhaemoglobin to the respiring tissues.
Here the partial pressure is low, since oxygen is continually being used up in respiration.
Under these conditions, oxyhaemoglobin gives up its oxygen to the respiring cells. We say that it **dissociates**.

So the properties of haemoglobin ensure that at high partial pressures of oxygen, it combines with large amounts of the gas.
And at low partial pressures of oxygen, it combines with very little.

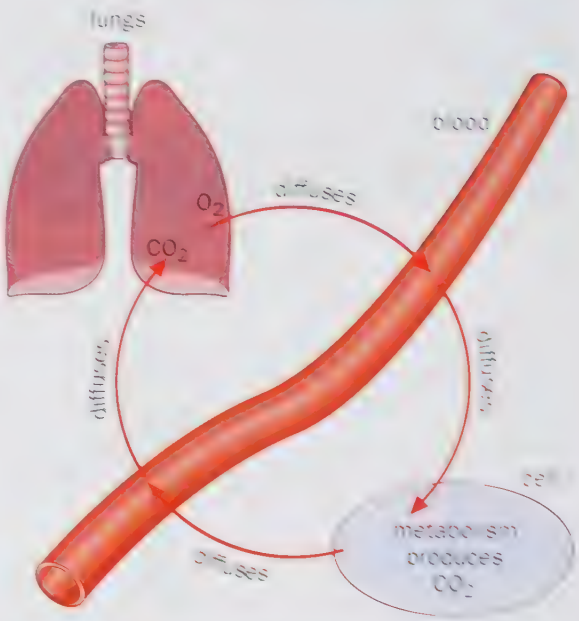
Samples of haemoglobin can be exposed to different partial pressures of oxygen.
The amount of oxygen combining with haemoglobin at the different partial pressures can then be estimated.
The percentage saturation of each sample can be plotted against the partial pressure to give an **oxygen dissociation curve**.

Look at the graph.
Can you see that haemoglobin becomes almost fully saturated with oxygen at high partial pressures (like the conditions in the lungs).
Oxyhaemoglobin dissociates under conditions of low partial pressures (like the conditions in the respiring tissues, which are using up oxygen in respiration).

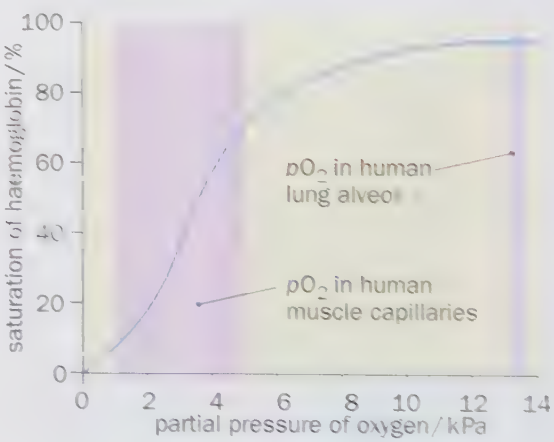
So why are oxygen dissociation curves S-shaped?
Remember that each molecule of haemoglobin has four haem groups.
When the first oxygen molecule combines with the first haem group, the shape of the haemoglobin molecule becomes distorted.
This makes it easier for the other three oxygen molecules to bind with the other haem groups.



Computer simulation model of haemoglobin

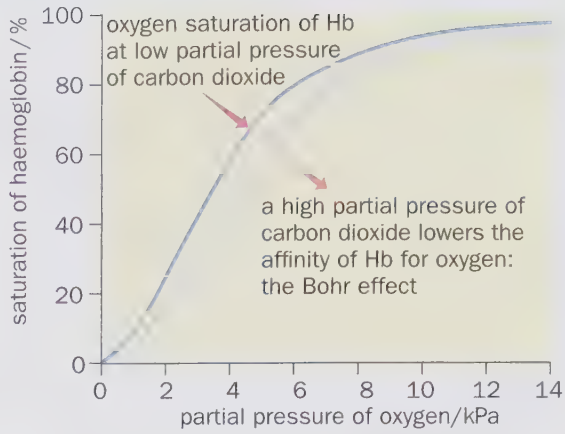


Gas exchange and transport



The Bohr effect

Haemoglobin picks up oxygen in the lungs and delivers it to the tissues. In fact, it is even more efficient than the dissociation curve suggests, because the amount of oxygen carried by haemoglobin depends not only on the partial pressure of oxygen but also on the ***partial pressure of carbon dioxide***.

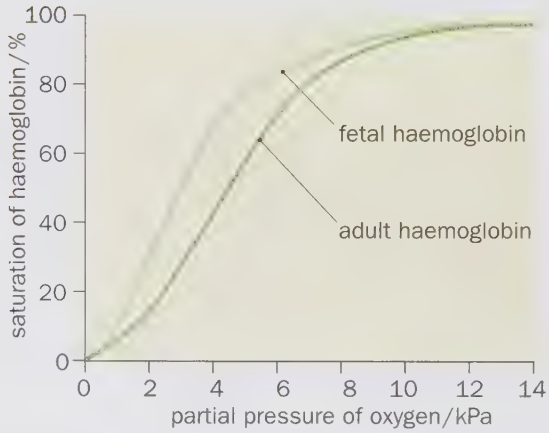


Look at the graph.
Can you see that, at higher carbon dioxide partial pressures, the oxygen dissociation curve moves to the ***right***?
This is known as the **Bohr effect**.
Higher partial pressures of carbon dioxide increase the dissociation of oxyhaemoglobin.
So, when oxyhaemoglobin reaches the tissues, the high partial pressure of carbon dioxide resulting from respiration makes oxyhaemoglobin respond by giving up its oxygen even more readily.
So oxyhaemoglobin releases its oxygen where it is most needed: to the actively respiring tissues.

The further a dissociation curve moves to the right, the more readily haemoglobin gives up its oxygen.
The further a dissociation curve moves to the left, the more readily haemoglobin picks up oxygen.

Fetal haemoglobin

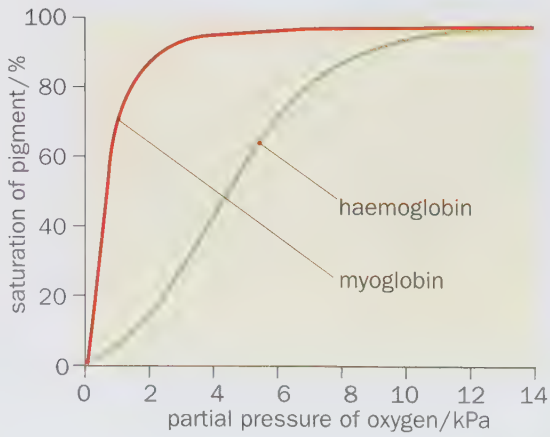
The developing fetus obtains oxygen from its mother.
The fetal blood and the mother's blood flow closely side by side in the placenta but never mix.
This allows materials to diffuse from the blood of the mother into the blood of the fetus and vice versa.



Look at the oxygen dissociation curve for fetal haemoglobin.
Can you see that it is to the ***left*** of that for adult haemoglobin?
How do you think this could be an advantage to the fetus?
The fetal haemoglobin combines with oxygen more readily than adult haemoglobin does.
We say that it has a ***higher affinity*** for oxygen.
So at the placenta the fetal haemoglobin can 'steal' oxygen from the mother's haemoglobin.

Myoglobin

There is an oxygen-binding molecule in muscle called **myoglobin**.
Oxymyoglobin is far more stable than oxyhaemoglobin.
It will only give up its oxygen at very low oxygen partial pressures.



Look at the dissociation curve for myoglobin.
Can you see that it is to the left of the haemoglobin curve?
This means that at each partial pressure of oxygen, myoglobin has a higher percentage oxygen saturation than haemoglobin.

This enables myoglobin to act as an oxygen store.
Usually the respiring muscle gets its oxygen from oxyhaemoglobin.
But if the oxygen partial pressure becomes very low (as a result of exercise), the oxymyoglobin gives up its oxygen.

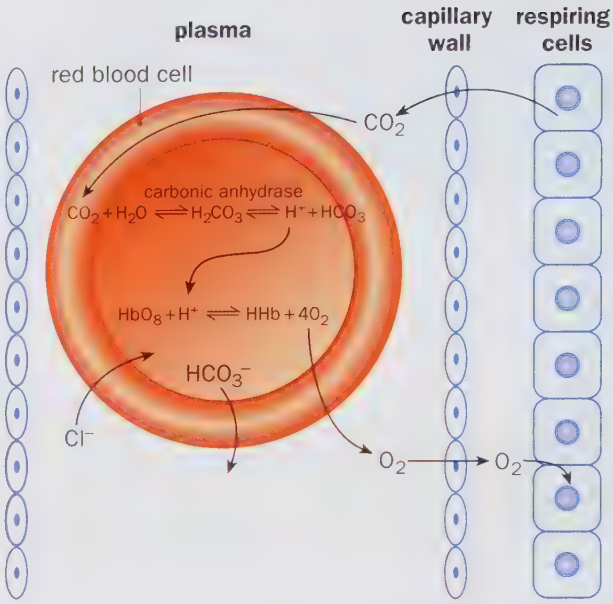
Carbon dioxide transport

Carbon dioxide is carried in the blood in ***three*** ways.

- About 5% is carried ***in solution*** in the plasma as carbon dioxide.
- About 10% combines with amino groups in the four polypeptides that make up the haemoglobin molecule. The compound formed is called **carbamino-haemoglobin**.
- About 85% is carried in the form of **hydrogencarbonate**.

The sequence of events in which hydrogencarbonate is formed is significant for a number of reasons and is worth looking at in detail.

- Carbon dioxide produced by the respiring tissues diffuses into the red blood cells in the tissue capillaries. As you have seen, some of it combines with amino groups to form carbamino-haemoglobin.
- The red blood cells contain an enzyme called **carbonic anhydrase**, which catalyses a reaction between the rest of the carbon dioxide and water to form carbonic acid.
- The carbonic acid dissociates into negatively charged hydrogencarbonate ions and positively charged hydrogen ions. The hydrogen ions tend to increase the acidity.
- The hydrogen ions combine with haemoglobin to give **haemoglobinic acid** (**HHb**). The hydrogen ions bind to amino acid side-chains in the haemoglobin molecule. This brings about a distortion of the molecule, which decreases its affinity for oxygen. So more oxygen is released to the tissues. (Remember the Bohr effect, where oxygen is given up under high partial pressures of carbon dioxide?)
- The build-up of hydrogencarbonate ions causes them to diffuse out of the red blood cell, leaving the inside of its membrane positively charged.
- In order to balance the electrical charge, chloride ions diffuse into the red blood cell from the plasma. This is known as the **chloride shift**.



Carbon dioxide transport in the plasma and red blood cell

Can you see that haemoglobin is acting as a buffer? It helps to maintain the blood pH by removing hydrogen ions from solution.

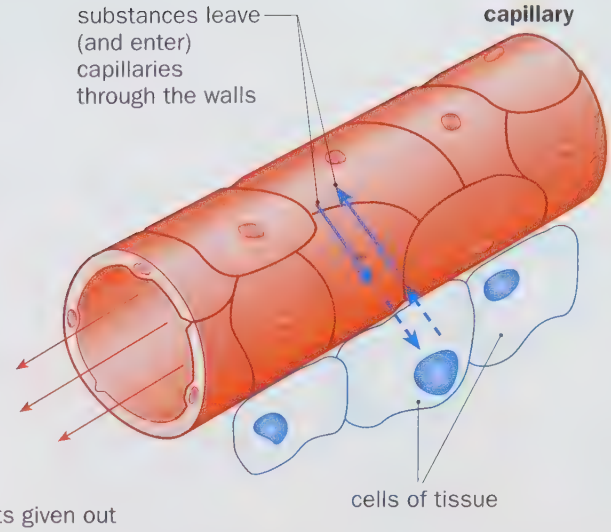
When the blood reaches the lungs, all the reactions described above are reversed.
Carbamino-haemoglobin breaks down to release carbon dioxide.
The hydrogencarbonate and hydrogen ions recombine, forming carbon dioxide molecules as the chloride shift is reversed.
The carbon dioxide diffuses out of the blood into the air in the alveoli.
The haemoglobin molecules are now free to pick up more oxygen and form oxyhaemoglobin.

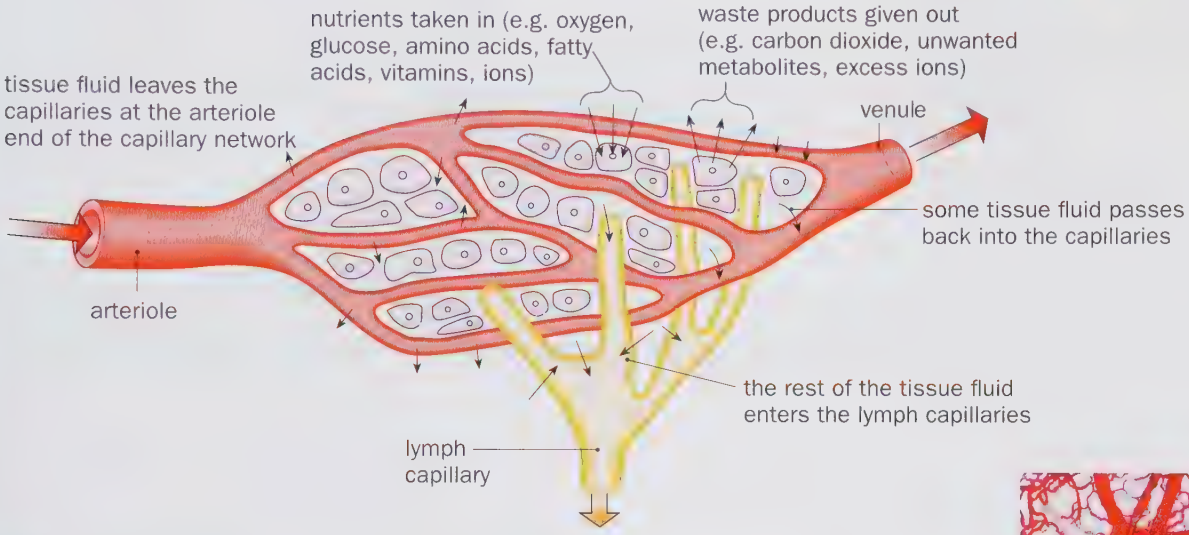
'OK folks, let's do the chloride shift!'

Exchange across capillaries

Capillaries are adapted to allow the exchange of materials between the blood and the cells.

- They have very thin, permeable walls, only one cell thick.
- They provide a huge surface area for exchange, because there are so many of them.
- Blood flows through them very slowly.
- The body cells are never far from a capillary.





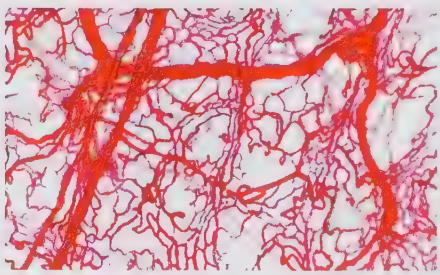
Formation of tissue fluid

As blood flows through the capillaries, some of the plasma passes out into the tissues.
This **tissue fluid** consists of plasma without the large plasma proteins, which are too big to pass through the capillary wall.
The tissue fluid 'bathes' the cells, supplying them with glucose, amino acids, fatty acids, salts and oxygen, whilst removing carbon dioxide and other waste materials from the cells.

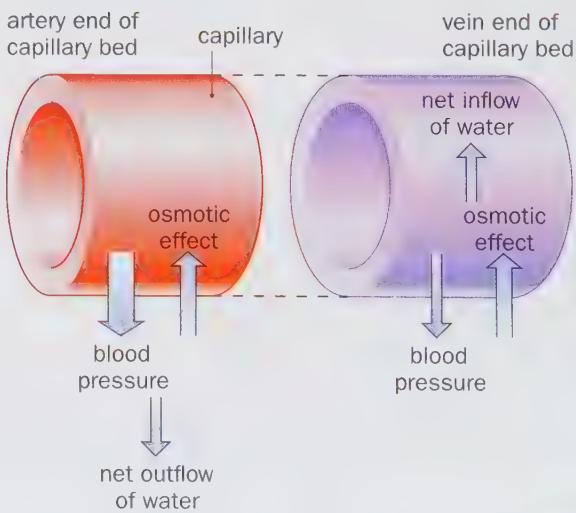
As blood enters the narrow capillaries from the arterioles, a build-up of blood pressure occurs.
This forces water through the capillary walls into the cells.

Other components of tissue fluid enter the cells from the capillary by diffusion or by active transport.
As fluid leaves the capillary, the blood pressure starts to fall.
As the blood has lost much of its solute cargo, it now has a higher concentration and lower water potential than the tissue fluid.
So water passes back into the capillary by osmosis down a water potential gradient.
Waste products leave the cells and enter the capillary by diffusion.

This ***net*** movement of tissue fluid back into the capillary can only happen when the pressure of the blood has dropped and is countered by the osmotic effect.



Capillaries in a frog's foot



Lymph

Not all of the tissue fluid returns to the blood capillary.
About one tenth of it enters a separate system of capillaries called the **lymph capillaries**.
The lymph capillaries are part of the **lymph system**.

Lymph capillaries have tiny valves that allow the tissue fluid to enter but won't let it leak out again.
Once inside the lymph system, the tissue fluid is called **lymph**.

So what's the difference between tissue fluid and lymph?

They both consist of plasma minus the large plasma proteins, but it's largely a matter of where they are found.

Tissue fluid surrounds the tissues.
Lymph is found only in the lymph system.

The tiny lymph capillaries join up to form **lymph vessels**.
These have a structure very similar to veins.
They are thin-walled and contain semi-lunar valves.

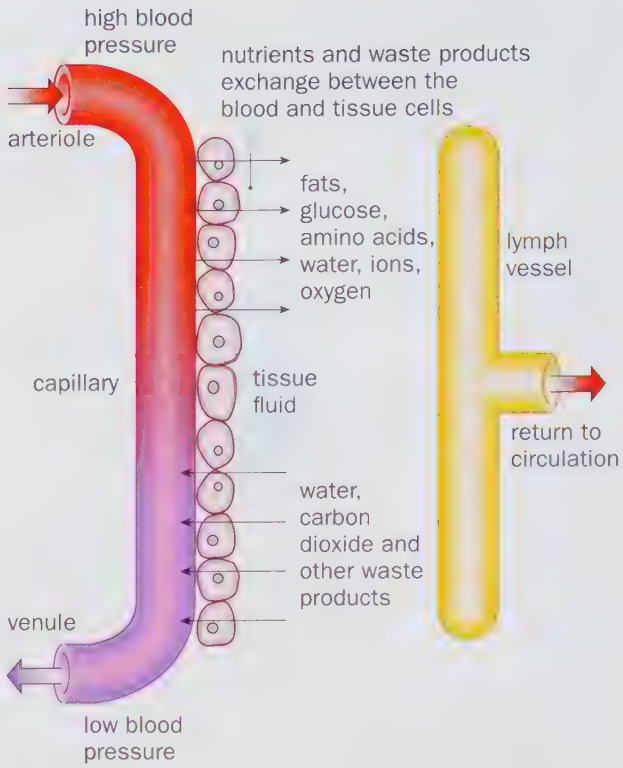
Perhaps not surprisingly, the flow of lymph is very slow.
It relies upon pressure from nearby muscles, the action of valves, and the negative pressure created in the chest when we breathe in, which helps to draw the lymph along.

Unlike blood, lymph is transported in one direction only, from the tissues towards the heart.
The smaller lymph vessels join up to form two large lymph vessels.
These empty the lymph into the **subclavian veins**, under the collarbones.
Here the lymph mixes with blood before joining the vena cava just before it enters the heart.

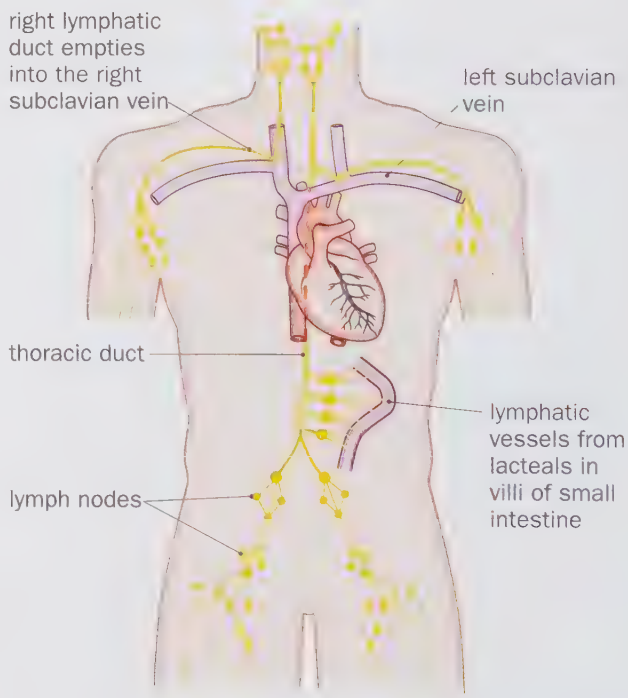
Lymph is a milky looking fluid.
It contains fats absorbed by the lymph capillaries in the villi of the small intestine.
These lymph capillaries are called **lacteals**.
The walls of lymph vessels are more permeable than the walls of blood capillaries, so large molecules such as fats can pass through them.

At intervals along the length of the lymph vessels are structures called **lymph nodes**, which are an important part of your defence system.

Lymphocytes are produced in the lymph nodes.
They have an important role in producing antibodies.
Lymphocytes are released from the lymph nodes and eventually find their way into the blood.
So the lymph system is an important part of the body's immune system (see Chapter 16).
Your lymph nodes often swell up if you have an infection.



The relationship between blood, tissue fluid and lymph at a capillary network



The lymph system

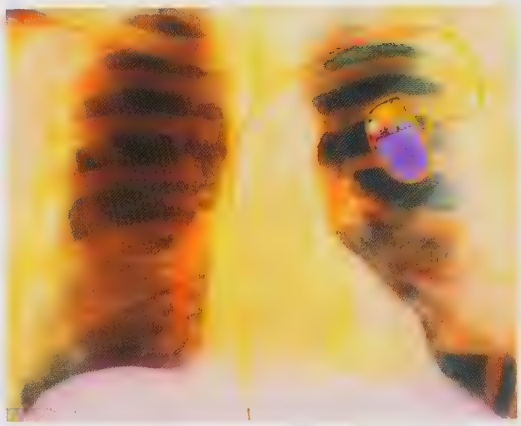
Biology at work: Pacemakers

As you know the cardiac cycle is controlled by a specialist group of cells in the wall of the right atrium known as the sino-atrial node (SAN). This is often called the **pacemaker** of the heart, and it works by sending out an electrical impulse that causes the atria to contract.

If the impulses from the SAN are disrupted then a patient can be fitted with an artificial pacemaker. This is a small box containing a battery, a computer circuit, a pulse generator, and leads to carry the impulses to the heart muscle. Almost all modern pacemakers work on demand. This means that they can be programmed to adjust their discharge rate in response to a body's needs. Pacemakers are usually inserted just under the skin near the collarbone on the left side of the chest.

Patients thought to be at risk of a heart attack may have both a pacemaker and an implantable **cardioverter defibrillator** (**ICD**). The ICD sends a larger impulse to the heart that effectively reboots the heart to get it pumping again.

Scientists hope that the next generation of pacemakers will be leadless and about the size of a small battery. These devices would be inserted directly into the heart itself.



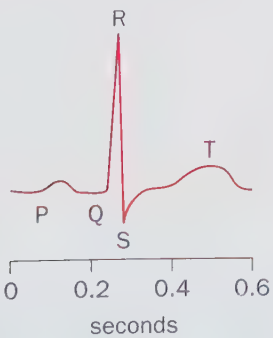
An X-ray of a pacemaker in situ

Biology at work: The electrocardiogram (ECG)

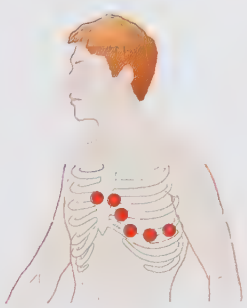
As you have already seen, the control of the heartbeat depends upon electrical activity. A variety of cardiac disorders can produce irregularities in this activity. An ECG is a useful diagnostic tool in that it can detect these irregularities.

Electrodes are taped at various positions on the body and the electrical activity of the heart is then displayed on a monitor.

The resultant trace can be compared with a normal ECG with its characteristic P, QRS and T waves.
The P wave shows atrial depolarisation, which is immediately followed by atrial contraction (systole). The QRS 'spike' shows depolarisation of the ventricles, which immediately precedes the contraction of the ventricles. The T wave shows repolarisation of the ventricles while they are in a state of relaxation.

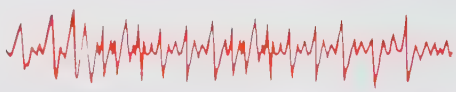


A normal ECG trace



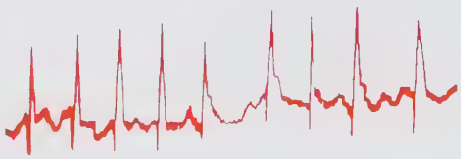
ECG lead positions

A normal ECG trace



ventricular fibrillation
the contractions of the ventricles are extremely irregular

complete heart block
the atria and ventricles are beating independently



atrial fibrillation
caused by the atria beating fast and irregularly

Some abnormal ECG traces

Biology at work: Treatment of hypertension

Constant high blood pressure or **hypertension** is harmful. It makes the heart work harder and the arteries to narrow. High blood pressure is linked to many cardiovascular diseases including stroke and heart attack. It can also damage the small blood vessels in the kidneys.

Category	Blood pressure systolic/diastolic (mmHg)
low blood pressure	below 90/60
ideal blood pressure	between 90/60 and 120/80
pre high blood pressure	between 120/80 and 140/90
high blood pressure	over 140/90

Blood pressure chart suitable for adults of any age

Symptoms

High blood pressure usually has no obvious symptoms and many people have it without knowing.
The only way to know if you have high blood pressure is to have your blood pressure measured, ideally every 12 months if you are an adult.

The lack of clear symptoms and the potential to cause death has led to hypertension being called the 'silent killer'.

Incidence

The risk of high blood pressure increases after the age of 35, and more than 25% of adults over 55 suffer from the condition.

Hypertension is often referred to as a 'disease of affluence' because the key risk factors are:

- obesity,
- excessive salt intake,
- excessive alcohol consumption and, in particular,
- smoking.

Treatment

Hypertension can be treated through lifestyle changes and, if necessary, drugs. A healthy diet, regular exercise, relaxation and stopping smoking will all help to lower blood pressure. If hypertension continues the patient will be offered medication, the type of which will depend upon their age.

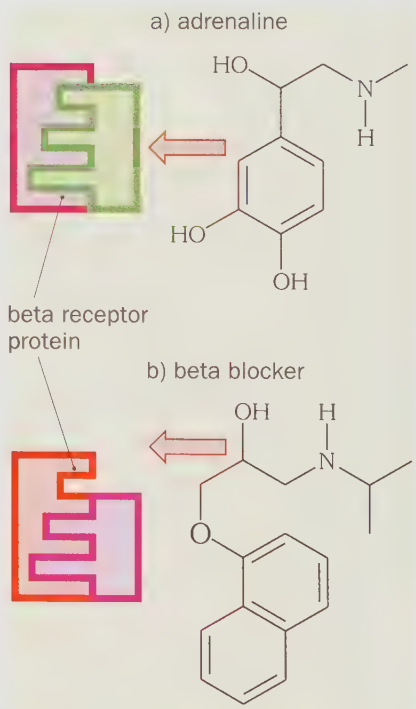
- ***Those under 55*** are usually offered an angiotensin converting enzyme (ACE) inhibitor or an angiotensin receptor blocker (ARB).
- ***Those over 55*** are usually offered a calcium channel blocker.

ACE inhibitors reduce the amount of water in the blood and widen the arteries. They block the body's ability to produce the chemical angiotensin II. When angiotensin II enters the bloodstream the blood vessels become narrower. So by blocking its production the blood vessels can relax and widen.

Calcium channel blockers work by reducing the contraction of the heart muscle and/or muscles in the walls of arteries. They have this effect because rising calcium levels in muscle cells are part of the mechanism that causes contraction.

Many patients receive a combination of different drugs, and a third example is **beta blockers**. These drugs work by relaxing the smooth muscle cells that control the size of blood vessels.
Beta blockers competitively inhibit the action of the hormones adrenaline and noradrenaline. These drugs bind with receptors on the cell-surface membrane of the heart muscle and the smooth muscle lining the blood vessels. As a result they bring about:

- dilation of the blood vessels,
- a reduction in heart rate and a decrease in blood pressure.



Adrenaline fits the receptor and activates it. Beta blocker also fits the receptor but blocks the access for adrenaline

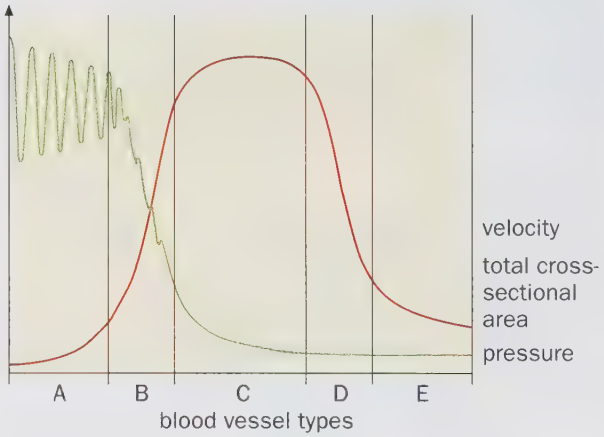
Summary

- The blood transports oxygen, carbon dioxide, dissolved food, waste materials and hormones around the body. The blood also provides immunity, distributes heat around the body and acts as a buffer.
- Arteries contain a lot of muscle and elastic tissue to maintain the flow of blood under high pressure.
- Veins have relatively thin walls. They have semi-lunar valves to prevent the backflow of blood.
- Capillary walls are only one cell thick, allowing exchange of materials to take place easily.
- Plasma and some white blood cells are forced out of the capillaries under pressure to form tissue fluid. Most tissue fluid passes back into the capillaries; the rest enters the lymph system.
- Blood is made up of liquid plasma, red blood cells, white blood cells and platelets.
- The red blood cells contain haemoglobin, which combines with oxygen in the lungs to form oxyhaemoglobin.
- The oxygen dissociation curve for haemoglobin shows that it becomes fully saturated with oxygen in the lungs and releases oxygen to the tissues.
- At high concentrations of carbon dioxide, oxyhaemoglobin responds by releasing more oxygen.
- Fetal haemoglobin and myoglobin have a greater affinity for oxygen than adult haemoglobin.
- Carbon dioxide is mainly carried in the form of hydrogencarbonate ions in the plasma.
- The heart consists of two muscular pumps. One pumps deoxygenated blood to the lungs where it picks up oxygen; the other pumps the oxygenated blood all around the body.
- There are three main phases of the heartbeat: atrial systole, ventricular systole and diastole.
- The heartbeat is initiated by the heart itself (myogenic). This involves the sino-atrial node, the atrio-ventricular node and the Bundle of His.
- The heartbeat can be modified by the cardiac acceleratory and cardiac inhibitory centres in the brain, which respond to sense receptors in the carotid body and stretch receptors in the large arteries.

Questions

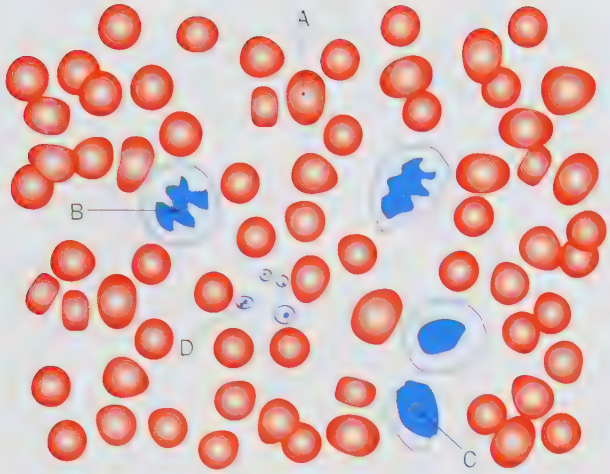
1 Copy and complete the following passage.
Blood consists of a pale yellow liquid called ___, in which are found a number of different kinds of cells. The plasma contains various proteins, such as albumins, which help to maintain the ___ ___ of the blood: ___, which are antibodies; and ___, which is involved in blood clotting. The red blood cells, or ___, transport ___, which combines with the conjugated protein ___. White blood cells, or ___, are of two main types: ___, which engulf bacteria and cell debris, and ___, which produce antibodies as part of the body's ___ response. ___ are cell fragments involved in the ___ of blood.

2 The graph shows the blood pressure, blood velocity and cross-sectional area of different types of blood vessels.



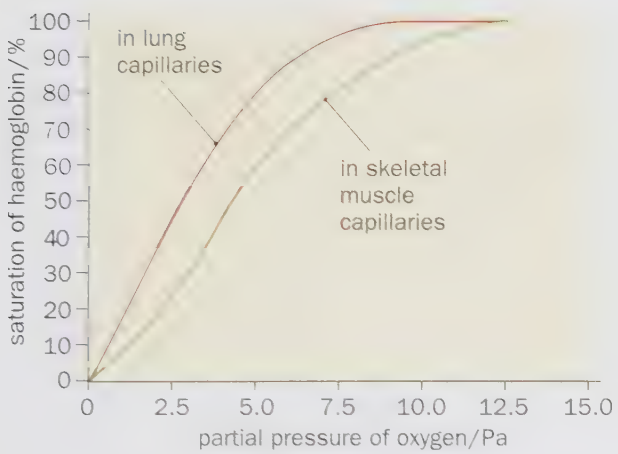
a) Name the blood vessels A, B, C, D and E.
b) Which of these vessels has the highest proportion of muscle tissue in its wall?
c) i) Explain the variations in blood pressure shown in vessel type A.
ii) What is the reason for the rapid drop in blood pressure in vessel type B?
d) What are the advantages of the relationship between blood velocity and cross-sectional area of vessel type C?
e) Suggest two reasons for the increase in blood velocity in vessel types D and E when the blood pressure is low.

3 Look at the diagram at the top of the next page of the blood of a mammal, magnified about ×1000.
a) What are the cells labelled A, B and C called?
b) Approximately how many red blood cells are there for every white blood cell?
c) Describe the main functions of cell types A, B and C.
d) In what process is structure D involved?
e) Outline the main stages of this process.

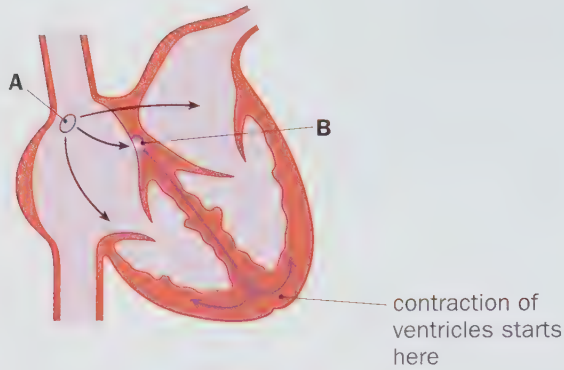


4 a) Explain how increased haemoglobin concentration can lead to increased performance in endurance events.

b) The graph shows the oxygen dissociation curve for haemoglobin as blood passes through capillaries in the lungs and skeletal muscle of an athlete. Explain how features of the oxygen dissociation curves for haemoglobin in the lungs and skeletal muscle benefit the athlete.



5 The diagram shows a vertical section through the human heart. The arrows indicate the direction of movement of electrical activity, which starts muscle contraction.



a) Name structure A.

b) Explain why each of the following is important for pumping blood through the heart:

i) There is a slight delay in the passage of electrical activity that takes place at point B.

ii) The contraction of the ventricles starts at the base.

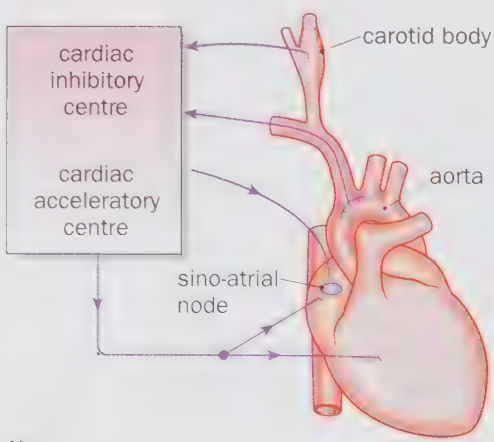
c) Describe how stimulation of the cardiovascular centre in the brain may result in an increase in heart rate.

6 The diagram shows some nerves associated with the heart and the blood system.

a) In which part of the brain are the cardiac inhibitory and acceleratory centres found?

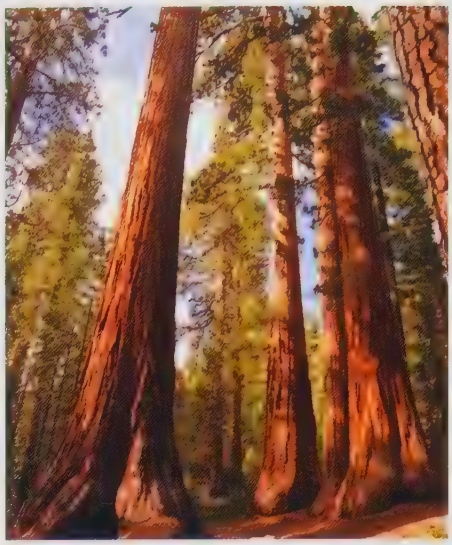
b) Give two stimuli, detected in the aorta and carotid body, that may result in a change of heart rate.

c) Suggest two ways in which stimulation of the heart by nerve X leads to an increase in the amount of blood pumped out of the ventricles.



11 Transport in plants

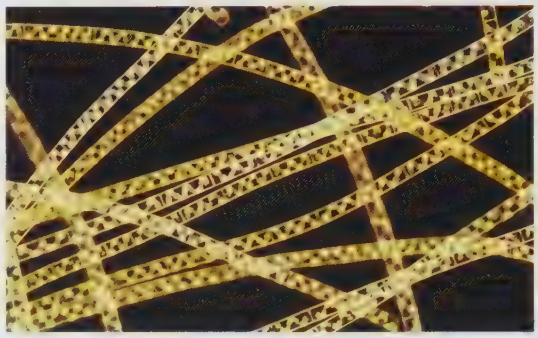
These North American redwoods can grow to a height of 60 m. So how does water get up to such a height from the roots? And how are the products of photosynthesis transported away from the leaves?



Try to imagine one of these giant redwoods on a summer's day. The leaves will be photosynthesising and will need carbon dioxide, water, nutrients and light.

As you have seen, carbon dioxide is able to diffuse into the leaves from the atmosphere through the stomata.
Water and mineral nutrients have to reach the leaves from the roots.
Photosynthetic products, such as sugars and amino acids, need to be transported away from the leaves to maintain concentration gradients.

In simple photosynthesising organisms such as algae, these processes take place by diffusion.
Spirogyra, for example, has most of its cells close to the surrounding water.



Spirogyra: a simple filamentous green alga

But in complex, multicellular plants, a **mass flow system** is needed. In larger plants diffusion would simply be too slow to supply:

- carbon dioxide for photosynthesis during daylight,
- oxygen for respiration,
- inorganic nutrients, such as nitrates and phosphates,
- sugars made during photosynthesis for those plant cells that cannot photosynthesise, such as root cells.

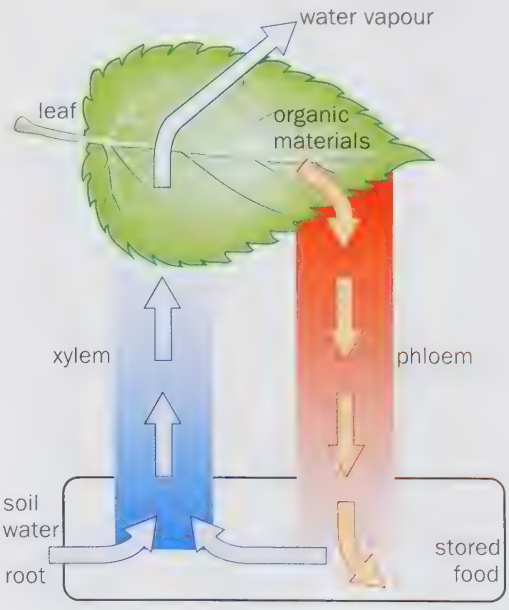
Plants are able to develop good transport tissue as they are made from a column of plant cells with strong, supporting cellulose cell walls.
If these cells lose their end-walls, they form tubes.
And this is exactly what happens.

Simple **parenchyma** cells either lose their end-walls altogether or the end-walls become perforated to form a system of tubes. There are two such systems:

- the **xylem** tissue, which transports water and mineral salts up the stem from the roots to the leaves,
- the **phloem** tissue, which transports the materials made in photosynthesis, for example sugars and amino acids, to all other parts of the plant.

In each case the walls of the tubes are further thickened by the addition of cellulose and **lignin**, a woody material.

So in plants there are ***two*** distinct transport tissues.

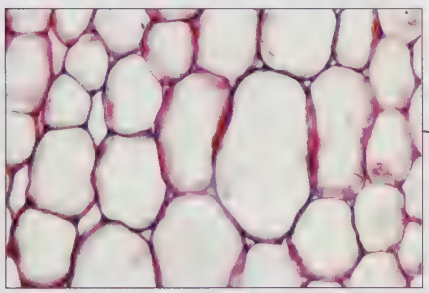


Tissues inside the stem

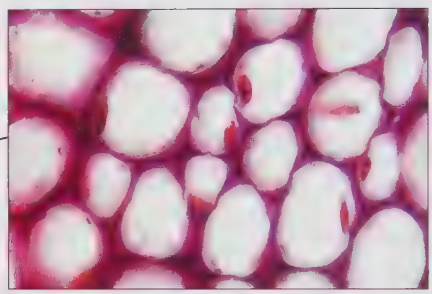
On page 142, we looked at the different tissues that make up the internal structure of a leaf.
A transverse section of a stem reveals a ring of **vascular bundles**.
Each vascular bundle consists of:

- a cap of **sclerenchyma** fibres for mechanical support,
- outer phloem tissue for transporting organic materials,
- inner xylem tissue for transporting water and mineral salts and also for mechanical support,
- a thin layer of tissue called the **cambium**, between the phloem and xylem.

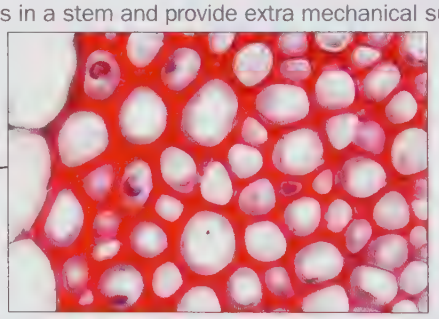
Cambial cells divide to cut off new phloem cells (secondary phloem) to the ***outside*** and new xylem cells (secondary xylem) to the ***inside***.
A region of dividing cells like this in a plant is called a **meristem**.
In older stems a complete ring of secondary xylem and secondary phloem forms to give additional mechanical support.



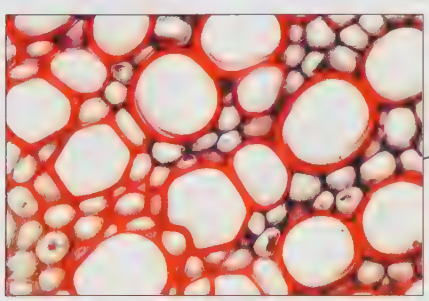
Parenchyma are unspecialised plant cells. They have unthickened walls and provide the packing around other tissues. They are able to store food and their turgidity provides support in non-woody stems.



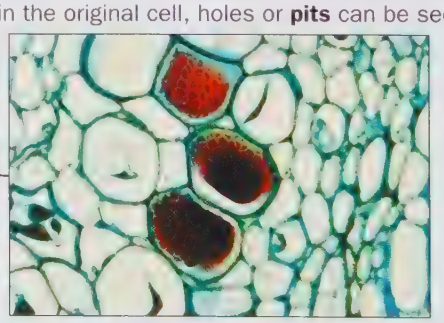
Collenchyma are living cells that have been reinforced by the addition of extra cellulose. They are often found below the epidermis in a stem and provide extra mechanical support.



Sclerenchyma have thickened walls due to the addition of lignin. These cells are dead since lignin is impermeable to gases and liquids. Sclerenchyma provides immense mechanical support in stems and leaves. In places where plasmodesmata were found in the original cell, holes or **pits** can be seen.



Xylem vessels transport water and mineral salts. Lignin is deposited in their wall to give greater mechanical support. (Details of xylem structure are on page 183)



Phloem is made up of large **sieve tubes** which transport organic materials and adjacent **companion cells**. (Details of phloem structure are on page 191)

Structure of xylem tissue

There are two main types of water-conducting tissue: ***vessels*** and ***tracheids***.

Xylem vessels form when parenchyma cells in a column lose their end-walls.
The walls of these tubes become strengthened by the addition of lignin.
This increased mechanical strength is important if the vessels are to withstand the strong pressures that occur during water transport.
As lignin is impermeable, materials cannot pass into xylem cells and so the protoplasm dies.
This means that the cells are hollow and there are no cell contents to restrict the flow of water.

No lignin is laid down where plasmodesmata were present in the original cell walls.
These non-lignified areas are known as **pits** and they allow water to pass sideways between one xylem vessel and the next.

Primary xylem is the first xylem tissue in a young stem, root or leaf.
There are two types of primary xylem: **protoxylem** and **metaxylem**.

Protoxylem is the first xylem to develop behind root and shoot tips.
Lignin is added in rings or spirals to form annular vessels (rings) and spiral vessels (spirals).

Metaxylem is more mature and the walls are fully lignified (with the exception of pits).

Secondary xylem is formed from the ring of cambium in a stem.
Each year woody plants are able to increase their girth in this way as new secondary xylem is formed.
This is known as **secondary thickening**.

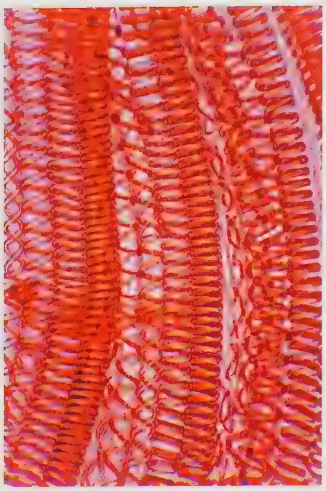
The seasonal growth of the xylem shows up as **annual rings**.
A ring formed in the previous year transports little water, its main function being to support the plant's increasing biomass.

How do you think that you could find out the age of a tree?

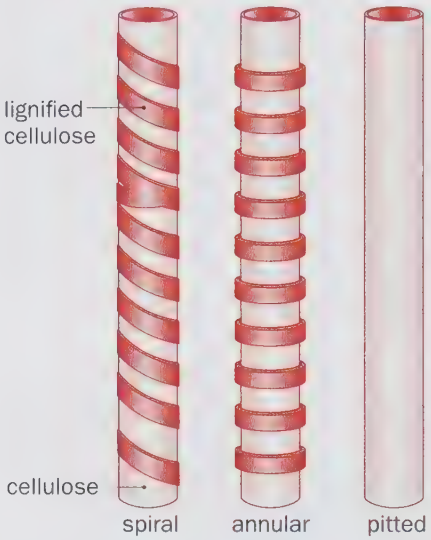
Tracheids are elongated cells with tapering ends.
They also conduct water but are less well adapted than vessels.
Unlike large vessels, tracheids do not have open ends so that water has to pass from cell to cell via the pits.
Tracheids are usually found in the finest branches of the xylem tissue in the leaves and in the roots.

Other xylem tissue includes:

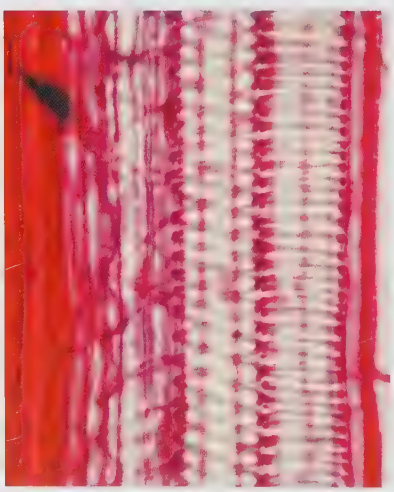
- **fibres**, which are similar to sclerenchyma fibres.
  Their function is solely support; they have no role in water transport.
- **xylem parenchyma**, the packing tissue that keeps the other xylem elements in place.



Can you see different types of xylem vessel in this micrograph?



Primary xylem vessels



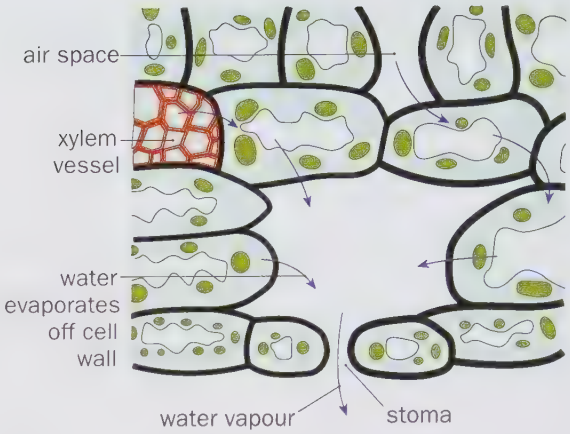
Longitudinal section (LS) of xylem tissue

Transpiration

Transpiration is the loss of water from the surface of land plants. About 99% of the water that a plant absorbs from the soil is lost in transpiration.

- Most water passes out through the stomata of the leaves.
- Only a small amount is able to pass out through the cuticle.
- A very small amount is lost through the lenticels in woody stems. The water is lost as water vapour to the air.

Water loss through the stomata is a consequence of gas exchange. If gases are to diffuse in and out of the internal tissues of the leaf, they pass through stomata on the lower leaf surface. Inevitably, water vapour will escape when the stomata are open. Since plants are able to open and close the stomata, there is some control over this loss of water.
The sun provides the energy that evaporates the water.



Water evaporates out of a stoma pore

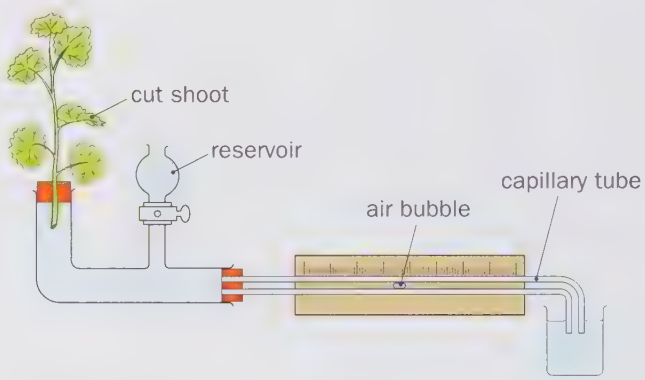
Diffusion pathway

As you have seen, the spongy mesophyll of a leaf consists of loosely-packed cells with many air spaces between them. Water evaporates from their moist cell walls into the air spaces. So the air spaces soon become saturated with water vapour. Connecting the air spaces to the atmosphere are the stomata. If the water potential of the air outside is ***lower*** than that in the air spaces, then water will diffuse down the gradient in water potential, out of the leaf.

Measuring the rate of transpiration

You have probably used a potometer before.
It actually measures the ***rate of water absorption***, but this is virtually the same as the rate of transpiration, since nearly all the water taken in is lost.
A very small amount of water is used in the leaf for photosynthesis.

- A leafy shoot is cut under water to prevent air bubbles from entering the xylem vessels.
- The cut shoot is attached by means of a rubber bung. Vaseline is used to ensure an airtight seal.
- The inside of the apparatus is flooded with water.
- An air bubble is introduced at the end of the capillary tube.
- The distance moved by the air bubble per unit time is measured.



A simple potometer

A potometer can be used to investigate the rate of transpiration under different conditions.

What do you think would happen to the rate of transpiration under these conditions?

- high humidity,
- high wind speed,
- high temperature,
- high light intensity.

In each case, say how you could simulate these environmental conditions in a laboratory situation.

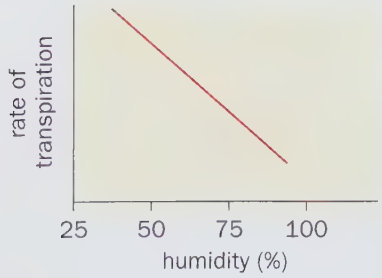

A potometer in use

Factors affecting the rate of transpiration

Anything that changes the gradient in water potential between the air spaces inside the leaf and the air outside will affect the rate of transpiration.

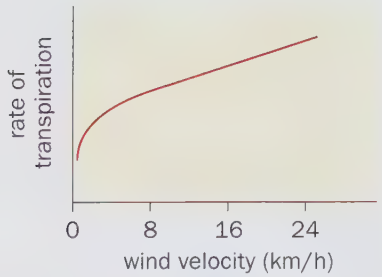
Humidity

When the atmosphere is humid, it contains a lot of water molecules.
This reduces the water potential gradient between the air spaces and the atmosphere.
So the rate of transpiration will decrease.
Low humidity will increase the rate of transpiration.



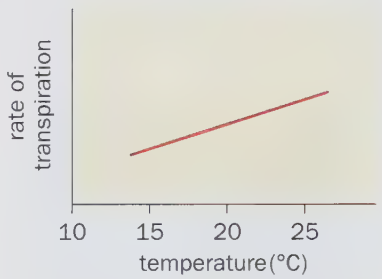
Wind speed

If the air is still, water vapour diffusing out of the leaf will tend to accumulate around the stomata pores.
This reduces the water potential gradient and slows down the rate of transpiration.
But windy conditions disperse this water vapour, increasing the gradient in water potential and thus increasing the rate of transpiration.



Temperature

Increasing temperature increases the kinetic energy of water molecules so their rate of diffusion through the stomata pores increases.
In addition, the air is able to hold more water molecules at higher temperatures.
We say that the relative humidity of the air is lower.
Both these effects result in an increase in the rate of transpiration.



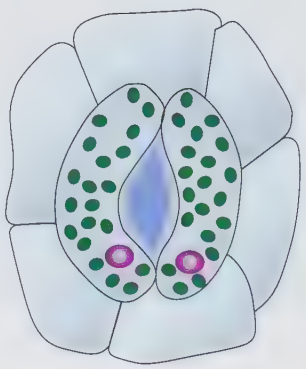
Light

As you will see, light influences the opening and closing of the stomata, so it has an indirect effect upon the rate of transpiration.

How do the stomata open and close?

You know that stomata open during the day and close at night.
Closing the stomata will of course reduce the transpiration rate.
Two guard cells lie either side of the stoma pore.
They control the size of the stoma pore by changing their shape.
As you saw in Chapter 8:

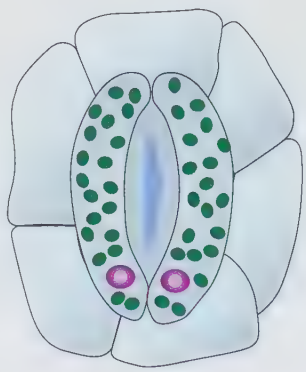
- If water enters the guard cells, they swell up and the stoma pore opens.
- If water leaves the guard cells, they become flaccid and the stoma pore closes.



Guard cells turgid: stoma opens

These changes are thought to be due to a reversible uptake and loss of potassium ions by the guard cells.

- During the day, the chloroplasts inside the guard cells photosynthesise.
- As a result they produce ATP, which fuels an active transport mechanism that takes up potassium ions from the surrounding epidermal cells.
- This lowers the water potential in the guard cells and water enters by osmosis, the guard cells become turgid and the stoma pore opens.
- At night, the chloroplasts in the guard cells do not photosynthesise.
- Less ATP is available for the active uptake of potassium ions, which then start to leave the guard cells by diffusion.
- This loss of potassium ions raises the water potential in the guard cell, water passes out into the epidermal cells, which have a more negative water potential.
- The guard cells become flaccid and the stoma pore closes.



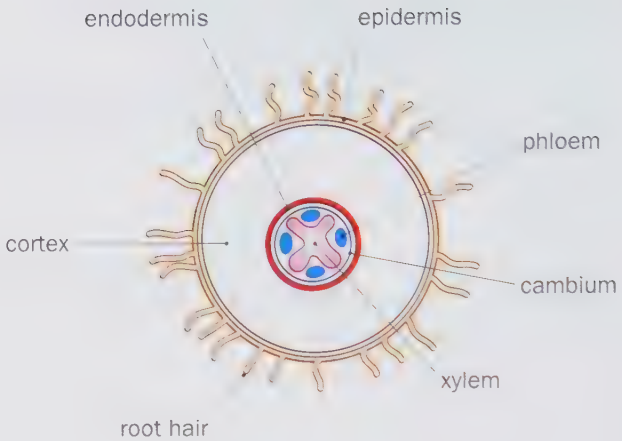
Guard cells flaccid: stoma closes

Water uptake by roots

If you look at a transverse section of a root, you will be able to see many of the plant tissues that are found in a stem but they are arranged in a different way in a root.

Can you see that the transport tissue (the xylem and phloem) is concentrated into a central core in a root?
Roots are subjected to ***vertical*** stresses; that is, they have to be able to resist being pulled out of the soil.
A central core of strong xylem tissue gives ideal resistance to being uprooted and at the same time gives economy of space.

Behind the root tip are numerous tiny **root hairs**.
Water enters the root hairs from the soil and passes across the root cortex and into the xylem tissue.
From here, water passes up the xylem vessels in the stem to the leaves.



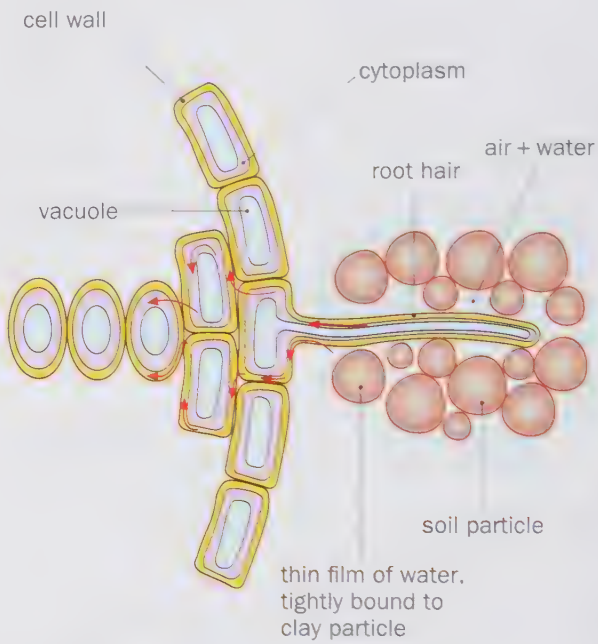
Transverse section (TS) through a young root

Root hairs are well adapted for absorbing water and mineral ions. They have very thin walls, giving a short diffusion pathway, and their shape provides a large surface area to volume ratio for absorption.

Routes through the root

The water in the soil contains a very weak solution of mineral salts, so it has a high water potential (near to zero).
Inside the root hair vacuole, there is a relatively strong solution of sugars and other dissolved substances, giving the contents of the vacuole a low water potential (more negative).
So water passes into the root hair cell down a water potential gradient, from a region of high water potential (in the soil) to a region of lower water potential (in the root hair vacuole) by osmosis.

The water potential in the xylem is lower than that in the root hairs. So water taken in by the root hairs passes across the cortex and enters the xylem tissue in the centre of the root.



Absorption of water by a root hair

There are ***two*** main pathways along which water travels:

- The **apoplast pathway** involves water passing through the ***cell walls*** of the cortex cells.
  The cell walls are made up of minute cellulose fibres and water is thought to pass along the spaces between them, a bit like soaking up water with a paper towel.
  As the water seeps along the micro-spaces between the fibres, the cohesive forces between the water molecules mean that more water is pulled along the apoplast route.
- The **symplast pathway** suggests that water passes along through the ***cytoplasm*** of the cortex cells.
  There will be a gradient in water potential across the cortex.
  The cytoplasm in the cells nearest the xylem will have a lower water potential than those nearer the outside of the root.
  Between adjacent cells there are strands of cytoplasm called **plasmodesmata**.
  These connections allow water molecules to pass between the cytoplasm of one cortex cell and the next.

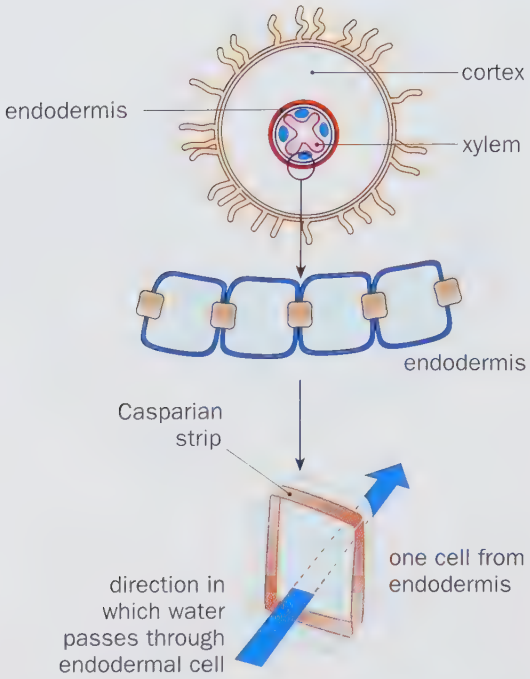
The apoplast pathway

The symplast pathway

The endodermis

Around the central core of transport tissue in a root is a layer one cell thick called the **endodermis**.
It is here that the apoplast pathway becomes blocked.

The cell walls of the endodermis are impregnated with a waxy material called **suberin**.
This forms a band of wax around the cells called the **Casparian strip**.
Suberin is waterproof, so the Casparian strip effectively stops water passing along the cell walls (apoplast route).
The only way that water can pass across the endodermis is by the symplast route, by crossing the cell membrane and passing through the cytoplasm.



It is thought that, as the water passes through the cytoplasm of the endodermal cells, they actively secrete mineral salts into the xylem tissue.
This lowers the water potential in the xylem, causing water to be drawn through the endodermis.
This 'pulling' of water into the xylem from the surrounding cells is thought to produce a positive hydrostatic pressure inside the xylem, forcing water upwards.
This positive pressure is known as **root pressure**.
Root pressure can be demonstrated by cutting the stem of a potted plant near the base. Water can be seen to seep out of the cut.
Root pressure is thought to be a minor force in the movement of water up the stems of plants.

A demonstration of root pressure

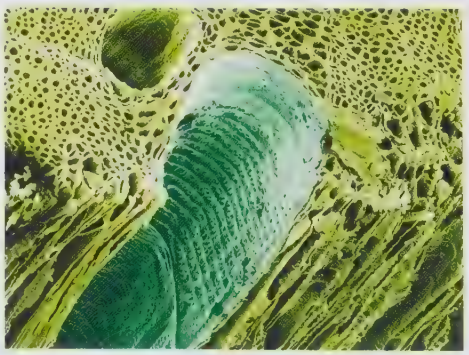
Mineral uptake

As you saw in Chapter 1, many mineral ions are needed for plant metabolism.
Mineral ions enter through the root hairs either passively or actively.

- Mineral ions enter the root hair by simple ***diffusion*** provided their concentration is greater outside the root hair than inside.
- If the concentration of mineral ions in the soil is lower than it is inside the root hair, then energy from ATP is required to enable ***active transport*** to take place.

Active transport (as you saw in Chapter 5) enables cells to selectively take up ions against a concentration gradient, into the symplast pathway.
It is thought that most ion uptake by roots occurs in this way.

Any active transport mechanism requires energy from respiration.
Consequently, anything that affects the rate of respiration will affect the rate of ion uptake by the roots.
Such factors include temperature, oxygen supply, and the presence of respiratory inhibitors such as cyanide.
Once inside the root hairs, mineral ions may move across the cortex by the apoplast route (by diffusion or mass flow in solution).
Otherwise, ions pass via the symplast route (by diffusion or active transport).
Either way, mineral ions have to pass across the endodermis by the symplast pathway and are then thought to enter the xylem by a combination of active pumping and diffusion.



SEM of xylem vessels: note the thick bands of lignin

How water passes up the stem

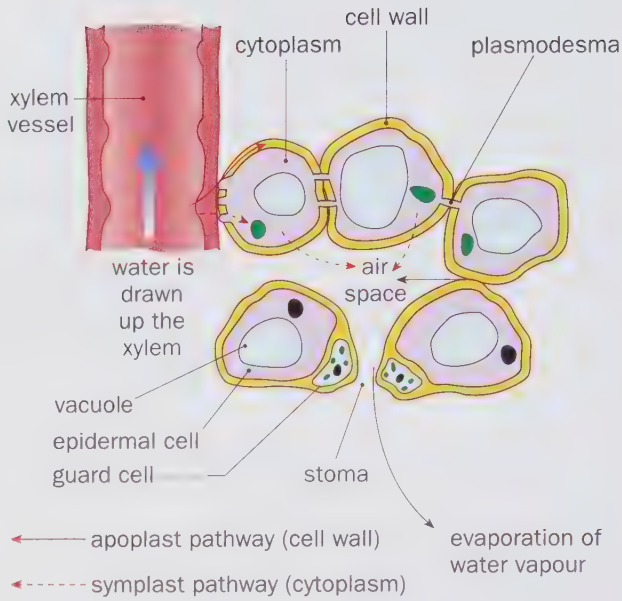
So how is water able to reach the tops of 60 m high Californian redwoods?

To find the answer we need to look at what goes on in the leaf.

- Water evaporates from the walls of the spongy mesophyll cells and the air in the air spaces becomes saturated with water.
- Usually the air outside the stomata is not saturated with water. So water diffuses from a region of high water potential (in the air spaces) to a region of lower water potential (in the air outside).

As more water evaporates from the spongy mesophyll walls, more water is drawn in to replace it.
Water passes from the xylem through the spongy mesophyll cells in ***two*** ways, which we have already looked at.

- Water can pass from cell to cell along the cell walls by the apoplast pathway.
- Water can pass along a water potential gradient in the cytoplasm of the cells by the symplast pathway.



The route by which water passes out of the leaves

Cohesion-tension theory

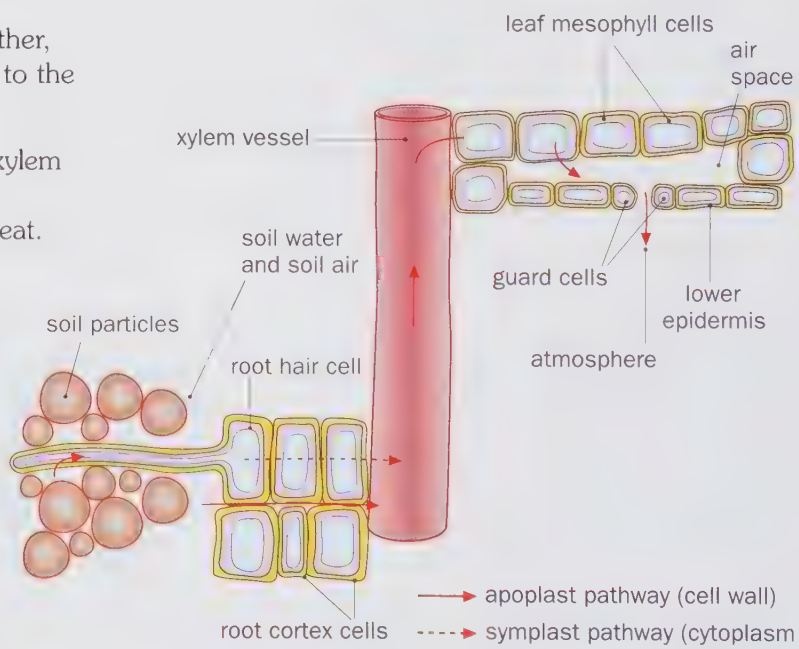
As molecules of water are removed from the xylem, more water molecules are 'pulled up' to replace them.
This pulling force is known as **transpiration pull**.
The negative pressure produced is rather like sucking a fluid up through a straw.
When a fluid is sucked up a straw, it is under a tension; and it is the same with the water inside the xylem vessel.

This mass flow of water through the xylem relies upon ***two*** important properties of water.

- **cohesion** – the water molecules tend to stick together,
- **adhesion** – the water molecules also tend to stick to the inside of the xylem vessel.

This drawing of a continuous column of water up the xylem vessel is known as the **cohesion-tension theory**.
The force needed to break the water column is very great.
The walls of the xylem vessels have to be thickened in order to withstand the tension in the water column, otherwise they would collapse.
The cohesion-tension theory is regarded as the main way in which water reaches the leaves of plants from the roots.

As you have seen, root pressure is thought to contribute a positive pressure, or push, to the water column.
Capillarity is a third force that is also thought to contribute to the rise of water in the xylem.
Water tends to rise inside narrow tubes by capillary action.
In plants, capillarity relies upon the tendency of water molecules to stick to the walls of xylem vessels by adhesion.
This force may be important in the upward movement of water in small plants but it is of little relevance in large trees.

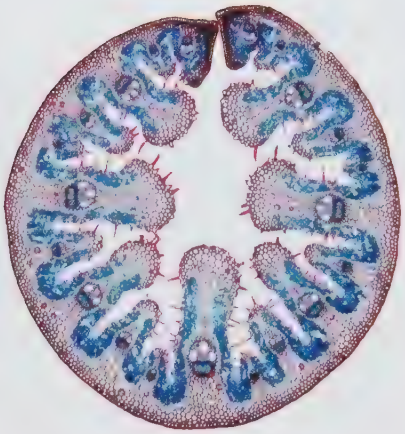


The cohesion-tension mechanism accounts for the passage of most water up a stem

Reducing water loss

All plants have to balance water uptake with water loss.
It is important that they maintain the turgor in their cells, or they will wilt.
Excessive transpiration can lead to the death of a plant if it passes its **permanent wilting point** and cannot regain its turgor.

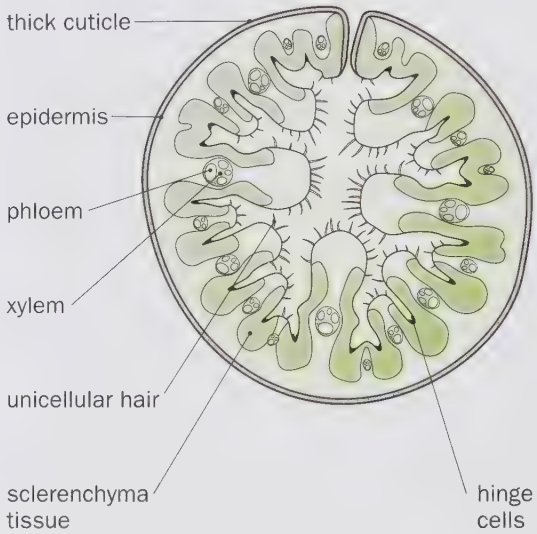
Xerophytes are plants that live in conditions where water is scarce.
These will of course include hot, dry, desert conditions.
But not all xerophytes live in these sorts of regions.
Many plants can be deprived of water in winter when the soil water freezes.
They can experience ***water stress*** because they continue to transpire but cannot take up water from the soil.
Xerophytic plants are also found in exposed, windy areas.

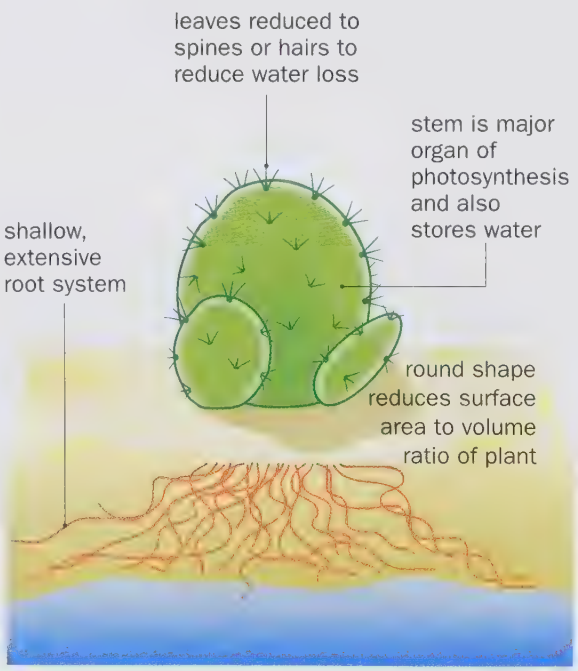


TS through a leaf of marram grass

Xerophytic adaptations enable a plant to reduce its water loss in number of different ways.

- A ***very thick waxy cuticle*** cuts down evaporation from the upper epidermis, as in the leaves of evergreen shrubs such as *Oleander*.
- Having ***smaller leaves*** reduces the surface area to volume ratio, so there is less area over which water is lost, as in *Pinus*.
- ***Rolling up*** of leaves so that the lower surface faces inside and traps humid air next to the stomata reduces the rate of evaporation from the leaf surface, as in marram grass (*Ammophila*).
- ***Sunken stomata*** can be found in grooves in some xerophytic leaves, for example *Bryophyllum*. Humid air accumulates in the grooves above the stomata, reducing the rate of diffusion of water molecules.
  Sunken stomata have the effect of reducing the amount of air movement over the surface of the stomata.
- ***Leaf hairs*** are outgrowths of the epidermal cells of leaves. They are also able to trap damp air close to the leaf surface, reducing the amount of air movement and cutting down transpiration, as in marram grass and *Oleander*.
- Some plants have ***succulent leaves*** in which they are able to store water, for example *Bryophyllum*.
- Other plants have ***succulent stems*** for water storage, for example cacti.
  These stems also take over the role of photosynthesis from the reduced leaves.
- Some plants have the ability to ***close their stomata during daylight*** to significantly reduce transpiration, for example most cacti and pineapple.
- Many xerophytes have ***shallow, extensive root systems*** to quickly absorb water from rain and overnight condensation, for example most cacti.
- The ***development of sclerenchyma*** tissue in the leaf prevents it from collapsing in times of drought, as in the case of *Hakea*.

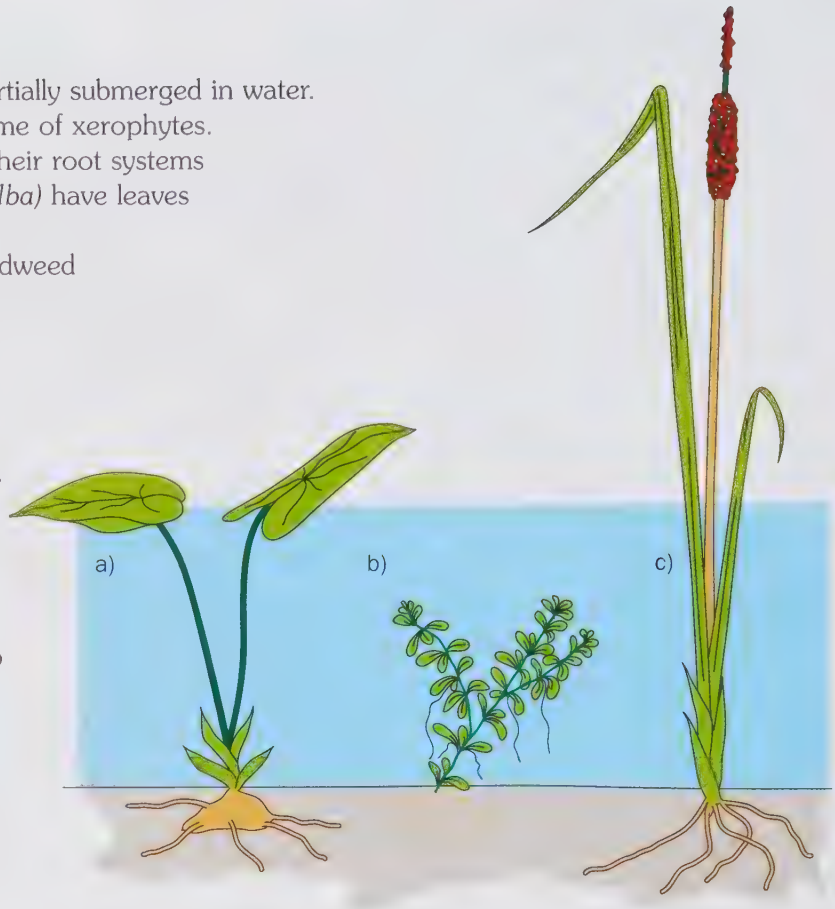




Hydrophytes

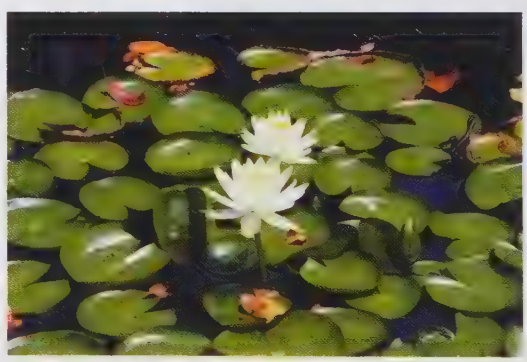
Hydrophytes are plants that grow submerged or partially submerged in water. So they inhabit conditions that are at the other extreme of xerophytes.
In some marginal plants, like rushes and irises, only their root systems are flooded. Others such as water lilies (*Nymphaea alba)* have leaves that float on the water surface.
In more extreme hydrophytes, such as Canadian pondweed *(Elodea canadensis)*, the entire plant is submerged.

Living in water has both its costs and its benefits.
Surrounded and buoyed up by water support is not a problem, and the absence of water transport mechanisms is not a disadvantage.
Floating plants save energy since they produce little or no xylem or sclerenchyma.
Roots, if present, are for anchorage, and root hairs are absent as it is unnecessary for roots to absorb water or mineral salts.
The leaves and stems of hydrophytes have little or no cuticle since conservation of water is not a problem.

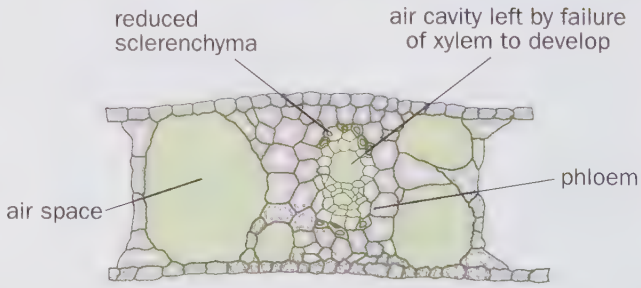


Hydrophytes: a) water lily (Nymphaea alba); b) Canadian pondweed (Elodea canadensis); c) bulrush (Typha latifolia)

The main disadvantage to plants that exist exclusively in water is that of gas exchange.
This is particularly true in the case of carbon dioxide, which is needed for photosynthesis.
Carbon dioxide diffuses 40 000 times more slowly through water then it does through air. So in the still water of a pond, carbon dioxide may be in short supply.
The same applies for oxygen which is needed for plant respiration.
It is only sparingly soluble in water and diffuses 300 000 times more slowly in water than it does in air.
So many pond plants have evolved an extensive system of air spaces in their stems and leaves through which gases can diffuse quickly.
These interconnecting air spaces provide a reservoir of oxygen and carbon dioxide, which also provide buoyancy to the plant tissues when submerged.
Buoyancy in many species of hydrophytes with floating leaves, such as water lilies, is assisted by the waxy covering on the upper surface of the leaves.
This prevents the upper surface from holding water, so helping the leaves to float.


Water lilies (Nymphaea alba)

The penetration of light through water can be a problem to some hydrophytes.
Submerged plant leaves show many features similar to those of 'shade' leaves found deep in the canopy of woodland trees.
Their thinness increases the ratio of outer photosynthetic tissue to inner tissue.
Also chloroplasts are often present in the epidermis of shade and submerged leaves.
This is an advantage because photosynthetic tissue is found closer to the limited light source where the pathway of diffusion of carbon dioxide from the water is shortest.



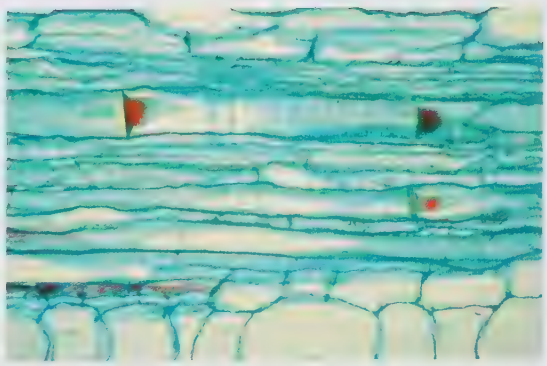
TS of the leaf of Potamogeton (pondweed), note the large air spaces

Structure of phloem tissue

As you have seen, transport of water through the xylem is a ***passive*** process and the cells that make up the xylem vessels are dead.

The transport of materials such as sugars and amino acids made in photosynthesis is an ***active*** process, that is, it requires energy for it to work.
Not surprisingly then, phloem is living tissue.
The movement of substances such as sugars and ions through the phloem is called **translocation**.

The most important phloem tissues in terms of transport are **sieve tubes** and **companion cells**.



Photomicrograph of LS of phloem

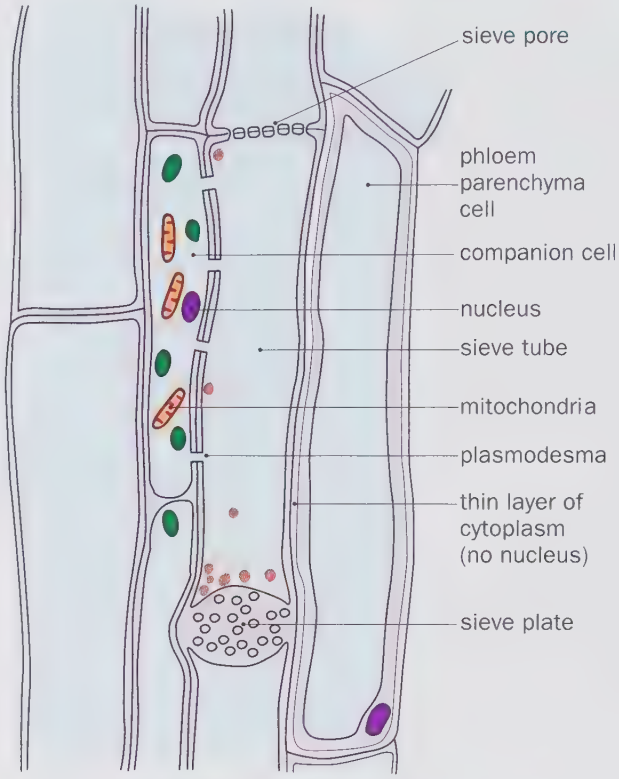
Sieve tubes

Phloem tissue is made from columns of parenchyma cells.
Each parenchyma cell is adapted to form a **sieve element**.
A column of sieve elements joined together forms a sieve tube.
Sieve tubes allow the mass flow of materials.
But they are very different to the xylem vessels.

The sieve tube is alive, though as it matures it loses several of the usual plant cell organelles.
The nucleus, ribosomes and Golgi bodies all degenerate.
So the sieve element ranks with the red blood cell as one of the few cells that does not contain a nucleus.
Presumably, the loss of these structures allows materials to flow more easily through the cells.

The sieve elements ***do*** have a cell wall, a cell-surface membrane, and cytoplasm containing endoplasmic reticulum and mitochondria.
There is, in fact, only a small amount of cytoplasm lining the inside of the cellulose wall.

The end-walls, where two sieve elements meet, together form the **sieve plate**.
Here the end-walls are perforated by a number of large pores, unlike in xylem vessels where the end-walls completely break down.
It is through these pores that materials have to pass during translocation.

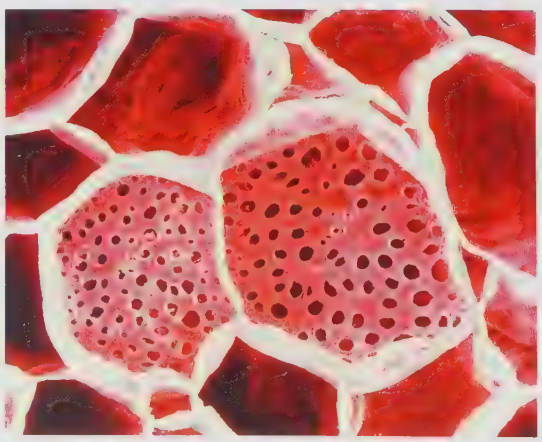


LS of phloem tissue to show structure

Companion cells

Each sieve element has at least one companion cell adjacent to it.
Companion cells have a more typical plant cell structure, with all the usual components.
However, they have many more mitochondria and ribosomes than the usual plant cell.
This reflects the fact that they are metabolically very active.

Companion cells are linked to sieve elements by numerous plasmodesmata.
The fact that the sieve element has lost so many of its organelles means that it needs a companion cell with a nucleus to help it to survive.



Sieve plates and sieve tubes are clearly visible in this TS of phloem

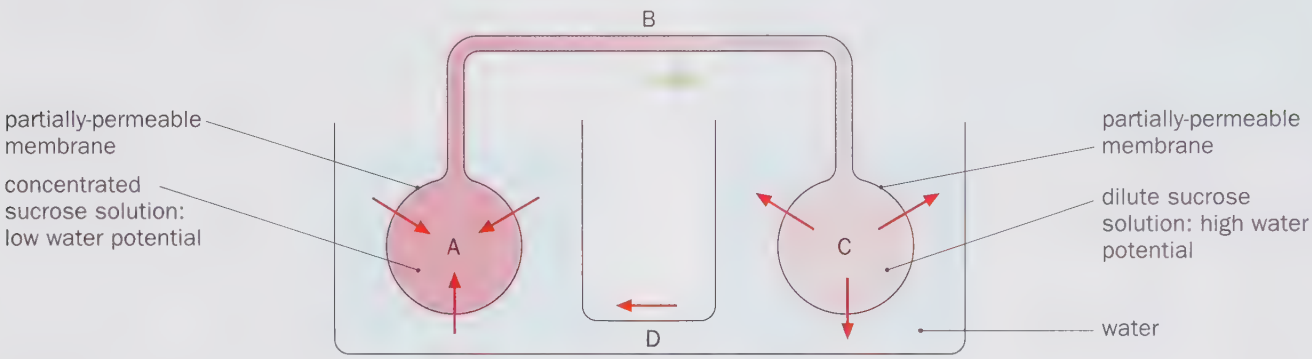
Transport of substances in the phloem

Organic substances, such as sucrose (30%), amino acids, minerals and hormones, are transported along the sieve tubes.
The speed at which these substances move through the sieve tubes is far too rapid to be explained by diffusion alone.

Movement of water through the xylem is due to a water potential gradient between the soil water and the air. The process is purely ***passive***.
The mass flow of materials through the sieve tubes is an ***active*** process.

The mass flow theory

Areas in a plant where sucrose is loaded into the phloem are called **sources**.
The usual source of sucrose is the photosynthesising leaf.
Areas where sucrose is removed from the phloem are known as **sinks**.
A lot of sucrose is removed from the phloem to form starch in the root.
Look at this mass flow model.



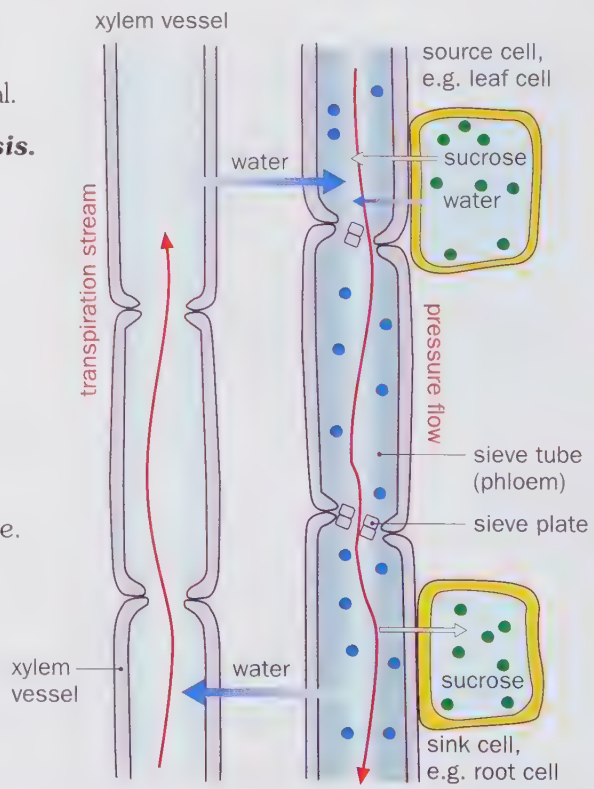
Region A represents a source.
Sugar is added at A and so the solution in A has a low water potential.
Region C represents a sink.
Sugar is removed from the solution at C, so it has a higher water potential.

There will be a tendency for water to pass into both A and C by ***osmosis.***
But this tendency will be far greater in A, since it has a much lower water potential than C.

As water enters A, hydrostatic pressure builds up, forcing the solution out into B.
Mass flow of solution occurs along the hydrostatic gradient B into C.
This forces water out of C into D.

In our model, A represents the leaf cells, a source of sugar made by photosynthesis.
C represents a sink, an area where sugar is removed.
This could be the roots, where sucrose is converted to starch for storage.
Channel B, joining the source to the sink, would be the phloem.
Channel D, bringing water back to the leaves, represents the xylem.

The use of radioactive tracers has shown that materials can move up and down in the phloem, depending upon the needs of the plant.
For instance, sugars made in older leaves (sources) can be transported to young leaves (sinks) nearer to the shoot tip.
We need to look at the nature of the sources and sinks to establish the ***active*** mechanism involved in translocation.



Possible sources and sinks in the mass flow theory

The loading and unloading of sucrose

If our mass flow model is to work, then sucrose must be

- constantly ***added*** at A, and
- constantly ***removed*** at C.

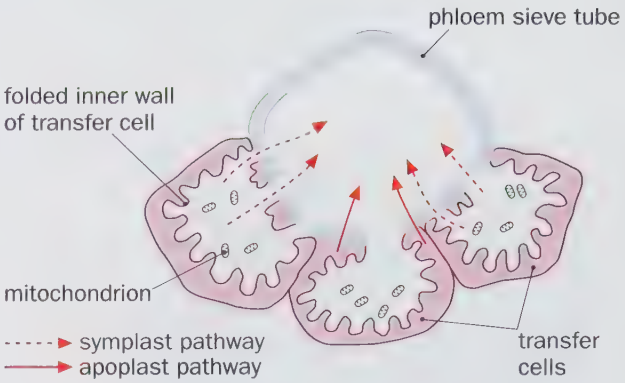
Otherwise the set-up would eventually come to equilibrium.

Loading sucrose

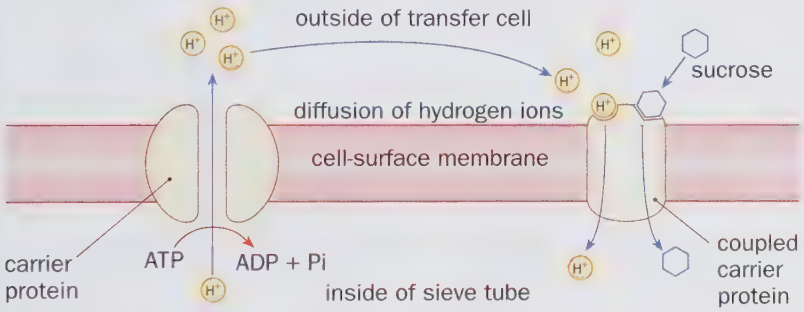
Sucrose is produced in the leaf mesophyll cells as a result of photosynthesis. These are the sources.
But how does the sucrose get into the phloem sieve tubes?

It may move by the apoplast pathway, passing along the cell walls.
It can also travel by the symplast pathway, moving from cell to cell via the plasmodesmata.
It is thought to be a combination of these two methods.

The sucrose is loaded into sieve tube elements using an active transport mechanism.
Specialised donor **transfer cells** are responsible for this.
The transfer cells are modified parenchyma cells with very folded inner walls.
This folding increases the surface area of cell-surface membrane over which active transport can take place.
The transfer cells contain large numbers of mitochondria, which provide the ATP needed for the active transport of sucrose.



Transfer cells load sucrose into sieve tubes by active transport

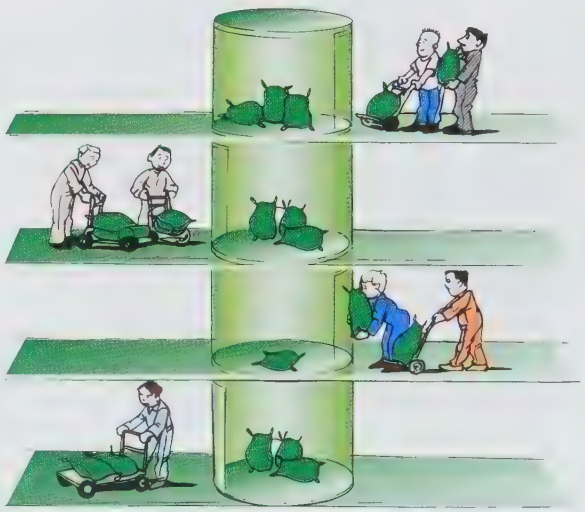


ATP from respiration is used to pump sucrose into the sieve tube element against a concentration gradient.
To enter, the sucrose has to cross the cell-surface membrane.
Once inside, the sucrose molecules are too large to diffuse out passively, so they stay inside.

Unloading sucrose

Unloading of sucrose will occur anywhere in the plant where sucrose is needed (at the sinks).
This could be in the root or at the stem apex and flower buds.
So phloem transport may be up or down the stem, unlike xylem transport which is only in one direction, from the roots up to the leaves.

At these sinks, there will be a low concentration of sucrose.
But sucrose molecules are too large to diffuse across the cell-surface membrane out of the sieve tube.
So transfer cells are again involved in the active transport of sucrose out of the sieve tubes into the surrounding tissue.
As soon as sucrose enters the tissue it is converted to something else, for example starch in the roots, or cellulose in the cells of the stem apex.



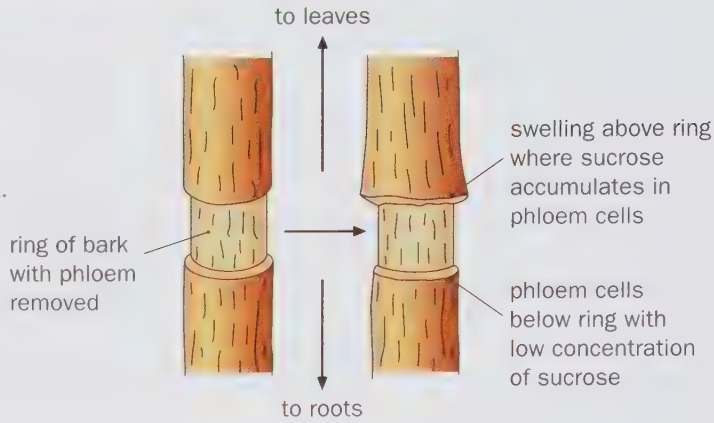
'Sucrose sir? At your service'

Evidence that translocation occurs in the phloem

There are several different pieces of evidence to suggest that photosynthetic products made in the leaves are distributed to the rest of the plant via the phloem.

Ringing experiments

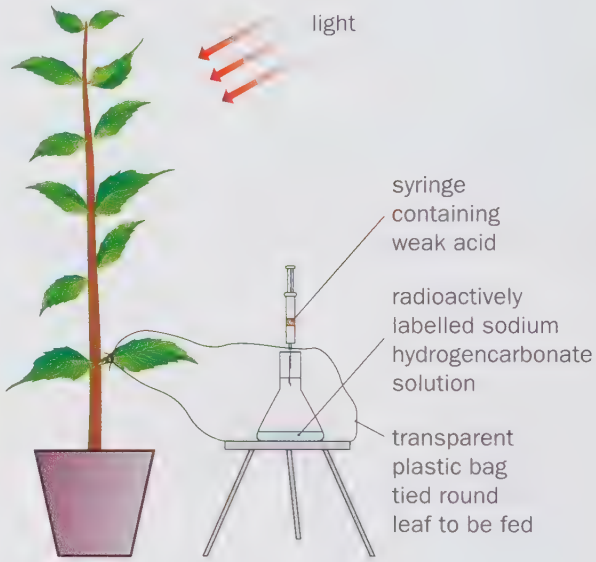
Phloem tissue in woody plants is situated underneath the bark.
If a complete ring bark is removed from a tree trunk or stem the phloem is removed with it.
In time the tissue just above the ring swells, but that below the ring tends to wither.
Analysis of the fluid in the swollen tissue shows that sugars and other organic compounds made in the leaves have accumulated above the ring.
Ringing interrupts the flow of organic materials down the stem and the roots are deprived of carbohydrate. Water transport from roots to the leaves continues as the xylem is unharmed.
Bark ringing will eventually kill the plant because the phloem cannot function, and the roots are deprived of sugar from the leaves.
Ringing experiments help explain why trees in unprotected young woodlands can be damaged or killed by deer, rabbits and grey squirrels gnawing the bark for nutrients.



A ringing experiment

Radioactive tracers

The radioactive isotope of carbon (^{14}C) can be used to trace the pathway taken by the photosynthetic products made in the leaves.
If a particular leaf is given radioactive carbon dioxide, the isotope is incorporated into the sugars that are formed.
The movement of these radioactively-labelled sugars through the stem can be traced using photographic film.
The film blackens where it is exposed to the radioactive isotope.
If sections of the stem are taken these radioactively-labelled sugars show up in areas corresponding to the phloem tissue. The technique shows that sugars made in the leaves eventually accumulate in the growing points, the new leaves and other sinks around the plant.
This technique is called **autoradiography**.



Exposing a leaf to radioactive ^{14}C

Evidence supporting mass flow theory	Evidence against mass flow theory
The sap in the sieve tubes is under pressure and seeps out if they are cut	The presence of sieve plates in the sieve tubes is questionable and would seem to obstruct any mass flow
The concentration of sucrose is higher in the leaves (sources) than in the roots (sinks)	Not all solutes move at the same speed, but they should do if under the same pressure from mass flow
Increases in the level of sucrose in the leaf are followed by similar increases in sucrose level in the phloem	Sucrose is delivered to all parts of the plant (sinks) at the same rate, rather than passing more quickly to areas with the lowest sucrose levels as the mass flow theory would suggest
The rate of translocation in the phloem can be inhibited by a lack of oxygen or by metabolic poisons suggesting some active transport process is involved	
The companion cells contain many mitochondria which produce ATP	

See further evidence that translocation occurs in the phloem on page 195.

Biology at work: Aphids and plant viruses

Aphids such as greenfly and blackfly are probably the most common pests in British gardens.
Viral diseases such as **tobacco mosaic virus** (**TMV**) are also all too familiar to plant growers.

What is the connection between these tiny, often wingless, insects and diseases that can cause considerable economic loss ?

The link lies in the feeding mechanism of the aphid.
Aphids feed from the phloem tubes in plant stems.
They have specially adapted mouthparts that are formed into a narrow tube called a **stylet**.

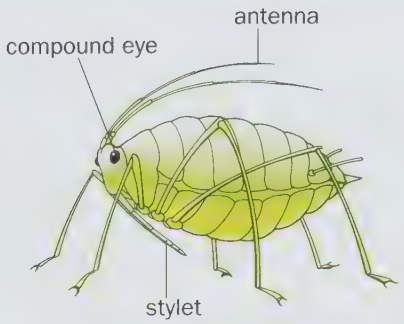
The aphid uses the stylet to pierce the phloem sieve tube cells.
It then feeds on the sugary sap that is forced under pressure up the stylet.
Scientists have used this feeding method to study the composition of the sap. A feeding aphid is anaesthetised and its body removed, leaving the stylet in place.
The stylet acts as a handy micropipette from which sap can be collected.

Although plant viruses can enter through wounds caused when planting or pruning, the main transmitters (vectors) of viruses between plants are aphids.

The tiny viral particles live in the salivary glands of the insects.
In the act of piercing the plant tissue, saliva is introduced into the plant cells.
Once inside the cells, the viral particles proceed to reproduce.
Within about 24 hours of infection, it has been found that viral particles can start to spread throughout a plant, carried by the phloem vessels.

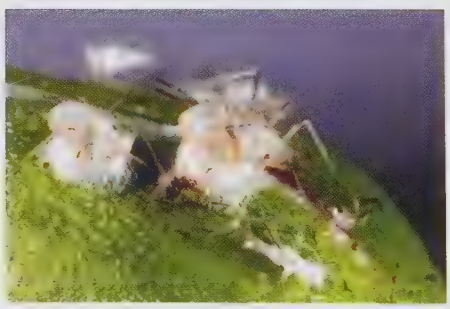
Once a plant is infected by a virus, the only solution is to dig it up and burn it.
Aphids themselves can be eliminated by a variety of chemicals, including systemic pesticides that enter the phloem and kill the insect when it feeds.

Alternatively, a **biological control** could be used, whereby a predator is introduced.
Biological control is mainly used in glasshouses.
This is because the predators require warm temperatures in order to breed more rapidly than the pests.
There are a wide range of biological controls for aphids.
Some, such as the larvae of ladybirds, hoverflies and lacewings, can be collected from garden plants and released into a glasshouse.
Others, such as species of parasitic wasps, can be bought commercially.
These lay eggs in aphid nymphs, and the eggs hatch into larvae which kill the aphid by eating it from the inside out.



Tomato plant affected by tobacco mosaic virus

Aphids feeding



Parasitised aphids

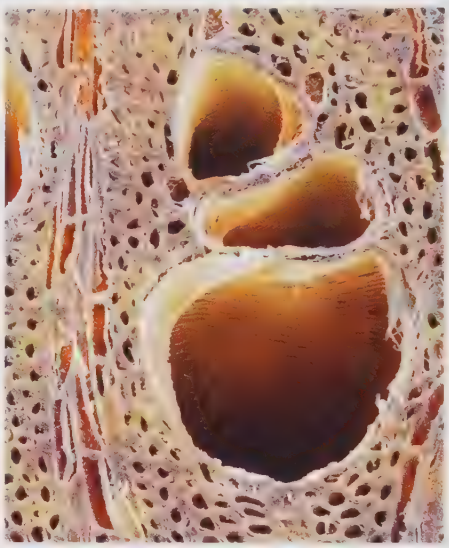
Biology at work: Dutch elm disease and ash dieback

Dutch elm disease (DED) is a wilt disease caused by a fungus called *Ophiostoma ulmi*. It is one of the most devastating tree diseases in Europe and North America, and research indicates that serious outbreaks can be expected roughly every 20 years. The first aggressive outbreak in the UK was in the late 1960s and by 1976 some 9 million elms (out of a population of 23 million) had been killed in southern England. A second wave of destruction was subsequently seen in the 1990s. DED is transmitted by the elm bark beetle when it feeds on new elms in the spring.

Hedgerow elms in an area severely attacked by Dutch elm disease

What causes DED?

After an elm tree becomes infected the spores rapidly reproduce in the vascular system of the tree. They begin to block the xylem vessels which transport water, and the elm tree starts to show visible signs of wilting. As more xylem vessels are blocked, parts of the tree begin to die before, eventually, the whole tree dies. Initially, fungal growth is sustained by nutrients in the xylem sap, but eventually the fungus produces enzymes that digest the cell walls. This leads to death of the parenchyma cells. These same enzymes penetrate the pits in the vessel walls, further spreading the fungus through the plant.



Xylem vessels in an elm tree. These vessels become blocked due to the growth of fungal spores

Combating DED

There are three main types of treatment for DED:

- cultural control, breeding of DED-resistant cultivars and monitoring of trees during the breeding season combined with sanitation felling,
- biological control, for example using other species of fungi, which controls the beetle by restricting its feeding and breeding in elm bark,
- chemical control, applying insecticides to the bark prior to beetle activity.

Elm trees also have a degree of natural defence against the less aggressive forms of DED.
One example is the production of rings of impermeable **tylose**, this is a compound that seals off the xylem vessel to localise the infection.

Ash dieback

In 2012 the emergence of another tree disease was widely reported in the UK. Ash dieback is caused by the fungus *Chalara fraxinea*. This fungus is thought to have reached England by being blown over the English Channel or imported via nurseries. It causes the crown of ash trees to blacken and wither, and can kill younger trees. Just under 5% of the UK's woodlands are made up of ash, and it is a valuable timber, as well as being the sole habitat for around 45 other species.

As with DED the fungus causing ash dieback also leads to a blocking of the xylem vessels.

The effect of ash dieback disease

Combating ash dieback

Although there is no known cure for ash dieback, in 2014 scientists announced progress in the development of a new fungicide designed to stop the growth of *Chalara*. Other strategies include:

- the removal of young ash trees to be replaced with different species,
- identification of *Chalara*-resistant strains of ash.

Summary

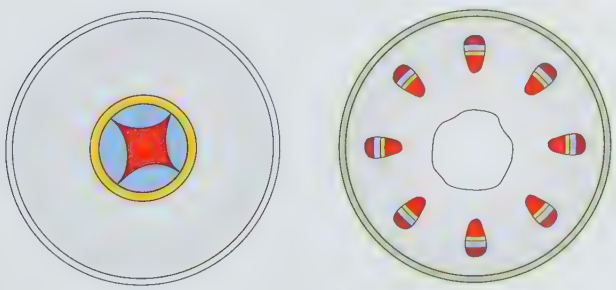
- Water is transported through the plant in one direction — from the roots, up the stem, to the leaves.
- This is a passive process and water passes down a water potential gradient from the soil water to the air.
- Water enters the root through root hairs, which are thin and have a large surface area to volume ratio.
- Water passes across the root cortex by the apoplast and symplast pathways.
- The endodermis prevents passage by the apoplast route, so water has to pass through the cytoplasm of the epidermal cells.
- Salts are actively added to the water in the xylem, which lowers the water potential and draws in more water. This produces a positive pressure known as root pressure.
- Loss of water by evaporation from the leaves is called transpiration.
- Most transpiration takes place through the stomata, which tend to open during the day and close at night.
- When water vapour is lost from the leaves, a water potential gradient is set up that draws water out of the xylem vessels.
- Transpiration pull is the main method by which water rises in the xylem: it is dependent upon a water potential gradient between root and leaf, cohesive forces between water molecules, and adhesive forces between water molecules and the inside of the xylem vessel walls.
- Xylem vessels have developed lignified walls to withstand the pressures involved in water transport.
- Xerophytes are plants that live in environments short of water. They have developed many adaptations to reduce water loss, particularly adaptations of the leaves.
- Translocation of organic materials takes place in the phloem sieve tubes. This is an active process involving the use of energy from ATP.
- The mass flow theory explains how materials are transported from sources to sinks.
- Sucrose is loaded into companion cells by active transport.

Questions

1 a) What are the main cell types that make up xylem tissue?
b) Explain how the structure and distribution of xylem in a stem is related to:
i) transportation of water and mineral salts,
ii) mechanical support.
c) Explain how movement of materials in the xylem takes place.

2 Describe how water transport in a flowering plant takes place:
a) from its uptake from the soil by root hairs,
b) its transport up the stem to the leaves,
c) its evaporation from the leaves into the atmosphere.

3 Diagrams A and B show transverse sections through the stem and root of a flowering plant, to show the distribution of tissues.



A B

a) Which diagram (A or B) is a stem? Explain your choice.
b) Copy and complete the section of the stem indicating the tissue ***most*** involved in the following processes:
i) upward transport of water (W),
ii) transport of sugars (S),
iii) cell division (D).

4 Copy and complete the table, which refers to xylem and phloem tissues.
If you think that the statement is correct, put a tick (✓) in the appropriate box.
If you think that the statement is incorrect, put a cross (✗) in the appropriate box.

Statement	Xylem	Phloem
may contain tracheids		
contains cells with living contents		
contains lignified cells		
transports organic products of photosynthesis		
unidirectional transport		
transport inhibited by metabolic poisons		

5 a) Copy and complete the table, which compares the xylem and phloem.

Feature	Xylem	Phloem
name of conducting cells		
direction in which materials are transported		
one possible mechanism by which materials are transported		

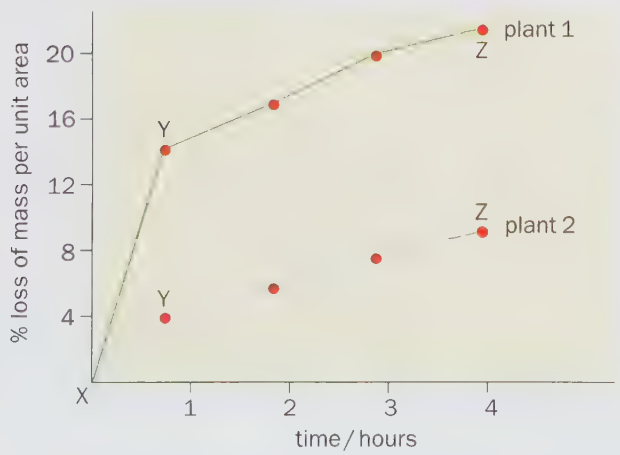
b) Briefly describe one way of demonstrating the movement of fluid in the phloem.

c) The table shows the concentrations of two chemicals in the phloem and xylem saps of the white lupin, *Lupinus albus*.

Chemical	Xylem (mg dm^{-3})	Phloem (mg dm^{-3})
sucrose	not detected	154 000
magnesium	27	85

Why do you think that the concentrations of sucrose and magnesium differ in xylem and phloem?

6 *Phaseolus* has leaves with a thin cuticle and hairless epidermis.
Pelargonium has leaves with a thicker cuticle and hairy epidermis.
These plants were used to carry out an investigation into water loss from detached leaves, measuring the change in the mass of leaves over several hours.
The results of the investigation are shown in the graph.



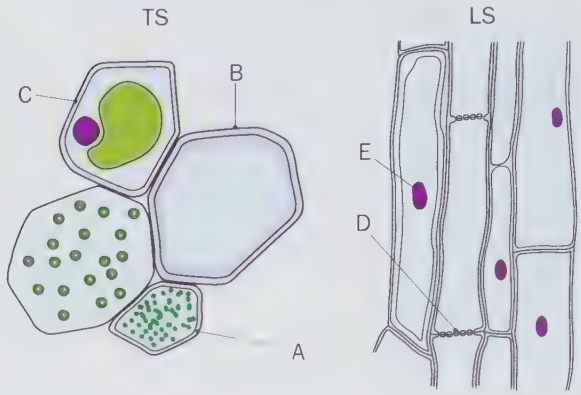
a) Describe how you would carry out this investigation.

b) i) Suggest why percentage loss of mass was rapid from both plants between X and Y.

ii) Why does the rate of loss decrease between Y and Z?

iii) Deduce, giving reasons, whether you think *Pelargonium* was plant 1 or plant 2.

7 The diagram shows details of phloem structure in transverse section (TS) and longitudinal section (LS).



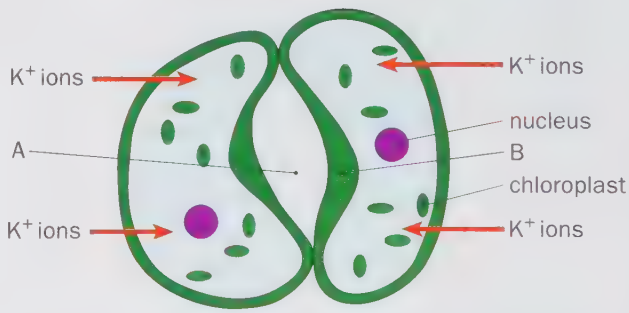
a) i) Name the cell types labelled A to C.

ii) Name the structures labelled D and E.

b) i) The phloem tissues translocate organic materials from sources to sinks. Explain the meaning of the terms 'sources' and 'sinks', giving an example in each case.

ii) Give two ways in which the phloem sieve tubes are adapted for translocation.

8 The diagram shows structures found on the lower epidermis of a leaf.



a) What are the features labelled A and B?

b) One theory suggests that the space labelled A becomes larger as a result of the influx of potassium (K^+) ions into the cells shown in the diagram.

i) Name the process that results in the movement of these potassium ions.

ii) Describe how this influx of potassium ions results in an increase in the size of A.

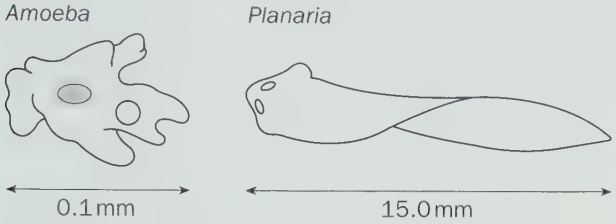
Gas exchange

1 a) State *three* characteristic features of gas exchange surfaces. [3]

b) Describe how the process of inspiration (breathing in) takes place in mammals. [3]

Edexcel (formerly London) **[6]**

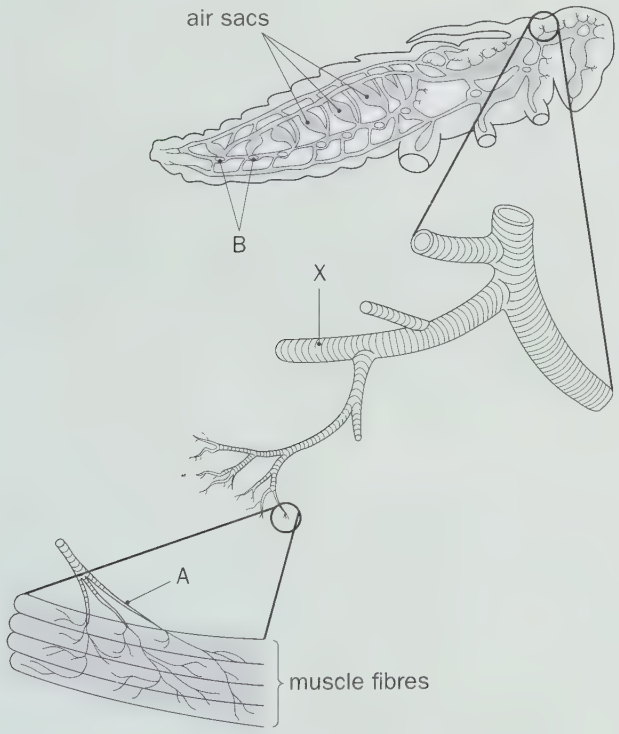
2 The drawings below illustrate the size and shape of *Amoeba* (a protoctist) and *Planaria* (a platyhelminth).



For each animal, explain why simple diffusion provides adequate gas exchange between the organism and its environment. [4]

WJEC **[4]**

3 The drawing below shows structures in the breathing system of an insect.



a) Name the structures labelled A and B. [2]

b) Describe the mechanism by which the respiratory surfaces of an insect are ventilated. [2]

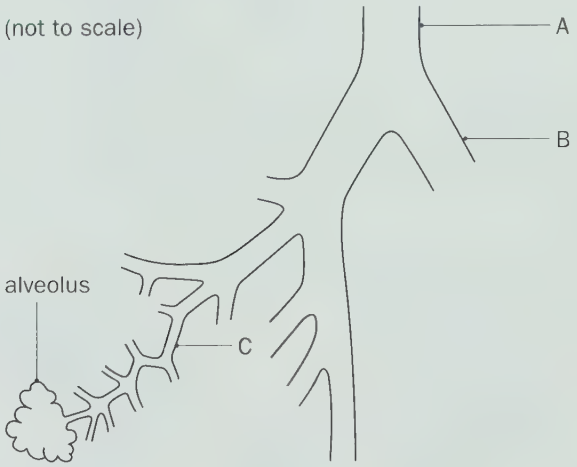
c) Suggest the function of the bands of thickening in structure X. [1]

d) Give *two* features which are common to the respiratory surfaces of an insect and of a mammal. [2]

AQA (formerly NEAB) **[7]**

Further questions on exchange and transport

4 a) The diagram below shows part of the human airway system.



Name the parts labelled A, B and C, and for each part, give *one* distinctive feature. [6]

b) The table below shows how different types of breathing affect ventilation.

Each of the headings A, B, C, D and E represents:

A Tidal volume / cm^3 $breath^{-1}$

B respiration rate / breaths min^{-1}

C dead space volume / cm^3

D pulmonary ventilation / cm^3 min^{-1}

E alveolar ventilation / cm^3 min^{-1}

The breathing types are all at rest.

Breathing type	A	B	C	D	E
Quiet	500	12	150	6000	4200
Deep, slow	1200	5	150	6000	5250
Shallow, rapid	150	40	150	6000	0

i) Suggest what is meant by the term *dead space volume*. [1]

ii) **Pulmonary ventilation = Tidal volume × Respiratory rate**

Using the data above, derive a similar word equation to show how the rate of alveolar ventilation has been calculated. [1]

iii) Explain why alveolar ventilation decreases with shallow rapid breathing. [1]

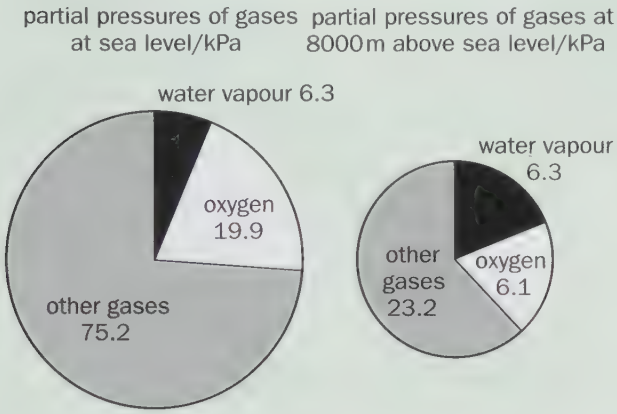
iv) What will happen to a person who continues to ventilate by shallow, rapid breathing? [2]

OCR **[11]**

5 Mountaineers often experience problems with gaseous exchange when they climb to high altitudes. This is because of the low pressure, low temperature and low humidity of the air.

a) How is the diameter of the trachea maintained at low pressures? [1]

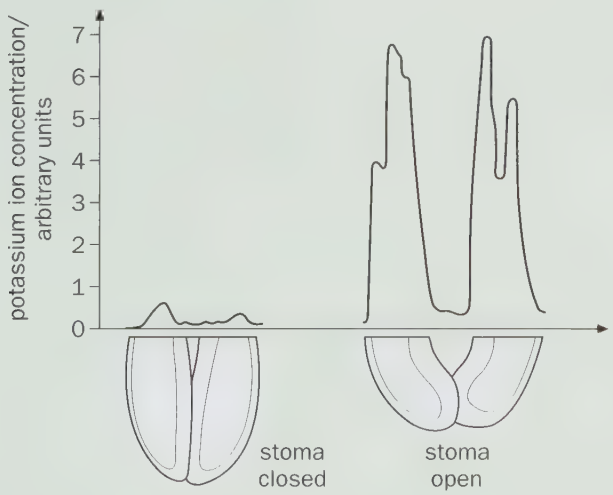
b) Air is inhaled through the trachea, bronchi and bronchioles. As it passes along these tubes, the air is warmed and humidified.
The diagram below shows the partial pressures of oxygen, water vapour and other gases entering the lungs at sea level and at 8000 m above sea level.



i) Comment on the differences in the air entering the lungs at sea level compared with that at 8000 m above sea level. [3]
ii) Suggest how oxygen absorption by the lungs at sea level would differ from that at 8000 m. [2]
iii) Suggest how the concentration of carbon dioxide in the blood at sea level would differ from that at 8000 m. [2]

OCR (formerly Camb) **[8]**

6 Plant scientists measured the potassium ion concentration in sections taken through closed and open stomata on the leaves of a bean plant. Their results are shown in the diagram below.



a) The scientists found an increase in the concentration of potassium ions in the guard cells when the stomata were open. Suggest where these potassium ions came from. [1]
b) Potassium ion concentration affects the water potential of the guard cells. Explain how an increase in potassium ion concentration causes the stomata to open. [3]
c) When stomata are open, the increase in potassium ion concentration involves active transport. The scientists treated the guard cells with a respiratory poison and the stomata started to close. Explain why. [3]

AQA (formerly NEAB) **[7]**

Digestion

7 The flow chart below represents the breakdown of starch in the human gut.

starch $\xrightarrow{amylase}$ maltose $\xrightarrow{maltase}$ glucose

a) Name *two* organs which produce amylase in humans. [1]
b) Describe how the release of amylase from each of these organs is controlled. [3]
c) Describe the precise location of maltase in the human gut. [2]

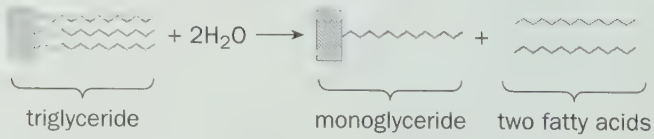
AQA (formerly AEB) **[6]**

8 The table below refers to some enzymes involved in the digestion of carbohydrates in the human digestive system. Copy and complete the table by writing the correct word or words in the empty boxes. [4]

Name of enzyme	Site of production	Products of reaction
	Wall of intestine	Glucose 1 galactose
Sucrase		
	Pancreas	Maltose

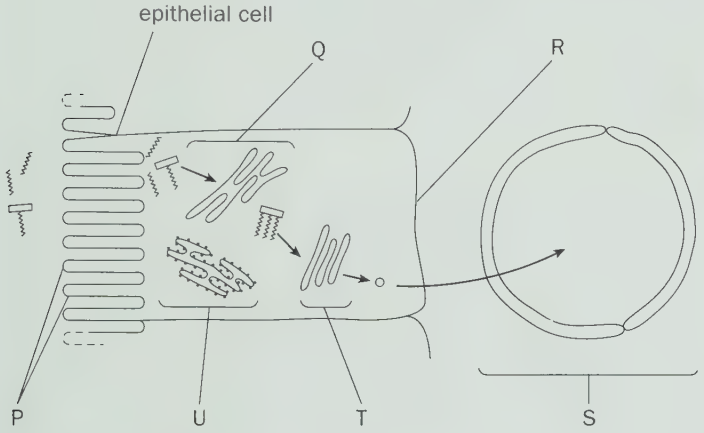
Edexcel (formerly London) **[4]**

9 The dietary recommendation is that lipids should be 30% of energy intake. The recommended energy intake for most women aged 19–49 is 8100 kJ day^{-1}. The energy content of lipid is 37.8 kJ g^{-1}.
a) Calculate the recommended lipid intake per day for these women. Show your working. [2]
Triglycerides are the main form of dietary lipids in humans. The end-products of lipid digestion are absorbed by the small intestine.
The diagram below summarises a reaction in the digestion of triglycerides.



b) i) Name the type of reaction shown. [1]
ii) Bile plays a part in the digestion of lipids. Describe how. [3]

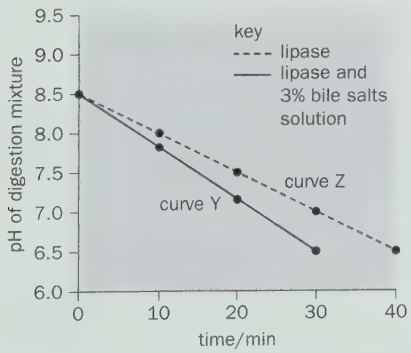
The diagram below shows the events that occur in the absorption of monoglycerides and fatty acids.
These molecules enter the epithelial cells of the small intestine by diffusion.
Once inside they are reassembled into triglycerides in organelle Q.
The triglyceride molecules are modified to form chylomicrons in organelle T.
Chylomicrons are made from many triglyceride molecules surrounded by protein molecules.
The chylomicrons leave the cell and enter vessel S.



c) i) Explain the importance of the structures labelled P. [1]
ii) Name R and S. [2]
iii) Name organelle U. Describe its role in the formation of chylomicrons. [2]
iv) Suggest how chylomicrons leave the epithelial cell. Explain your answer. [2]

AQA **[13]**

10 Scientists investigated the effects of lipase and lipase with a 3% bile salts solution on the digestion of triglycerides. They mixed the lipase, and lipase and bile salts, with the triglycerides and measured the pH of the mixture.
The diagram below shows their results.



a) Describe what curve Y shows about the effect of lipase and bile salts on the pH of the mixture. [2]
b) The concentration of lipase did not change during the course of the reaction. Explain why. [1]
c) One of the scientists decided to repeat the investigation at 10 °C below the original temperature. Describe how you would expect his plotted curve to be different from curve Z. [1]

The scientists also incubated triglycerides with different concentrations of bile salts. After 30 minutes they measured the diameter of the triglyceride droplets. They used the results to calculate the mean radius of the droplets at each concentration. The table below shows their results.

Concentration of bile salts (%)	0	1	2	3	4	5
Mean radius of triglyceride droplet (μm)	6	5	4	3	2	1

d) The ratio of mean radius of triglyceride droplets in bile salts at a concentration of 0% to the mean radius in bile salts at a concentration of 3% is 2 : 1.
You can calculate the surface area of a droplet using the formula:

$$A = 4\pi r^2$$

where A = surface area
r = radius
π = 3.14

Calculate the ratio of the surface area of triglyceride droplets in bile salts at a concentration of 0% to the surface area in bile salts at a concentration of 3%. Show your working. [2]
e) Use the data in the table to explain the difference between curves Y and Z in the diagram. [3]

AQA **[9]**

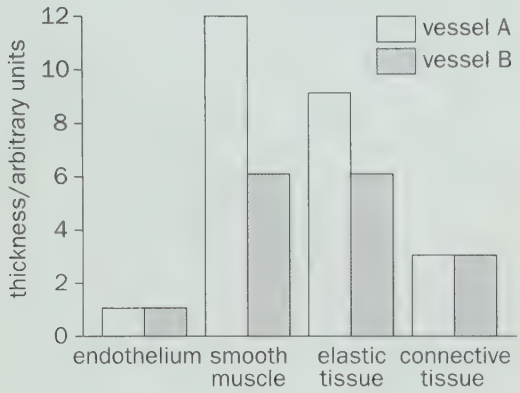
Transport in animals

11 Which sequence A–D shows the pathway a red blood cell follows from the heart to the lungs and back?
A Left ventricle, pulmonary artery, pulmonary vein, right atrium
B Left ventricle, pulmonary artery, pulmonary vein, left atrium
C Right ventricle, pulmonary vein, pulmonary artery, left atrium
D Right ventricle, pulmonary artery, pulmonary vein, left atrium

[1]

Further questions on exchange and transport

12 The bar chart below shows the relative thickness of parts of the walls of two blood vessels A and B. One of these blood vessels is an artery, the other is a vein.

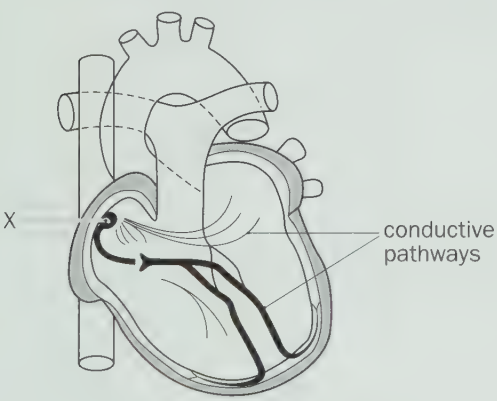


a) Which blood vessel is the artery? Explain the reasons for your answer. [2]

b) Explain how the structure of veins ensures the flow of blood in one direction only. [2]

AQA (formerly NEAB) **[4]**

13 The diagram below shows the pathways for the conduction of electrical impulses during the cardiac cycle.



a) i) Give the name of structure X. [1]

ii) Describe the role of structure X in the control of the cardiac cycle. [2]

b) The table below shows the pressures in the left atrium, left ventricle and aorta during one cardiac cycle.

Stage	Pressure/kPa		
	Left atrium	Left ventricle	Aorta
1	0.5	0.4	10.6
2	1.2	0.7	10.6
3	0.3	6.7	10.6
5	0.4	17.3	16.0
4	0.8	8.0	12.0

Using the table give the number of the stage for each of the following.

i) Blood flows into the aorta. [1]

ii) The valve between the atrium and the ventricle is open. [1]

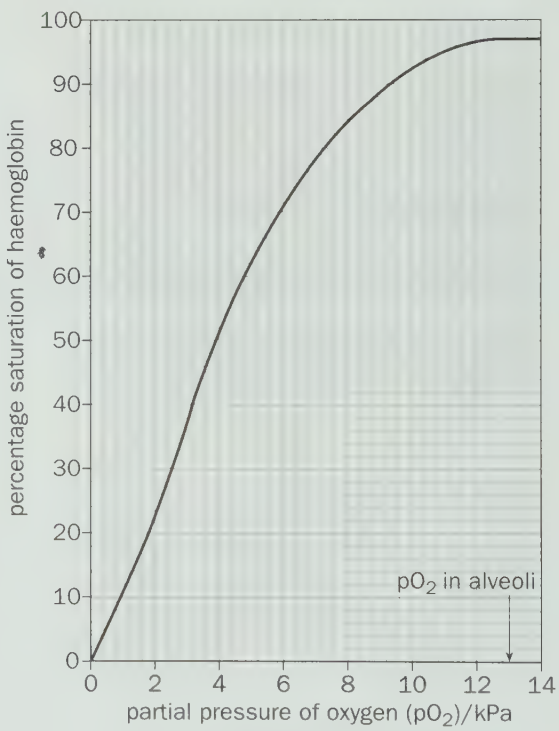
AQA (formerly NEAB) **[5]**

14 Copy and complete the table below which gives information about *three* types of mammalian blood cell. [4]

Appearance of blood cell	Name of blood cell	Function
A 		
B 		Makes antibodies
C 		Phagocytosis

AQA (formerly AEB) **[4]**

15 The graph below shows the dissociation curve for oxyhaemoglobin in the blood of a person at rest.

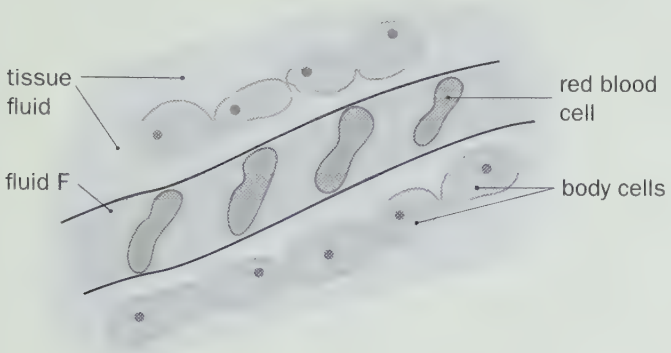


a) i) A typical partial pressure of oxygen in resting skeletal muscle is 2.5 kPa. Use the graph to determine percentage saturation of haemoglobin with oxygen in the blood leaving the resting muscle. [1]

ii) Blood leaving the lungs contains 20 cm^3 of oxygen per 100 cm^3 of blood. Calculate the volume of oxygen released per 100 cm^3 of blood as it passes through the resting muscle. [2]

b) i) Copy the graph. Sketch the dissociation curve expected when the person is exercising. [1]
ii) Give *two* changes in the blood of the exercising person which would have caused the change in the dissociation curve you sketched in i). [2]
AQA (formerly AEB) **[6]**

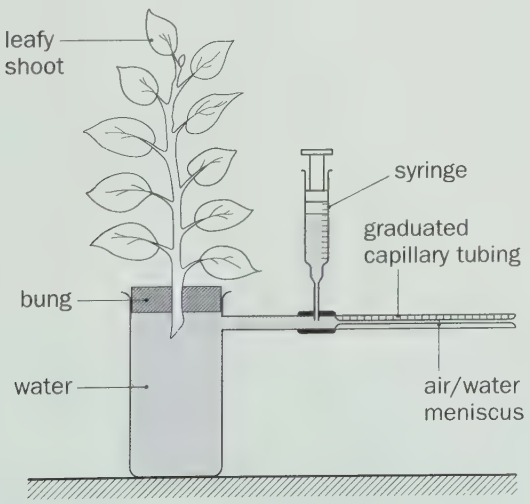
16 The diagram shows tissue fluid and cells surrounding a capillary.



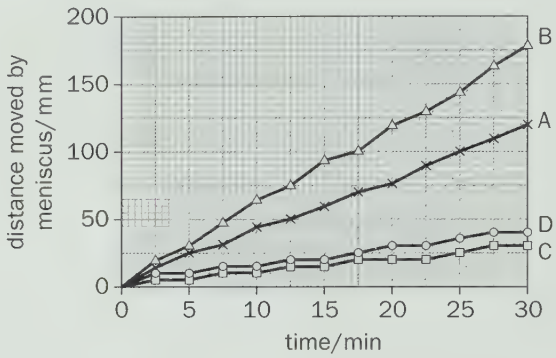
a) Name fluid F. [1]
b) Give *one* way in which fluid F is different from tissue fluid. [1]
c) i) The blood pressure is high at the start of the capillary. Explain how the left ventricle causes the blood to be at high pressure. [1]
ii) The blood pressure decreases along the length of the capillary. What causes this decrease in pressure? [1]
d) Some diets may result in a low concentration of protein in fluid F. This can cause the accumulation of tissue fluid. Explain how. [3]
AQA **[7]**

Transport in plants

17 The uptake of water by a leafy shoot can be investigated using a potometer, as shown in the diagram below.

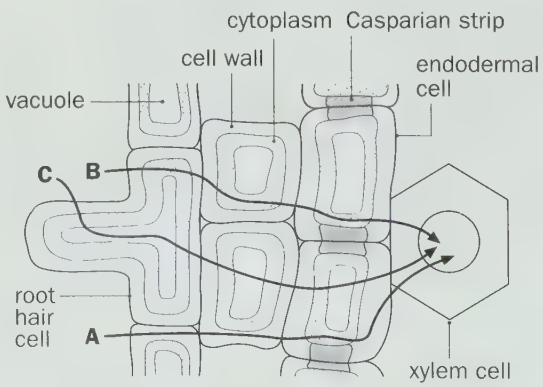


a) i) What assumption is made when this apparatus is used to investigate the rate of transpiration? [1]
ii) State *two* precautions which must be taken when setting up and using this apparatus. [2]
b) Using this apparatus, four experiments were carried out with the same shoot in the order given below.
A Still air, leaves untreated
B Moving air, leaves untreated
C Still air, lower surface of leaf covered with grease
D Moving air, lower surface of leaf covered with grease
Temperature and light intensity were kept constant during the investigation.
The results are shown in the graph below.



The mean rate of water uptake during experiment A was 3.2 mm^3 per minute. The cross-sectional area of the bore of the capillary tube is 0.8 mm^2. Calculate the mean rate of water uptake by the shoot during experiment B. Show your working. [3]
c) i) Describe and explain the effect of moving air on the rate of water uptake in experiment B. [3]
ii) Suggest an explanation for the different effects of moving air in experiments B and D. [3]
Edexcel (formerly London) **[12]**

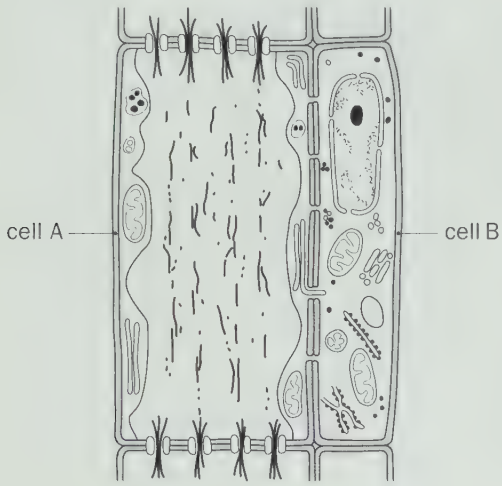
18 The diagram below shows three pathways along which water can pass from the soil into the xylem of a root.



a) Name the pathway labelled B. [1]
b) The Casparian strips shown in the endodermal cells are made of a waterproof material. Suggest the importance of the Casparian strip in the movement of water through the root. [2]
c) Explain in terms of water potential how water enters a root hair cell. [2]

AQA (formerly NEAB) **[5]**

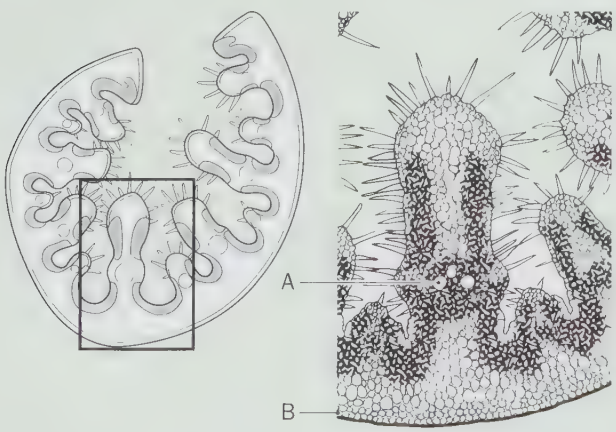
19 The diagram below shows a longitudinal section of two cells of phloem tissue in a plant stem.



a) Name the cells labelled A and B in the diagram. [2]
b) i) State the function of phloem in a plant. [1]
ii) Describe how aphids can be used to investigate the function of phloem. [3]

Edexcel (formerly London) **[6]**

20 The diagram below shows a transverse section of a leaf of *Ammophila arenaria*, which is a xerophyte. The photomicrograph shows the details of the area indicated by the box on the diagram.



a) Name the parts labelled A and B. [2]
b) Describe *two* xerophytic adaptations shown in this leaf and, in each case, indicate how the feature helps to reduce transpiration. [4]

Edexcel (formerly London) **[6]**

21 The 'Two-leaf Hakea' is a plant found in South-West Australia. Here the spring is relatively cool and wet, but the summer is hot and dry. The plant produces one type of leaf in spring and a second type in summer. The table shows the averages for a range of measurements taken from the leaves.

	Type of leaf	
Characteristic of leaf	A	B
Length/mm	33	55
maximum width/mm	10	0.8
Surface area/mm^2	292	144
Volume/mm^3	64	63
Cuticle thickness/μm	14	24

a) Calculate the surface area to volume ratio for each leaf type. [3]
b) Use the data to explain two ways in which leaf B is better adapted to summer conditions in South-West Australia than leaf A. [2]
c) What do you think are the advantages of the plant producing leaves of type A in spring? [1]

WJEC **[6]**

22 Scientists measured the rate of flow in the xylem of a tree. Over the course of one summer's day, they took measurements in a small branch at the top of the tree and in the trunk of the tree.

	Rate of flow ($m\,hour^{-1}$)	
Time of day	Branch	Trunk
02:00	1.3	1.0
04:00	1.4	1.1
06:00	1.5	1.2
08:00	1.6	1.3
10:00	5.0	1.4
12:00	7.7	2.6
14:00	6.0	3.3
16:00	4.0	2.9
18:00	2.0	2.4
20:00	0.2	0.7

a) The table provides evidence for the cohesion-tension theory of water movement through a plant. Explain how. [4]
b) Measurements of the diameter of the trunk were also made over the same time period. The maximum diameter was at 04:00 and the minimum at 16:00.
Suggest and explain what caused this variation in trunk diameter. [3]

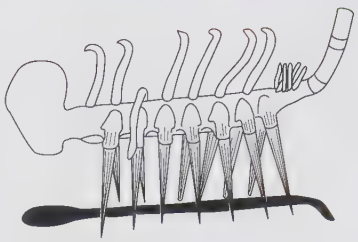
AQA **[7]**

12 Evolution and classification

Hallucigenia

Peripatus

Peripatus looks a bit like a caterpillar. It is an invertebrate with stout, fleshy legs and belongs to a small, obscure animal group called the Onychophora.
In 1911, fossils of ancient soft-bodied onychophorans were discovered in the Burgess Shale in British Columbia, Canada.
In 1977, Conway Morris described many of the weird organisms found in the Burgess Shale.
He named one particular animal *Hallucigenia*, because of its 'bizarre and dream-like appearance'.
It had a tubular body supported by seven pairs of long, pointed spines, and had a row of fleshy tubes running along its back.
It did not seem to fit into any existing animal group.
How could the creature move around on seven pairs of rigid spikes?
There was much debate, and in 1991 Ramskold and Hou suggested that *Hallucigenia* would make more sense if it were turned upside down.
The spines, which made no sense as walking appendages, could now function as protection.
The fleshy tubes now looked like the fleshy legs of *Peripatus*.
So by inverting *Hallucigenia*, Ramskold and Hou had turned the animal into an onychophoran! As you will see later in this chapter, the huge variety of life forms can make classification a tricky business.



Hallucigenia

Hallucigenia inverted

Variation

Phenotypic variation results from the effect of environmental factors on the genotype.
Phenotypic variation (V_p) = genetic variation (V_g) + environmental variation (V_e).
These environmental factors may include the following:

- diet – in many countries, children now grow taller and heavier,
- light, temperature and nutrients affect genetically identified plants,
- physical training can result in improved athletic performance.

But of course such changes are not passed on to the next generation.
More important to natural selection and evolution is inherited variation.
Genetic variation is caused by:

1. **the random distribution of chromosomes during metaphase I of meiosis,**
2. **crossing over between chromatids of homologous chromosomes during prophase I of meiosis,**
3. **random mating between organisms of the same species,**
4. **random fertilisation of gametes,**
5. **mutations.**

The first four result in a ***reshuffling*** of genes in the population.
New ***combinations*** of alleles are created in the offspring that are different from the parents and different from each other.
Mutations are different in that they do more than merely reshuffle existing genes. They create completely new alleles.

Mutations

A **mutation** is a change in the DNA of an organism.
There are two types of mutation:

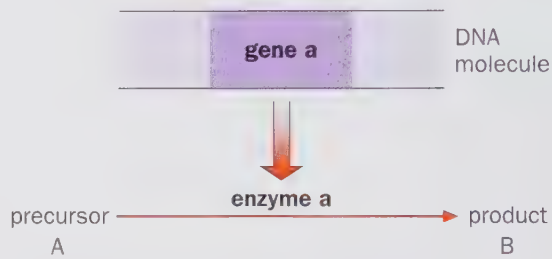
- a **gene** or **point mutation** – affecting a single gene,
- a **chromosome mutation** – affecting a single chromosome or set of chromosomes.

Both can occur in normal body cells or in the production of gametes.
Mutations that occur to body cells cannot be passed on to the offspring.
Cancers can develop if mutations occur in the genes that control cell division.
This can result in haphazard, unchecked growth leading to a tumour.
Often tumours turn out to be harmless, or **benign**, and may be destroyed by the body's own defence system.
But sometimes tumour cells can spread around the body and invade other tissues. This type of tumour is **malignant**.
A mutation can occur in the formation of an egg cell or a sperm cell.
If fertilisation occurs, the mutation will be passed on to all the cells of the new individual through repeated cell divisions.

Cancerous cells breaking away from a malignant tumour

Gene or point mutations

Gene or point mutations result from a change in the base sequence of the DNA of a gene.
As you know, a gene codes for the formation of a particular polypeptide. So if the sequence of bases is changed, a ***different*** polypeptide is produced. This new polypeptide may have different properties from the first. Since ***all*** enzymes are proteins, and enzymes control metabolic pathways, a change in an enzyme may block such a pathway. So a change in the sequence of DNA bases can lead to a change in an enzyme.



*If **gene a** mutates it no longer codes for **enzyme a** and the pathway will be blocked*

Let's say that one strand of DNA has the following 'normal' base sequence:
CCT AGT ATT CGC TGA GGC TAA TG
A **substitution** has occurred in the following strand, can you spot it?
CCT AGA ATT CGC TGA GGC TAA TG
An **inversion** has occurred in this strand:
CCT AGA TTA CGC TGA GGC TAA TG

Can you see that substitutions and inversions only alter one or two DNA codons?
The result is that only one or two amino acids in the protein are changed.
In some cases, the new amino acid does not alter the structure of the protein a great deal, so the mutation may not matter at all.

Now see if you can spot the **deletion** in this DNA strand:
CCT AGT TTC GCT GAG GCT AAT G
or the **insertion** in this strand:
CCT AGT AGT TCG CTG AGG CTA ATG

Only one base may be added or removed but this causes a shift in the whole sequence of bases and all the codons are changed after that point.

This is known as a **frame shift**. Such mutations can result in a completely different primary protein structure from the original. The three-dimensional shape of the protein also becomes altered and the protein is no longer able to do its job. So insertions and deletions can be far more damaging than substitutions or inversions.

Frame shifts are caused by deletions or insertions of a base. Every base triplet occurring after the mutation will now be changed. We can use sentences as an analogy.

THE CAT ATE THE BIG FAT RAT

The insertion of 'S' after CAT throws the whole sentence

THE CAT SAT ETH EBI GFA TRA T

A deletion also makes nonsense of the sentence.

THC ATA TET HEB IGF ATR AT

In a similar way, the sequence of base triplets coding for specific amino acids can be disrupted.

Chromosome mutations

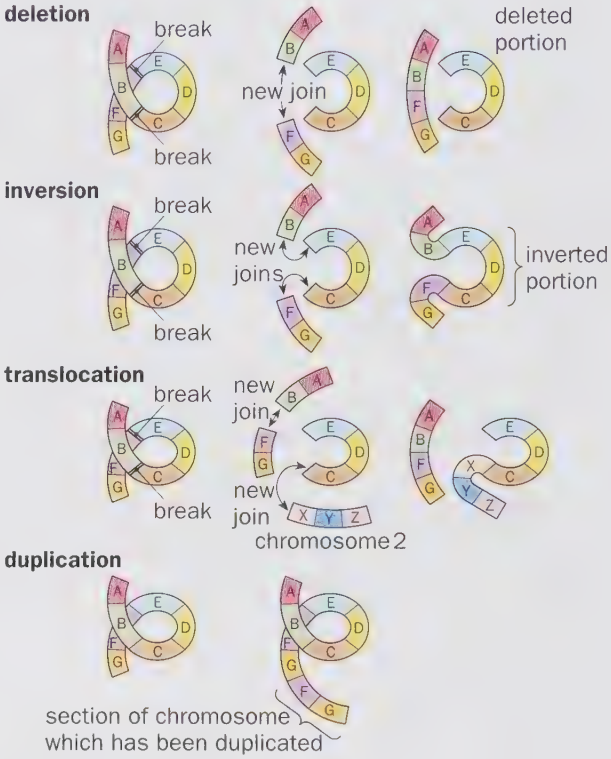
Chromosome mutations involve changes in entire chromosomes and are of two types:

- changes in the structure of a chromosome,
- changes in chromosome number.

Changes in chromosome structure

As you know, during prophase I of meiosis, homologous chromosomes pair up and exchange of chromosome material takes place at chiasmata. Perhaps it is not surprising that mistakes can occur, leading to major changes in the chromosome structure. There are four main types of changes:

- **Deletion** occurs when the chromosome breaks and a fragment of it is lost.
  The two ends join up to give a shorter chromosome.
  Since this involves the loss of genes, it can be lethal.
- **Inversion** – a deletion occurs but this time the chromosome fragment becomes reattached in an inverted position.
  So the correct genes are present but in the wrong order, so they will probably code for an incorrect protein.
- **Translocation** – a fragment of the chromosome becomes deleted and rejoins at a different position on the same chromosome.
  Reciprocal translocation occurs when the deleted section attaches to a different chromosome, as shown here.
- **Duplication** is when a portion of a chromosome is copied twice. As a result, the gene sequence is repeated.
  In our example, an extra length of chromosome has been added.



Whole chromosome mutations can be very disruptive because they involve entire blocks of genes.
Often the homologous chromosomes end up with a different gene sequence, which makes pairing up in meiosis impossible.

Changes in sets of chromosomes

Sometimes a mutation can affect whole sets of chromosomes. This is known as **polyploidy**.

A defect in meiosis may lead to a gamete getting two sets of chromosomes (diploid number).
When it is fertilised by a haploid gamete, the organism will be **triploid** (have three sets of chromosomes).
If two diploid gametes fertilise, then a **tetraploid** individual with four sets of chromosomes will be produced.
Tetraploidy can also happen after fertilisation if, during mitosis, the two sets of chromosomes double but fail to separate.
Tetraploids can be fertile, since there are two complete sets of homologous chromosomes that can pair up during meiosis.
Triploids cannot form complete homologous pairs, since one set is left isolated, so triploids are usually sterile.
Polyploidy is common in flowering plants and is associated with beneficial characters such as hardiness and disease-resistance.
Many important commercial crops, such as tomatoes, sugar beet, wheat and tobacco, are polyploids.

Chrysanthemums are polyploids

Changes in chromosome number

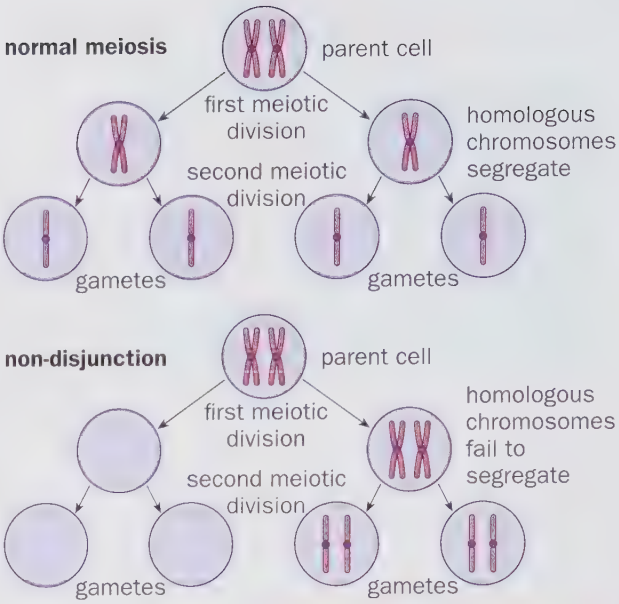
Changes in the number of individual chromosomes can also occur. Normally in humans the 23 homologous pairs of chromosomes form in meiosis I and then assort independently to give gametes each with 23 chromosomes.
But if just one of the 23 homologous pairs should fail to separate, then both chromosomes travel into one gamete.
This can result in one of the gametes having 24 chromosomes, and this is known as **non-disjunction**.

So what happens if the gamete with 24 chromosomes fuses with a normal gamete of the opposite sex with 23 chromosomes?

The resulting offspring will have 47 ($2n + 1$) chromosomes, in other words, ***three*** versions of the chromosome in question. This condition is known as **polysomy**.

Down's syndrome is a well known example of polysomy. Chromosome 21 fails to segregate from its homologous partner so the gamete produced has 24 chromosomes with two copies of chromosome 21. When fertilised with a normal gamete, the offspring has 47 chromosomes.
So the individual has three copies of chromosome 21.

Non-disjunction in most chromosomes is usually fatal.
Children with Down's syndrome are able to survive, though with varying degrees of disability.
In the case of Down's syndrome, the non-disjunction seems to occur in the ovary of the mother during egg production.
The incidence of the condition can be linked to the age of the mother. At the age of 20 years the risk is 1 in 2000, but by the age of 40 this has risen to 1 in 100. After 45 years, the incidence is three times greater than at 40.



Can you find the extra chromosome for this Down's female?

Mutagens

Mutations continually occur in nature at random.
The average rate at which a mutation occurs is about 1 in 100 000.
This is known as the spontaneous rate.
However, the spontaneous rate of a gene or chromosome mutation can be greatly increased by factors in the environment called **mutagens**.

Certain types of radiation, including ultraviolet light, X-rays and gamma rays, act as mutagens.
Emissions from radioactive substances are able to damage DNA.
High energy particles such as α and β particles are particularly dangerous.

A number of chemicals can act as mutagens.
These include **colchicine**, which can be extracted from crocuses.
It inhibits spindle formation during cell division, so the chromosomes fail to separate at anaphase and this leads to polyploidy.

Not all mutations are harmful. As you will see, some can be beneficial and produce an improvement in the phenotype.
Beneficial mutations are extremely rare, but they can give an advantage to an organism upon which natural selection can operate.

Many children with Down's syndrome surprise people with their achievements

Nuclear waste dump site

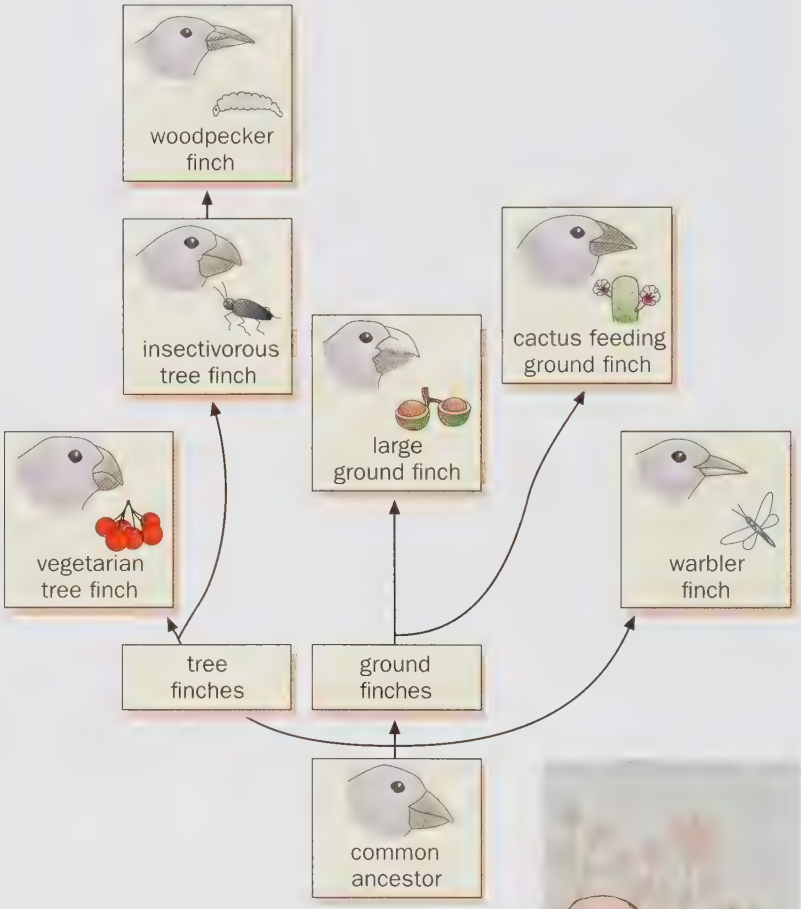
Charles Darwin and the theory of evolution

Charles Darwin (1809–82) was the naturalist on *HMS Beagle*, which sailed to South America and Australia on a scientific survey in 1832.
The voyage was a revelation to Darwin because he was able to study vast numbers of animals and plants that he never knew existed.
He collected fossils in the rocks which showed him that different life forms had gone through many changes.
What impressed Darwin most of all were the variations that existed between the species taken from a small group of volcanic islands about 600 miles off the coast of Ecuador.
These were the Galapagos Islands.
There was no life on these islands when they were originally formed by volcanic activity, so any life forms must have reached the Galapagos Islands by sea or air from the mainland.

HMS Beagle

Darwin's finches

Among the many animals that Darwin studied on the Galapagos Islands were the finches.
Darwin observed 13 different species of finch.
He suggested that one ancestral species of finch had reached the islands with the help of the prevailing south-east trade winds.
Since there were no other birds on the Galapagos, the original finches found many food sources not being eaten by other species.
Darwin noticed how individual finches differed from one island to the next.
One of the main differences was in the size and shape of their beaks. Some had short, strong beaks with which they could crack open seeds.
Others had long, thin beaks for catching insects.
There was even one species, the woodpecker finch, which was able to use a cactus spine to probe insect larvae out of the bark of trees.
It seemed that on each island, the characteristics that best suited a particular finch to its environment were passed on to the offspring.



Darwin suggested that the finches had all developed from a common ancestor and that each type of finch had, over time, developed a type of beak adapted to exploit a particular food supply.
This is a classic example of **adaptive radiation**.

This led Darwin to draw conclusions about how evolution came about.
At the same time, the British scientist **Alfred Russel Wallace** had come to similar conclusions about the theory of evolution.
For some time, Darwin was reluctant to publish his ideas, but eventually, persuaded by Wallace, they jointly published their findings in a paper to the Linnaean Society in 1858.
Soon after, Darwin published his famous book '*The Origin of Species*'.
At the time it caused consternation, suggesting as it did that humans and apes could have evolved from a common ancestor.
With the passage of time, more and more scientists came to accept Darwin's ideas.

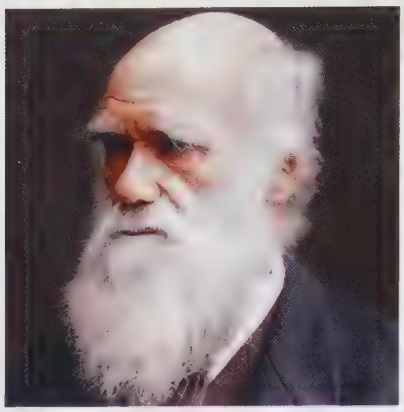
Darwin's ideas on the descent of man were ridiculed at the time

Natural selection

Darwin and Wallace jointly proposed that species evolve by a process of **natural selection**.
The basic principle behind natural selection is that those organisms that are better adapted to their environment are more likely to survive and reproduce to produce successful offspring.

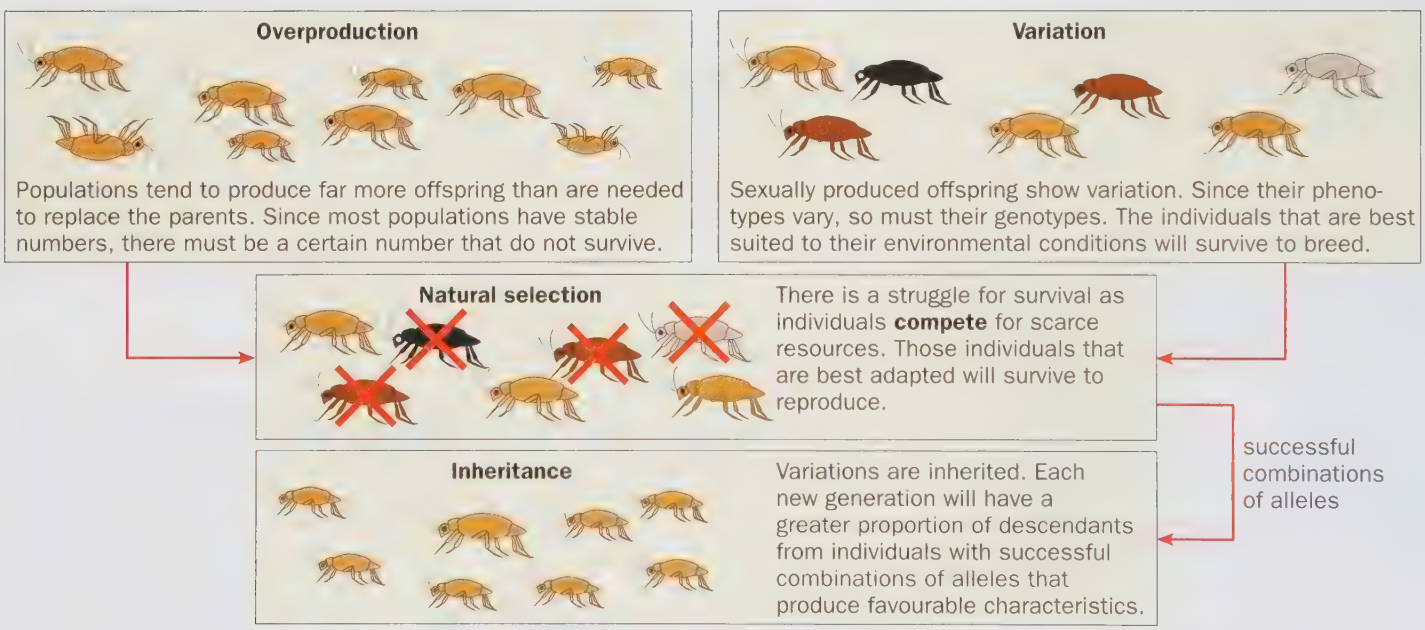
Natural selection can be summed up in a series of observations (O) and deductions (D).

- All organisms produce far more offspring than are needed to simply replace the parents (O).
- Despite this tendency to increase, most populations maintain fairly constant numbers (O).
- There must be a 'struggle for existence' between individuals of the same species. They compete with each other for the means of survival. A number do not live long enough to reproduce (D).
- There is variation among the offspring of any species (O).
- Those individuals that are best adapted to their environment will be more likely to survive than others – 'survival of the fittest' (D).
- The survivors will be able to pass on the favourable characteristics to their offspring (D).
- Over successive generations, the characteristics of the population will slowly change (D).



Charles Darwin, the great British naturalist

Alfred Russel Wallace (1823–1913)



Darwin was unable to explain the origin of variation or how it was passed on from one generation to the next, since there was no knowledge of genetics at that time.
The theory of evolution by natural selection is still generally accepted today.
The modern interpretation takes into account advances in modern genetics and is called **neo-Darwinism**.
The modern definition of evolution refers to changes in the allele frequencies of a population, which may or may not lead to the formation of a new species.

Types of selection

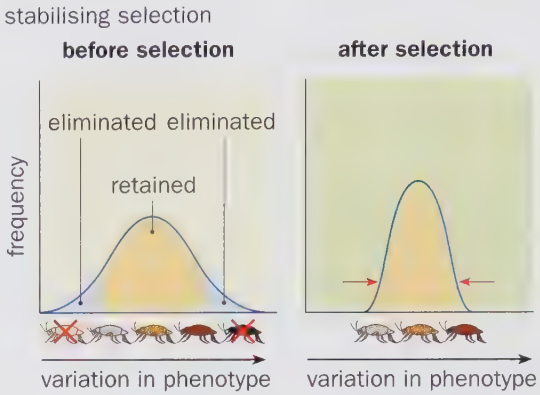
You may have gained the impression from the first part of this chapter that natural selection is just a mechanism for change. More often than not, the opposite is true: an organism that has a 'good' phenotype may well produce descendants very similar to itself. Sharks, crocodiles and ferns are examples of 'successful' organisms that have remained virtually unchanged for millions of years, in relatively unchanging environments.

Crocodiles have changed very little over millions of years

There are ***three*** different types of natural selection.

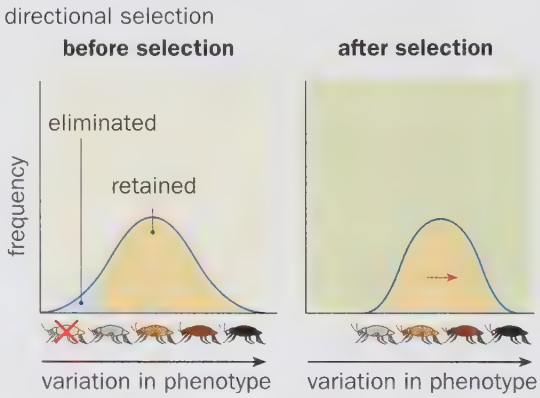
Stabilising selection

Stabilising selection tends to eliminate extreme variations from the population.
In a stable environment that does not change a great deal, the middle phenotypes tend to be ***selected for*** in greater numbers than the extreme ones.
Because it favours the average, stabilising selection tends to act to prevent change.
In our example, the light and dark beetles tend to be eliminated, since they are more conspicuous to predators, leaving medium shaded beetles.
A good example of stabilising selection is human birth weight. Studies have shown that particularly small or particularly large babies have a higher mortality rate than average-sized ones.



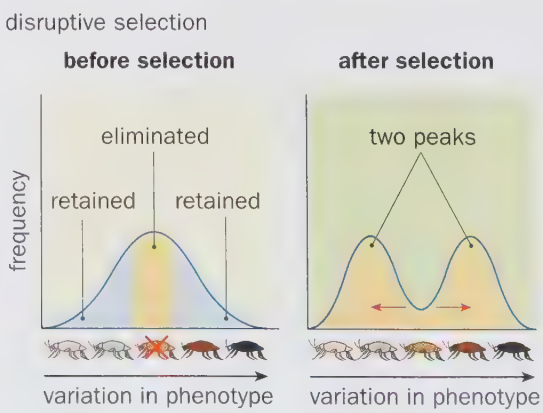
Directional selection

Directional selection can occur if an environmental change takes place. It may be that the change in the environment favours the organisms at one extreme of the phenotypes.
In our example, the darker beetles are selected because their environment has become darker.
There is a selection pressure on the lighter beetles. May be they now have less camouflage and are more easily seen by predators.
So there is a shift in the 'average' for the population.
A good example of directional selection is the peppered moth. As we shall see later in this chapter, there was an increase in the frequency of dark forms of the moth at the expense of the lighter forms as a result of industrial pollution darkening the environment.



Disruptive selection

Disruptive selection is the opposite to stabilising selection. Instead of favouring the mean, the extremes of the population are selected.
In our example, the light and dark beetles would be favoured at the expense of those with an intermediate shade.
This could happen if the beetles migrated to a new environment with a contrasting light and dark background.
This would probably result in selection of the light and dark beetles, since it would offer them better camouflage from predators.
Disruptive selection is uncommon compared with the other two forms. But it can be important in achieving evolutionary change, resulting in the formation of a new species.



Selection in action

The peppered moth

The process of evolution is generally slow, but there are examples of natural selection operating over a person's lifetime.
The peppered moth (*Biston betularia*) exists naturally in two forms:

- a pale, speckled form,
- a dark, melanic form, which arose as a mutation.

The moths feed at night and rest on trees during the day.

The light form is well camouflaged against the lichen-covered bark of trees in unpolluted areas.
This helps to prevent it from being seen by predatory birds.

The dark form is at a disadvantage in such areas as it tends to stand out against the light background.
Insect-eating birds, such as robins and hedge sparrows, eat dark individuals before they can reproduce and pass on the allele for dark colour.

Owing to the Industrial Revolution in the 1800s, the environment changed fairly rapidly in industrial areas.
The air quality declined and sulfur dioxide in smoke emissions killed the lichens that covered the tree bark.
The bark also became blackened by soot from the smoke.

Against the black background, the dark form was now better camouflaged than the light form.
The light moths were more easily seen against the dark background and were more frequently taken by birds before they could reproduce.
Before 1850, the dark variety of moth was rare.
By 1895, almost the entire population of moths in industrial centres, like Manchester and Birmingham, was dark.

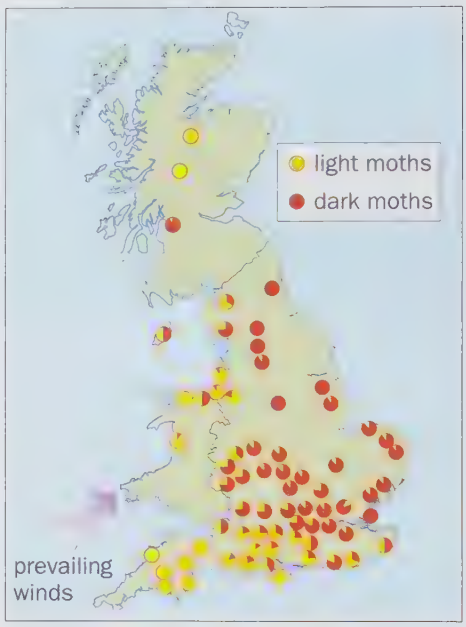
Studies using the 'release and recapture' technique have been carried out in Birmingham and rural Dorset. (See page 451.)
They confirm that the change in allele frequency for light or dark shading in the moth population is the result of natural selection.

Clearly the darker moth has a selective advantage over the light moth in industrial areas, whereas in non-polluted areas this advantage is with the light moth.

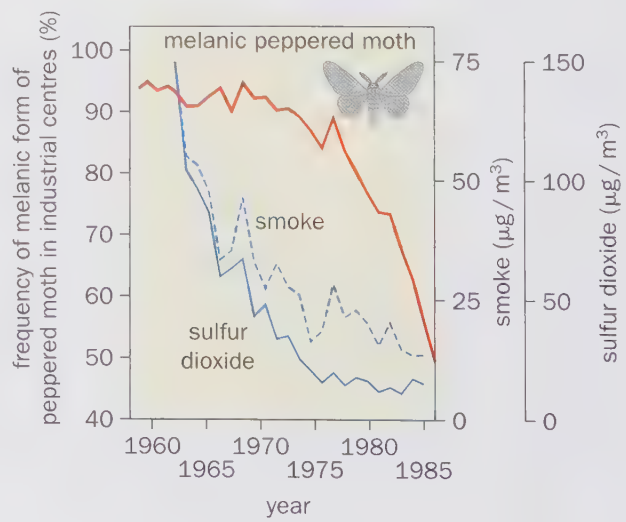
In the 1950s, coal-burning in industrial areas was still commonplace.
However, between 1960 and 1980 the environment changed again.
There was a decline in the number of coal-burning factories and the Clean Air Act was introduced.

Can you see what has happened to the levels of sulfur dioxide and smoke in the industrial centres?
In what two ways will this affect the environment?
How has this affected the frequency of light and dark moths in industrial areas?
Is this an example of stabilising, directional or disruptive selection?

The pale and dark forms of the peppered moth

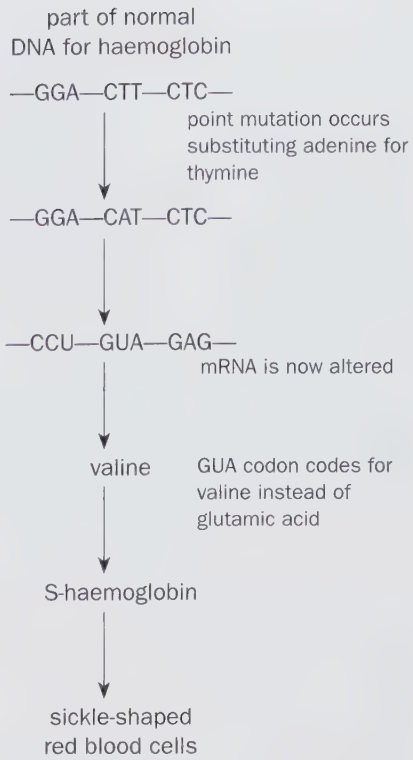


The proportions of light and dark moths found in Britain today



Sickle cell anaemia

Sickle cell anaemia (SCA) is a genetic disease resulting from a gene mutation.
The normal allele is partially dominant to the mutated allele for SCA, so only individuals with ***two*** SCA alleles will have the disease.
Such individuals produce abnormal haemoglobin, which causes red blood cells to become sickle-shaped.
This can prove fatal if the distorted red blood cells block blood vessels, or if the spleen destroys the abnormal sickle cells at a greater rate, so causing anaemia.
Heterozygotes who carry one SCA allele develop a much milder form of the disease called **sickle cell trait**.
The way in which gene mutation causes SCA is shown in the diagram.
Can you see that the replacement of just one amino acid in the haemoglobin molecule is responsible for the mutant gene?



Clearly, the possession of two SCA alleles puts a person at a great selective disadvantage.
The frequency of the SCA allele is much higher in some human populations, for instance in east Africa.

Malaria is a disease that leads to severe fever, convulsions and coma.
It can be fatal within a few days of the appearance of the first symptoms.
The cause is a unicellular parasite, *Plasmodium falciparum*, which enters the red blood cells and multiplies inside them.
Plasmodium is spread from person to person by female mosquitoes when they bite to take a blood meal. (See page 283.)

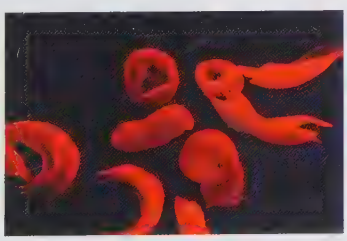
Look at the two maps of Africa.
The sickle cell allele is most common in those parts of Africa where malaria is found.

Sickle cells have low potassium levels, which kill off the *Plasmodium* parasite when it enters the cells.
Heterozygotes with sickle cell trait have sufficiently reduced potassium levels to protect them against the parasite and are less likely to suffer from malaria than people with two normal alleles.

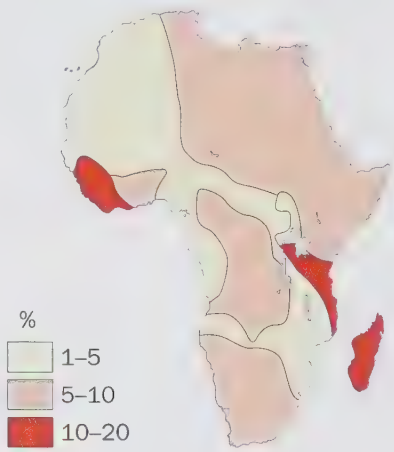
Natural selection is in operation in this situation.

- There is strong selection pressure against people who are homozygous for SCA, **Hb^s Hb^s**, because they may die from anaemia.
- There is strong selection pressure against people homozygous for the normal allele, **Hb Hb**, because they could die from malaria.
- But, in areas where malaria is common, there is a strong selective advantage for heterozygous individuals, **Hb Hb^s**, since they do not suffer badly from anaemia and they are protected from malaria.

So the sickle cell allele remains in the population in areas where malaria is an important ***selective agent***.



Sickle-shaped red blood cells



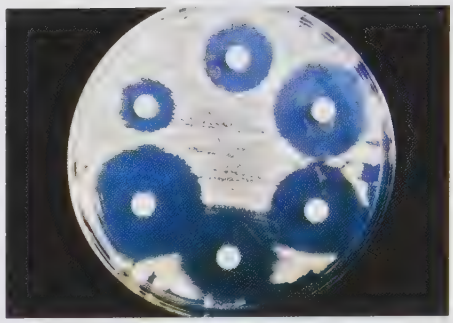
Distribution of people with at least one sickle cell allele

Distribution of malaria

Antibiotic resistance

Antibiotics are chemicals that kill bacteria or inhibit their growth. Soon after the introduction of antibiotics in the 1940s, certain bacteria were already developing a resistance to their effects.

If a population of bacteria, say *Staphylococcus*, were exposed to an antibiotic such as penicillin, most would be killed off.
But there may be just one or two individuals that by chance have a mutation that makes them resistant to the antibiotic.
The mutant bacterial cells are able to make an enzyme, in this case penicillinase, which breaks down the antibiotic.
These individuals have great selective advantage as they are unaffected and survive to reproduce very rapidly, passing on their resistant allele.
There is a constant search for new antibiotics as bacteria relentlessly develop resistance to existing ones. (See page 292.)



The effect of different antibiotics on the growth of E. coli. The clear areas around each disc show how much each antibiotic has inhibited bacterial growth.

Drug resistance

Resistance to pesticides such as DDT has occurred in a similar way.
DDT was introduced to control insect crop pests, and to kill those insects that spread diseases such as malaria and yellow fever.
But within 2 years of its introduction, many insects had become resistant to DDT.
In many such cases of pesticide resistance, the presence of the chemical switches on a gene that codes for an enzyme that inactivates the pesticide.

Another example comes from the use of warfarin as rat poison.
Warfarin is an anticoagulant. It kills the rat by inducing internal bleeding.
Mutant rats have an enzyme that allows their blood to clot in the presence of warfarin. The mutants survive to breed, so increasing the frequency of the resistant allele in the population.

Some rat colonies have developed resistance to warfarin

Myxomatosis

When the European rabbit was introduced to countries like Britain and Australia, its numbers soared owing to the lack of predation and competition.
The myxomatosis virus is spread by fleas and, when introduced into Britain, it succeeded in killing 99% of the rabbit population.
Resistance in the remaining 1% was the result of one or two mutant genes.
In the first case, the mutant gene coded for an enzyme that made the myxomatosis virus inactive.
More interestingly, the second mutant gene affected the rabbits' behaviour.
They spent more time above ground, out of the congested burrows in which the fleas were more likely to spread the virus.

Copper tolerance

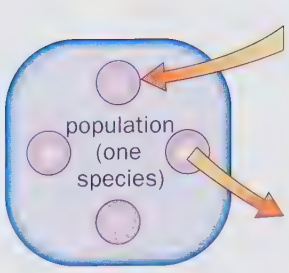
Copper is a toxic metal that can prevent plants growing on spoil heaps.
Resistant strains of the grasses *Festuca* and *Agrostis* have appeared.
The resistance seems to be linked to the plant's ability to transport copper out of the cell into the cell wall, so that it has less effect on cell metabolism.
These copper-tolerant grasses have a strong selective advantage at the sites of old mine workings, and have been used to reclaim land from spoil tips where previously no plants could grow.

All the examples on this page illustrate directional selection.

Copper-tolerant grass reclaiming the land of this disused mine

Species and speciation

Populations are groups of interbreeding individuals of the same species, occupying the same habitat at the same time.
But within each population there are breeding subunits called **demes**. Individuals within a deme tend to breed with each other more often than they do with individuals of other demes.
Although they remain part of the same gene pool, the flow of genes between separate demes slows, or may even cease.
Each deme may evolve along separate lines so that eventually the demes may become so different that, even if they were to be reunited, they would not be able to breed successfully with each other.
They would become separate species, each with their own gene pool.
This process is known as **speciation**.



deme (a group of interbreeding members of one species)

If the members of a deme are isolated to such an extent that their capability to interbreed with other demes is lost, it will lead to the formation of a new species

Allopatric speciation

Allopatric speciation is the development of a new species as a result of populations being physically separated.
A **physical barrier**, such as a river, mountain range or stretch of sea, prevents populations of the same species from interbreeding.
Let's look at the example on the right.

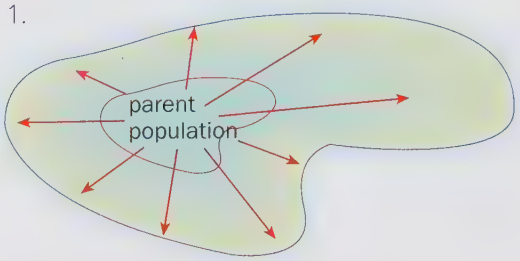
1. The parent population of a single species occupies an environment that does not alter a great deal.
   The population is able to move into new areas of the environment – we say it 'expands its range'.
   The population has a single gene pool and individuals are able to interbreed freely – we say that there is regular 'gene flow'.

2. Geological processes cause the environment to change.
   Physical barriers appear, such as a mountain range and a stretch of ocean.
   These barriers cut off parts of the population and gene flow is prevented.
   This partition of a population by such physical barriers is known as **geographical isolation**.

3. The isolated populations may be subjected to different selective pressures. For example, the more northern population B will live in a colder climate. So selection will favour animals that are larger and that have longer fur.
   The southern population lives in a warmer, drier climate so smaller individuals with shorter fur will have a selective advantage.
   Because of the effects of natural selection on each gene pool, the two populations may become genetically different enough to be reproductively isolated, so producing new species.

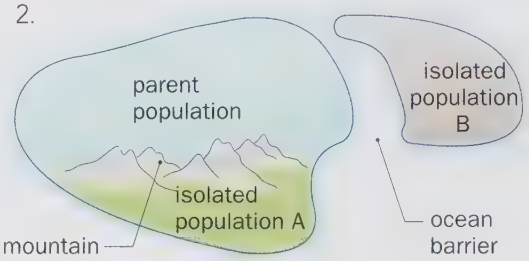
4. Geological forces act to break down the barriers dividing the two sub-species.
   They are able to mix, but by now each sub-species A and B has established a different gene pool and they are no longer capable of interbreeding. Two different species have evolved.

1.



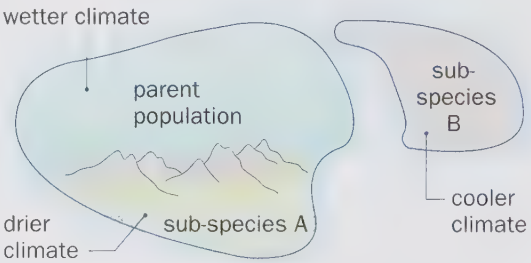
The parent population expands its range as it disperses to occupy new parts of the environment

2.



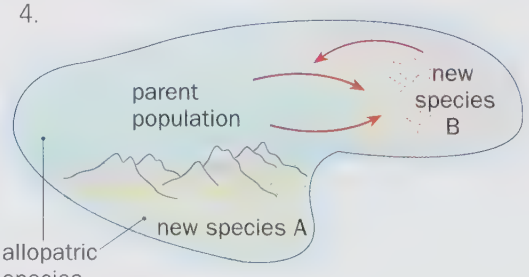
Geological processes cause the formation of physical barriers that prevent gene flow between the populations

3.



Each of the separated populations is subjected to different selection pressures by the environment

4.



Oceanic barrier breaks down and the population of the new species B is able to mix directly with the parent population again, but no interbreeding takes place

Sympatric speciation

Sympatric speciation is when new species are formed when there are no physical barriers.
It is usually caused when **reproductive isolation** occurs between individuals of a population. It can have a number of causes.

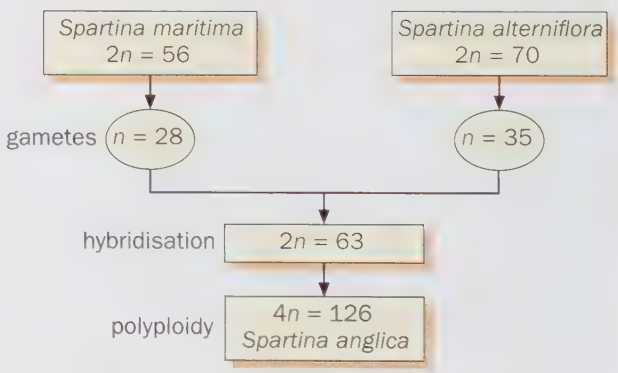
- **Behaviour**
  Animals attract mates with elaborate courtship displays.
  Calls, rituals, colour, markings, dances and body language may be used to attract the opposite sex.
  Speciation may result if the mating routine of one sub-species fails to trigger a response in a potential partner of another sub-species.
- **Structure**
  The sex organs of individuals in the population may not be compatible and so mating is prevented.
  For instance, many insects have a 'lock and key' arrangement for their genitalia.
  In the plant kingdom, pollen transfer can be affected by differences in flower structure.
- **Gamete mortality**
  Gametes may be prevented from meeting. In animals, the sperm may fail to survive in the reproductive tract of another sub-species.
  In plants, pollen may fail to grow on the stigma of a potential partner.
- **Hybrid inviability**
  Mating between two different sub-species may result in a zygote that fails to divide. This may be because the chromosomes no longer match each other, as is the case with polyploidy.
- **Hybrid sterility**
  Mating between two species results in a hybrid, but the species is reproductively isolated if the hybrid is sterile.
  A well known example is when a zebra is mated with a donkey.
  The resulting 'zebronkey' is sterile because of the failure at meiosis to produce viable gametes. The zebronkey has 53 chromosomes that are unable to form homologous pairs at prophase I of meiosis.
- **Polyploidy**
  Polyploidy can be due to a malfunction occurring during meiosis when the chromosomes fail to separate (see page 207).
  This can result in 'instant speciation' and the new polyploid species, though still in contact with the 'parent species', is reproductively isolated from it.
  Animals are rarely able to develop into a new species in this way, because they are usually sterile.
  Plants, however, can often self-pollinate or reproduce vegetatively.
  A successful example can be found in the cord grass *Spartina*.
  The diagram right shows how this polyploid hybrid was formed.
  Its vigorous growth and tolerance of varying salinities mean that it has been able to colonise estuarine mudflats.
  The cord grass fringes many harbours and estuaries in the UK, and if left unchecked can reach pest proportions.

Reproductive isolation can be important in preserving the uniqueness of a gene pool. It can prevent diluting effects of gene flow into the pool from other populations, which might be detrimental to existing good combinations that have resulted from natural selection.

Courtship in the great crested grebe

Zebra stallion (2n = 44) *Donkey mare (2n = 62)*

Karyotype of zebronkey offspring (2n = 53)



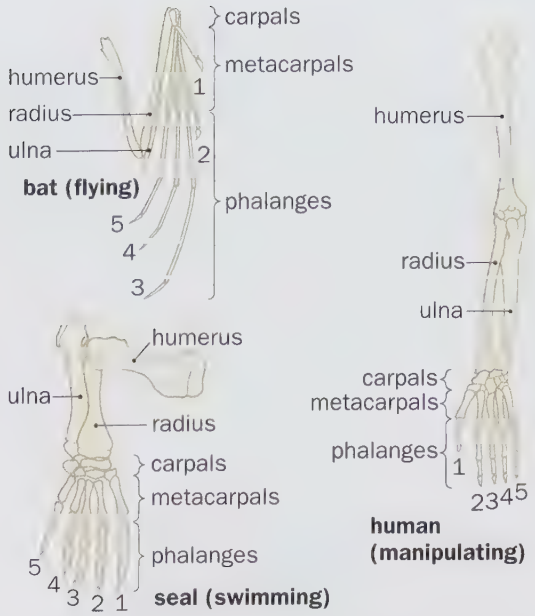
Evolution of Spartina anglica, a new species of cord grass

Cord grass colonising parts of an estuary

Classification

Estimates of the number of different species in the world vary from 3–30 million.
So far, over 2 million different kinds of organism have been described and identified.
The sorting of this vast array of living organisms into groups is known as **classification** or **taxonomy**.

Taxonomists look for differences and similarities between organisms.
Similarities between organisms may occur because they have evolved along the same lines.
For instance, the limb bones of mammals follow a similar pattern.
There is one upper limb bone, two lower limb bones and an arrangement of five digits.
These shared features are called **homologous structures**.
They can suggest how different organisms are related and the pattern of their evolution, a study called **phylogeny**.



The variations on the pentadactyl limb

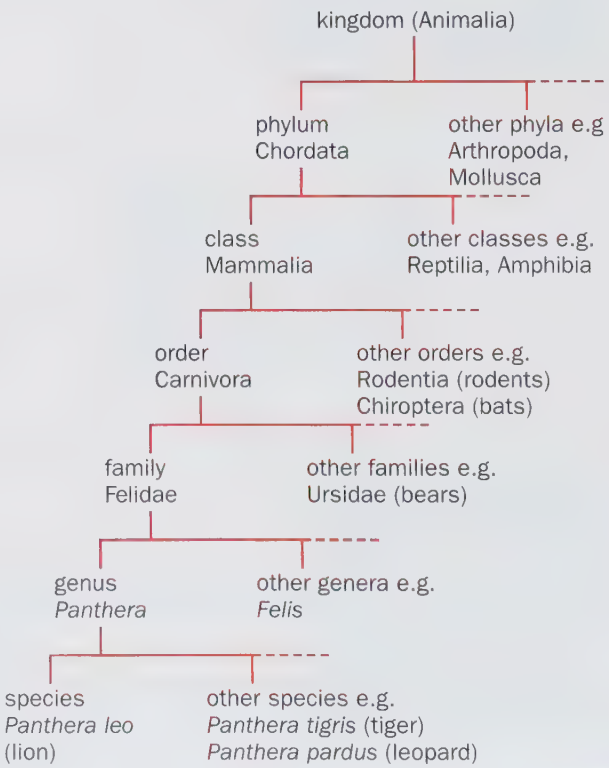
The system of classification that we use is based on the ideas of a Swedish botanist called Carl Linnaeus.
In the early 18th century, he decided that he could place animals and plants into **natural groups** based on their shared similar features.
He was the first person to produce an ordered system of classification.
Since then, his system of classification has been greatly modified.

A hierarchical system

Modern biology places organisms into **taxa**, a series of groups arranged in a hierarchy.
Each group is called a **taxon** and contains organisms sharing key features called **diagnostic characteristics**.

In any system of classification, taxonomists attempt to place similar organisms closely together and dissimilar ones further apart.
Large groups of organisms can be divided into successively smaller groups until you get to the smallest group: the **species**.
Such a classification that is ranked in ascending order is said to be **hierarchical**.
Starting with the smallest group we have:

- **species** – a group of similar individuals that can breed freely to produce fertile offspring, for example *Homo sapiens* (humans),
- **genus** – a group of species that are very closely related, for example *Canis latrans* (the coyote) and *Canis lupus* (the timber wolf),
- **family** – a grouping of similar genera (plural of genus), for example the Ranunculaceae family includes buttercup, columbine and larkspur,
- **order** – a grouping of related families, for example Falconiformes (the falcons),
- **class** – a grouping of similar orders, for example Pisces (fish),
- **phylum** – a large grouping of all the classes that share some common features, for example the Arthropoda includes crabs, spiders and insects,
- **kingdom** – the largest taxonomic grouping, for example plants and animals.



The name game

Living organisms often have common names. For instance, wall pepper, pennywort, roseroot and houseleek all belong to the same family of plants.

But some things have more than one name: the ox-eye daisy is also called dog daisy and Marguerite in different parts of the country.
It can get very confusing, especially if you want to describe a particular species to a person from another country.
Luckily, there is a system of naming living things that is universally understood by biologists, that is the **binomial system**.

The binomial system was first used by Linnaeus and was based upon Latin, a language widely used by educated people at that time.
It involved giving each organism ***two*** names: the name of its genus and the name of its species.
For instance, *Quercus rubra* is the red oak, and *Fagus sylvatica* is the common beech.

By using these 'scientific names', it is possible for a scientist in Germany to communicate accurately with a scientist in Japan about a particular species that they may both be working on. Furthermore, it allows biologists to be precise when talking about species, for example mosses, toadstools, beetles and worms, many of which do not have common names.

The binomial system is successful because each particular organism has its own unique scientific name and because we can often see straight away that two species are closely related.
For instance, *Gibbula umbilicalis* and *Gibbula cineraria* are the purple top-shell and the grey top-shell, both common on rocky shores.

Rules of the game

Any universally used system of classification, such as the binomial system, has to have rules so that people are consistent in using it. Here are some of them.

- The generic (genus) name is the first word and is always given a capital letter.
  Several species may share the same generic name, but each has its own unique specific name.
- The specific (species) name comes second and always starts with a small letter.
- Both names should either be written in italics (in printed text) or be underlined (if handwritten).
- The scientific name should be written out in full the first time it is used, for example *Culex pipiens*.
  But after that it can be abbreviated, for example *C. pipiens*.
- If the particular species is unknown, then the abbreviation 'sp' can be used, for example *Ectocarpus* sp.

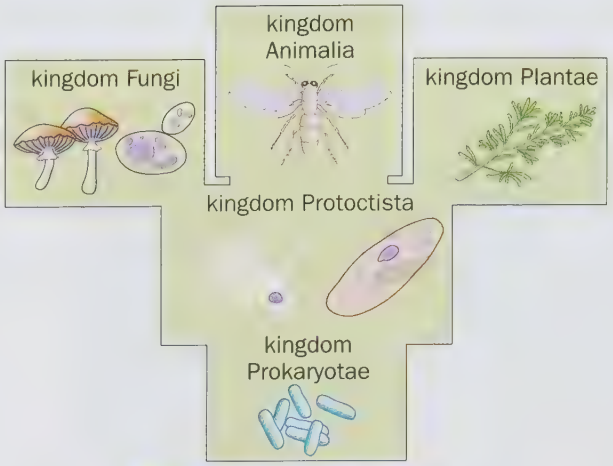
The purple top-shell (Gibbula umbilicalis)

Your classification is:

Kingdom:	Animalia
Phylum:	Chordata
Class:	Mammalia
Order:	Primates
Family:	Hominidae
Genus:	*Homo*
Species:	*Homo sapiens*

The five kingdoms

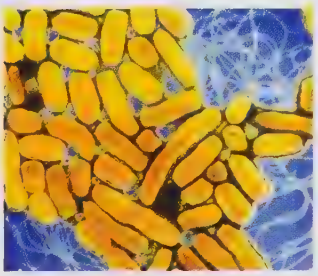
Older systems of classification tended to be based on a two kingdom plan: the plants and the animals.
Problems arose when groups of organisms could not fit into either category. For instance, into which group does the single-celled *Amoeba* fit?
The five kingdom classification has gained general acceptance.
It recognises two basic cell types: prokaryote and eukaryote.
The Prokaryotae includes all the bacteria and the cyanobacteria.
The Eukaryota includes the Protoctista, an odd grouping including algae, amoebae, flagellates and ciliates.
Also in the Eukaryota are the fungi, plants and animals.



Main features of the five kingdoms

Prokaryotae

Bacteria and cyanobacteria have prokaryotic cells.
The cell has no distinct nucleus enclosed by a nuclear membrane.
Neither do they have membrane-bound organelles such as mitochondria and chloroplasts.



Salmonella bacteria are prokaryotes

Protoctista

A collection of eukaryotic organisms including the algae (photosynthetic but not plants) to which the seaweeds belong.
Other protoctists include protozoans such as *Amoeba* and *Plasmodium*, ciliates such as *Paramecium,* and flagellates such as *Euglena*.
The protoctists seems to be a group in which organisms end up if they are not prokaryotes, fungi, plants or animals.
Many have eukaryotic cell features such as chloroplasts.

Euglena has chloroplasts but it is a protoctist not a plant

Fungi

Fungi are not able to photosynthesise.
Their cell walls are made not of cellulose, but of another polysaccharide called chitin.
Their bodies are composed of masses of filaments called **hyphae**, which are not divided up into separate cells.
Most are **saprobionts**, obtaining their food by the extra-cellular digestion of dead organic material. Some, however, are parasitic.
Fungi have membrane-bound organelles.

This bracket fungus grows from the bark of the tree

Plants

Plants are multicellular.
They have chlorophyll and are photosynthetic.
Their cells have a cellulose cell wall and a sap-filled vacuole.
Many have proper stems, roots and leaves.
Primitive plants reproduce by spores; more advanced forms, such as conifers and flowering plants, produce seeds.

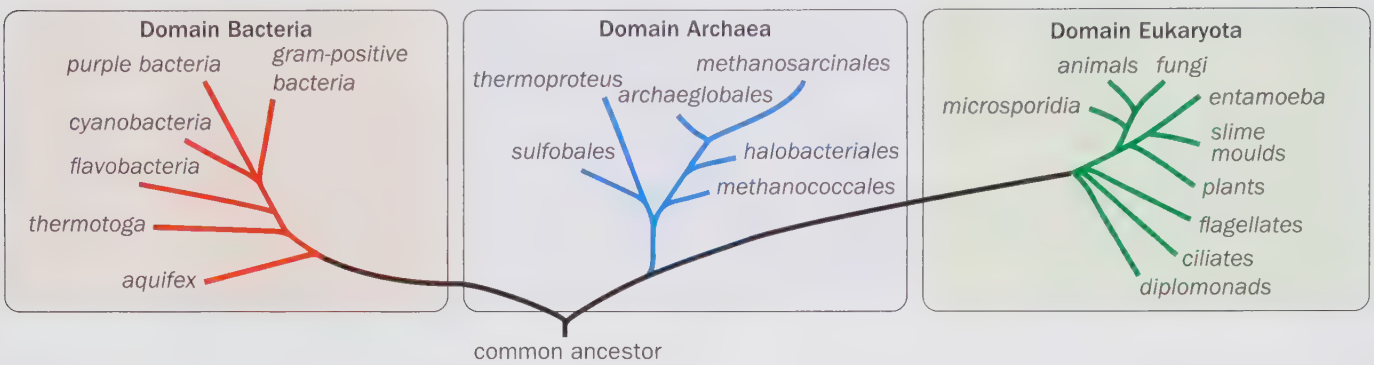
Animals

Animals are multicellular.
They are heterotrophic – they are unable to make their own food and need a supply of organic food material.
They have nervous systems and are able to move about.
Growth occurs throughout the body.

Vervet monkeys

Three domains?

Another classification system adds a taxon, the **domain**, above that of the kingdom.
In 1990, Carl Woese suggested the three domains on the basis of a detailed study of RNA.
It divides **Prokaryotae** into two groups: the **Bacteria** and the **Archaea**, all other organisms are placed in the **Eukaryota**.
This **three-domain system** of classification emphasises that the Bacteria and Archaea are two distinct groups that arose separately from a common ancestor.
Woese argued that these differences between the Bacteria and the Archaea are fundamental.
He even suggested that the two groups are more different from each other than the Bacteria are from the Eukaryota.
An accurate classification system must reflect this difference.



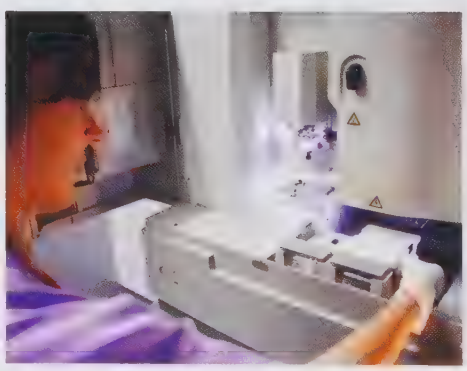
The three-domain system of classification

Classification using biochemistry

New techniques in biochemistry have helped determine how closely one species is related to another. Large biological molecules such as nucleic acids and proteins are found in all living cells, but they are not identical.
These differences can be used to reflect their evolutionary relationships.

Classification using DNA and amino acids

DNA and/or RNA are vital biochemicals in all living organisms.
New techniques enable molecular scientists to extract DNA from cells and use it to decipher the sequence of bases by means of sequencing machines.
It is then possible to compare the base sequences of DNA from different organisms to see how similar or different they are. The more similar the sequence, the more closely related the species.
As DNA is passed down from generation to generation it is possible to work out the evolutionary history of different species. (See DNA sequencing, page 416)
Coding DNA is used for classification because the gene sequences are very similar within a species, but different between species. It can also be used to work out the amino acid sequence of proteins.
As you know DNA base sequences code for amino acid sequences in a protein. The most often used proteins for this are **haemoglobin** and **cytochrome c** (an enzyme used in respiration). As with DNA base sequences, the more similar the amino acid sequence between two species, the more closely related they are.
For example, human haemoglobin contains 574 amino acids and the haemoglobin of a gorilla has 572 amino acids in common with those of humans.
A horse, on the other hand, has only 557 of its amino acids in haemoglobin in common with humans.
So the evolutionary relationships between a gorilla and a human are closer than between a human and a horse.



Loading samples into a DNA sequencer

Biology at work: Natural selection on the Underground

The idea of what a separate species is, is not as simple as we may think. Perhaps one of the best accepted definitions has been put forward by the respected zoologist Ernst Mayr:
'A species is a group of actually or potentially interbreeding natural populations that is reproductively isolated from other such groups.'

The London Underground, home to a new species of mosquito?

Recent research has indicated that a new species of mosquito could be well on the way to developing in the tunnels of the London Underground.
The tunnels have only existed since the mid-1800s, indicating that if conditions are right the development of a new species could be a much quicker process than previously thought.

One way a new species can develop is when part of a population becomes isolated from the rest of the population. The two populations live in different environments and are acted on by natural selection in different ways. Eventually, genetic differences build up to such an extent that members of the two populations can no longer interbreed.

Culex pipiens is the most widespread mosquito in the world and it thrives in stagnant water. In the late 1990s two British geneticists speculated that mosquitoes of the *pipiens* species had colonised the Underground when it was being built. In these tunnels the mosquito will have found lots of pools of stagnant water in which to lay eggs.

C. pipiens will have become adapted to its new environment. Two such adaptations are feeding on mammals, rather than birds, and mating in enclosed spaces rather than the more usual large open swarms.
Subsequently, a new form of the mosquito, known as *C. molestus* evolved in its new environment.
The geneticists also discovered that there is much less genetic variation in the Underground population compared with those living on the surface. This suggests that the original colonisers were small in number, and spread throughout the Underground as the network expanded.

Mosquito feeding

Perhaps the biggest indicator of *C. molestus* becoming a new species is that attempts to cross-breed it with its surface-living relative have failed.

Further research has also found genetic differences between *C. molestus* mosquitoes on different Underground lines. This may be due to the separation imposed by draughts blowing along and not between different tunnels.

Look back over the pages on species and speciation.
What process of speciation appears to be taking place on the Underground? Explain your decision.
Read this page once again and try to identify what selection pressures might be acting on the *C. molestus* population.
Ernst Mayr's definition of a species talks about reproductive isolation. Based on the information on this page what might be the cause of reproductive isolation between *C. pipiens* and *C. molestus*?

Biology at work: Recreating dinosaurs

In the film 'Jurassic Park' and its sequel 'The Lost World', dinosaurs were recreated from fragments of their DNA.
This came from the stomachs of ancient blood-sucking insects, which had been mummified inside amber.

Recently, American scientists claimed they had managed to extract insect DNA from amber that was up to 120 million years old.
It was thought that DNA could not last more than 100 000 years.
Scientists from the Natural History Museum in London spent over 2 years searching for fossil DNA from museum specimens.
They could not find any DNA in their insect specimens, but did find DNA from modern fungi and themselves!
This contamination of DNA samples explained the American's findings.
Molecular techniques are routinely used in the identification and classification of organisms.

A fly encased in Baltic amber

Systematics (or taxonomy as it is sometimes referred to) is the study of classifying or grouping organisms, both living and fossil.
It is concerned with:

- discovering and describing biological diversity,
- investigating evolutionary relationships between organisms,
- classifying so as to reflect these relationships.

Many biologists mistakenly think that all organisms have been classified and species identified.
The estimated number of species in the world is between 3 and 30 million.
Only 2 million have so far been identified.

The antiviral drug castanospermine is found in the Moreton Bay chestnut

Correct identification and classification has important applications.

- **Agriculture** – the control of the cassava mealy bug in Africa failed initially because of incorrect identification of the pest.
- **Medicine** – the antiviral castanospermine is a potential new drug found in an Australian plant, the Moreton Bay chestnut. A closely related Amazonian genus, *Alexa*, was identified and found to have greater quantities and less toxic forms of the drug.
- **Industry** – because fermentation products vary according to the strain of microbe involved, correct identification is essential for reliable production.
- **Forensic science** – sequencing the stages of corpse decomposition relies on the succession of decomposer communities. This is essential to reconstruct the events surrounding the cause of death.
- **Environmental assessment** – bio-indicators are used in pollution assessment. These include the use of diatoms to measure surface water acidification, the use of lichens for air pollution, and freshwater invertebrates for river pollution.

A related genus Alexa contains more of the drug

'Jurassic Park' and the resurrection of dinosaurs remain science fiction.

The mayfly Ecdyonurus is an indicator of clean water

Summary

- Variation is a result of both genetic change and environmental factors.
- Genetic change can be a result of the random distribution of chromosomes at metaphase I of meiosis, crossing over, random mating, random fertilisation of gametes and mutations.
- Gene or point mutations result from a change in the base sequence of DNA.
- Chromosome mutations result from a change in the chromosome structure or changes in the number of whole sets of chromosomes or individual chromosomes.
- Mutations can be induced by mutagens. These are certain types of radiation and certain chemicals.
- Natural selection results in individuals better adapted to surviving and passing their genes for beneficial characteristics on to the next generation.
- There are three main types of selection: stabilising, directional and disruptive.
- The separation of two populations as a result of geographical isolation or as a result of reproductive isolation can result in the formation of a new species. This process is called speciation.
- The sorting of living organisms into groups is called classification or taxonomy.
- Phylogeny is the study of how different organisms are related and the pattern of their evolution.
- The five kingdom system classifies organisms into prokaryotes, protoctists, fungi, plants and animals.
- A three domain classification has been proposed including Bacteria, Archaea and Eukaryota.
- DNA and amino acid sequencing is used to determine how closely one species is related to another.

Questions

1 Explain the process of natural selection in relation to each of the following: a) melanic moths, b) heavy metal tolerance, c) bacterial resistance to antibiotics.

2 There are 64 chromosomes in each body cell of a horse and 62 chromosomes in each body cell of a donkey.

Animal	Number of chromosomes in one of the nuclei formed at the end of		
	mitosis	first division of meiosis	second division of meiosis
horse			
donkey			

a) Copy and complete the table to show the number of chromosomes in the nuclei of these animals at the end of various stages of cell division.

b) A mule is the offspring of a cross between a horse and a donkey.

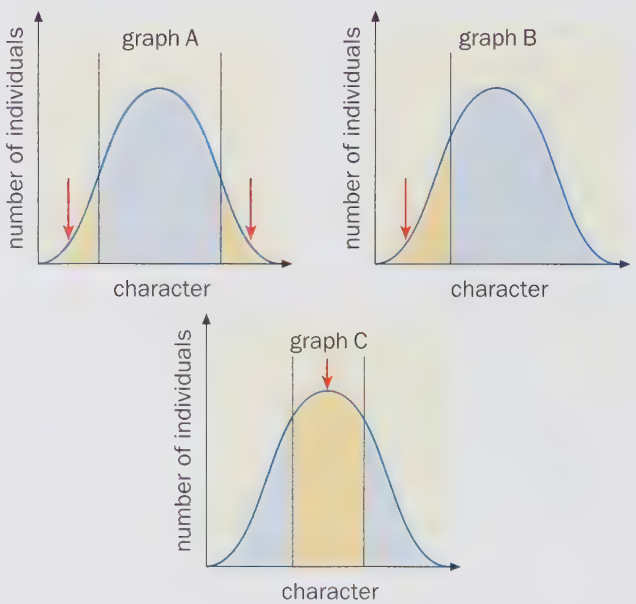
i) How many chromosomes are there in a body cell from a mule? Give a reason for your answer.

ii) Suggest why a mule is unable to produce viable gametes.

iii) Explain why a horse and a donkey are regarded as different species.

3 The graphs show three different types of selection. The shaded areas marked with arrows show the individuals in the population which are being selected against.

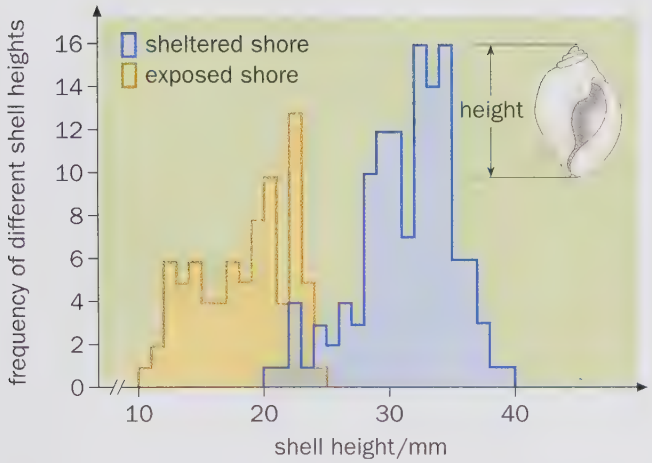
a) What name is given to the type of selection shown in graph A?



b) Describe one specific example of the type of selection shown in graph B. Be sure to name the organism and describe the character selected.

c) What will happen to the modal class in subsequent generations as a result of the type of selection shown in: i) graph B, ii) graph C?

4 The dog-whelk lives on rocky shores around Britain. The graph at the top of page 224 shows the variation in shell height in two different populations, one from a rocky shore exposed to strong wave action and the other from a sheltered rocky shore. Shell height was measured as shown in the diagram.



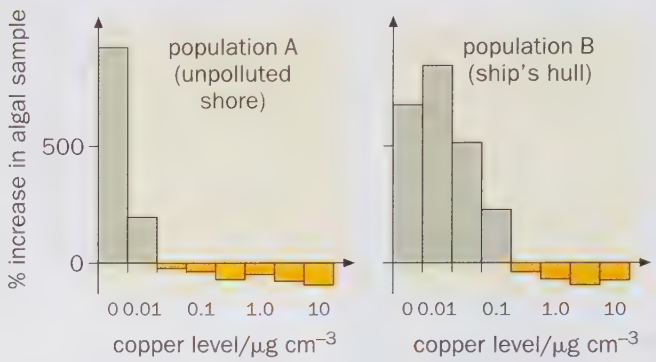
a) What types of variation are shown in the graph?
b) Describe two differences shown in the graph between dog-whelks from the exposed shore and dog-whelks from the sheltered shore.
c) In a follow-up investigation, it was found that dog-whelks on the sheltered shore had much thicker shells than those on the exposed shore. On the sheltered shore, there were more crabs, which are predators of dog-whelks. Describe how natural selection could account for this difference in shell thickness.

5 'The Chatham Islands lie 850 kilometres from mainland New Zealand. In 1980 the number of black robins, *Petroica traversi,* which live there dropped to 5. Today there are 200, all descended from a single breeding pair. DNA fingerprints from blood samples revealed that, for the genetic sequences used in the test, the robins appeared to be genetically identical. The robins are now prospering. Just over 70% of their young survive to fledgling state, compared with only 42% for their mainland cousin the bush robin, *Petroica australis.*'

Adapted from *New Scientist*, 31 May 1997

a) i) State the information conveyed in the scientific names about the taxonomic relationship between Chatham Islands black robins and mainland bush robins.
ii) From the information in the paragraph above, name the factor that may have been responsible for the divergence between the two populations of robin.
b) Suggest
i) the only way in which all robins on Chatham Islands may not be genetically identical,
ii) two reasons why low genetic diversity might be a disadvantage in the long-term survival of Chatham Islands robins,
iii) one reason for the difference in survival of young robins to fledgling stage in the two populations.

6 *Ectocarpus siliculosis* is a marine alga found growing on the shore and on the hulls of ships. Two populations of *Ectocarpus siliculosis*, A and B, were sampled. Samples of population A were collected from an unpolluted shore. Samples of population B were collected from the hull of a ship painted with antifouling paint, which slowly releases copper into the water. The diagram shows the percentage increase in algal samples collected from populations A and B and cultured, for identical periods of time, in media containing different concentrations of copper.



a) i) Describe two ways in which the data for the two populations differ.
ii) Explain how copper tolerance observed in this population may have evolved.
b) Despite thorough cleaning and repainting with antifouling paint, the hull of the ship rapidly recolonised. Suggest why shipping companies are prepared to spend money to prevent this recolonisation.

7 The classification system for living organisms is a hierarchy of phylogenetic groupings.
a) Explain what is meant, in this context, by
i) a hierarchy,
ii) phylogenetic.
b) Copy and complete the table to show the classification of the ocelot.

Kingdom	Animalia
	Chordata
Class	Mammalia
	Carnivora
Family	Felidae
	Leopardus
	pardalis

13 Biodiversity

Biodiversity is a general term used to describe the variety of life on Earth. It refers to the number and variety of living organisms and can be divided into three levels:

- **Genetic diversity** refers to the variety of alleles within a particular species.
- **Species diversity** refers to the number of different species and the number of individuals of each species found within a habitat.
- **Ecosystem diversity** refers to the variety of different habitats found within an ecosystem.

Coral reefs are highly diverse ecosystems

All three levels of biodiversity are important because living organisms interact with each other in numerous ways. Human activity often acts to reduce biodiversity at all three levels. Greater understanding of biodiversity has led to **conservation** in an attempt to conserve biodiversity worldwide.

Genetic diversity

Similarities and differences between organisms result from the variation in their DNA. It is these differences in DNA that lead to the vast genetic diversity that is found on Earth. Members of the same species have the same genes, but different combinations of alleles. These different combinations of alleles occur due to mutation, recombination during meiosis and random fertilisation by gametes. The number of different alleles within a species is called the genetic diversity of that species.

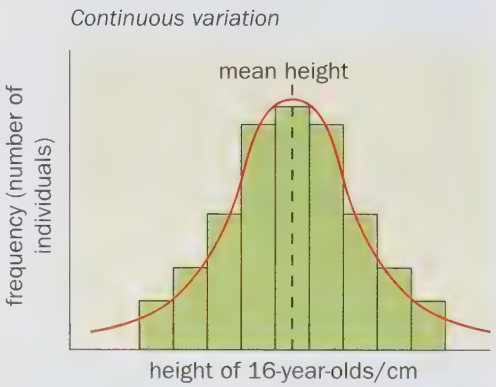
There are two types of **variation** that exist within species **continuous** and **discontinuous**.

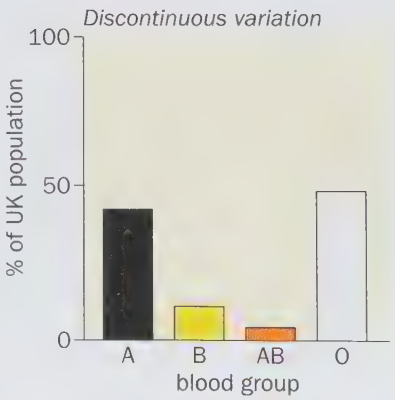
Continuous variation

Some characteristics show a continuous range of values, for example height. When these values are plotted, a frequency histogram will produce a smooth, bell-shaped curve. In continuous variation, there may be small differences between individuals which can be measured, for example length, mass and volume. These categories are therefore quantitative and each category is continuous with the next. Characteristics tend to be controlled by a large number of genes (polygenic characteristics). Continuous characteristics can be affected by the environment, for example body mass, muscular fitness, photosynthetic efficiency and growth rate.

Discontinuous variation

Here the characteristics can be divided into distinct categories in which individuals can be placed. In this instance, the histogram will assume a much different appearance. There is no overlapping between categories since the characteristics are distinctive and qualitative, for example hair colour. The characteristics tend to be one thing or another, with no 'in-betweens'. They tend to be controlled by one gene or a small number of genes and are generally unaffected by the environment, for example human blood groups, flower colour and seed shape. Continuous characteristics tend to be very common in humans and other organisms, whereas discontinuous characters are much less prevalent.





Percentage of the UK population with ABO blood groups

Loss of genetic diversity

Genetic diversity means that there are numerous different alleles present within a species. This is important because it is the basis upon which natural selection and evolution operate. A species with a high level of genetic diversity is likely to produce individuals with characteristics that enable them to survive environmental change. In contrast a species with a low genetic diversity is more likely to face extinction due to its inability to cope with changes in the environment. Genetic diversity tends to be higher in species with large populations, and lower in species with only small populations. A number of factors can contribute to a population having low genetic diversity, these include the **founder effect**, **genetic bottlenecks** and **selective breeding**.

Cheetahs are threatened by low genetic diversity

The founder effect

The founder effect occurs when only a few individuals from a population colonise a new region and start a new, isolated population. These few individuals will carry with them only a small range of alleles from the original population. These alleles may not be representative of the larger population. As a result the new population that develops from a few colonisers will show far less genetic diversity compared with the population from which it originated. Examples of founder effects can be seen when new volcanic islands arise out of the sea, for example the Galapagos and the Hawaiian Islands. Colonisation of these barren islands is difficult and rare.

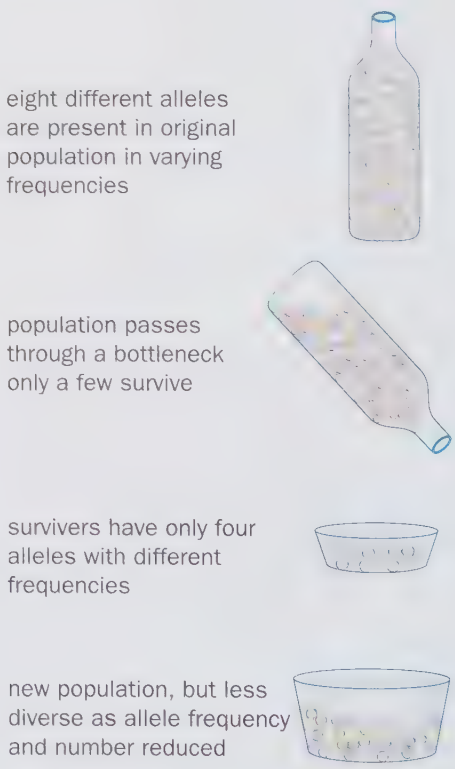
A few seeds may be carried there by winds, or perhaps animals may reach them by currents or on 'rafts'. But the few individuals that are able to colonise give rise to populations that are genetically distinct from the populations they left behind. In South Africa the Afrikaners have a high incidence of Huntingdon's disease, which is caused by a dominant allele carried by one of the original Dutch settlers.

Marine iguana on the Galapagos Islands

Genetic bottlenecks

Populations may from time to time suffer a dramatic decrease in size. This may be due to a chance event such as a volcanic eruption or human interference. The few individuals that are left will have a much smaller variety of alleles between them compared with the original population so their genetic diversity will be reduced. As these few individuals breed and re-establish a population, their genetic diversity will remain low. Many of the original alleles will have been lost in individuals that did not survive. At the end of the nineteenth century, the population of northern elephant seals was hunted almost to extinction by humans with a population of just 20 remaining in a colony on the coast of Mexico. The population has now recovered to around 30 000. However, the northern elephant seal has a far lower genetic diversity than the southern population in California, which was not intensively hunted.

Cheetahs are a threatened species partly due to their low genetic diversity. This is likely to be due to a genetic bottleneck at the end of the last glacial period, 10 000 years ago. Less diversity means fewer alleles, making it less likely that the population is able to adapt to major changes in its environment. There is, in fact, so little genetic diversity in the world's cheetah population that it has practically become a clone.



The bottleneck effect

Selective breeding

Selective breeding (or artificial selection) is the process by which animals or plants with characteristics useful to humans are allowed to breed. The aim is to produce offspring in which these characteristics are further enhanced.

In genetic terms, man rather than the environment determines which alleles pass to successive generations and which are lost.
This means that certain alleles are selected and others are rejected, so reducing the genetic diversity of these animals and plants.
The whole idea of selective breeding is to bring about change to a species so that it becomes more useful to humans. This can result in new breeds of animals and new varieties of plants.

Selective breeding has been practised for centuries and has been used to develop our modern crop plants, farm animals and domestic pets from their wild ancestors.

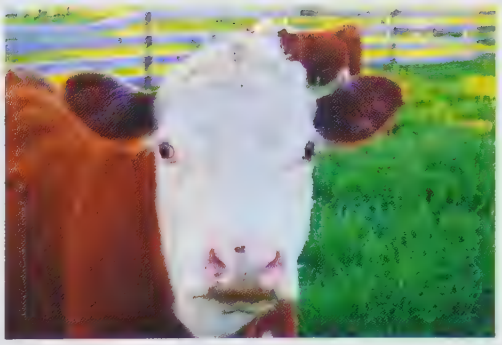
Cattle, for example, have been selectively bred for two reasons.
Breeds such as the Hereford and Aberdeen Angus have been selected for the quantity and quality of their meat, whereas other breeds such as the Jersey and Guernsey have been selected for their milk yield.

Techniques such as artificial insemination and embryo transplantation have increased the success of selective breeding in animals. However, more work has been carried out on breeding improved varieties of plants, as they have greater potential for providing food.
Increasing the yield of crops such as wheat, as well as breeding for disease resistance, are two important areas of research.

A very familiar example of selective breeding is the domestic dog.
There are many different breeds of dog, but they all belong to the species *Canis familiaris*.
The dog was selectively bred from the wolf (*Canis lupus*) around 13000 years ago.
The many breeds that exist have been selected for a whole host of characteristics such as speed, agility and the ability to follow scent.
Domesticated animals and plants will have a low genetic diversity.
This means that their chance of survival in the wild is reduced as they would have to compete with those wild species with greater diversity.
Low genetic diversity means that they are more prone to predation and disease, and more susceptible to abiotic factors, for example drought.

Another danger with selective breeding in animals is excessive inbreeding.
This involves selective reproduction between closely related organisms, for example offspring of the same species, which is common in pedigree 'show' dogs.
The danger is that there is a reduction in the variation of alleles within a population. This then increases the risk of homozygosity, and harmful recessive alleles being expressed in the phenotype.

As a result of selective breeding the development of physical problems can occur in domestic animals. Natural selection would mean that these particular animals would normally disappear in the wild due to competition.
This process of selection leads to an ethical issue as to whether animals are being harmed by this type of breeding.



Hereford cattle are bred for their meat

Guernsey cattle are selected for their milk yield

The wolf is the wild ancestor of many domestic breeds of dog

The bloodhound, bred for its tracking ability

The German shepherd, bred for speed and agility

Species diversity

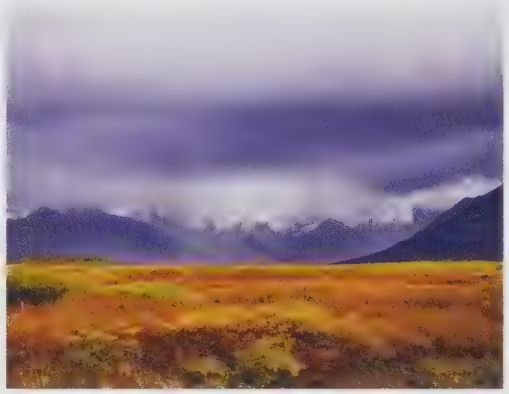
Species diversity is a measure of the number of different species in a habitat, and the proportion of the community that an individual species makes up. So species diversity takes into account not only how many different species there are in a habitat (**species richness**), but also the relative sizes of the populations and how well the species is spread through the habitat (**species evenness**). It may be that two communities have the same number of species, but differ a great deal in the proportion of the community that each species makes up. For instance, meadow or natural grassland and a field of barley may each contain 30 different plant species. But it may well be that the 30 species in the meadow are equally abundant, whereas in the field of barley, 90% of the plant species present consist of the barley only.

Tropical rainforests have high species diversity

Species diversity and ecosystems

Species diversity is an effective measure of the stability of an ecosystem. In general, the higher the species diversity, the more stable the ecosystem, and the less likely it is to be threatened by events such as climate change. Ecosystems with high species diversity are more likely to have individuals in the community that will be better equipped to tolerate change and maintain the community than an ecosystem with a low species diversity.

Hostile environments, such as deserts, are difficult for species to colonise and survive in. In such extreme conditions, only plants and animals that have evolved adaptations to enable them to cope with the lack of water and wide-ranging temperatures can survive. There are relatively few such organisms, and the population size tends to be fairly small. Hostile environments tend to have relatively low species diversity as only a few species are able to adapt and survive the harsh conditions, which leads to an unstable ecosystem. The distribution and abundance of organisms in this type of environment is largely determined by their ability to deal with the abiotic factors in the habitat. Biotic factors are relatively unimportant.



Barren sub-arctic tundra has low species diversity

In contrast, stable ecosystems, such as tropical rainforests, have high species diversity. In such environments, huge numbers of species are able to survive. This is a result of:

- high primary productivity and an increase in height and biomass of the vegetation,
- the forest ecosystem being able to support a large variety of organisms,
- a large number of different food chains,
- the height and density of vegetation providing numerous micro-habitats and niches,
- a high genetic diversity between the species present,
- a mature soil developing with increased depth and greater organic content,
- a stable ecosystem being far more able to tolerate changes in climate.

Low species diversity occurs in harsh environments, such as this desert near Petra in Jordan, because few species are adapted to survive

In a rainforest environment, abiotic factors are relatively unimportant in determining the distribution and abundance of species. The most important factors tend to be biotic ones, such as competition between plants for light.

Measuring species diversity

Various methods have been devised for quantifying the species diversity of a community. All of them are based on the relationship between the total number of organisms present and the number of individuals per species.

Since we are dealing with numbers, some method of **sampling** will have to be employed. It is not possible to count all the individual plants in a grassland habitat, and estimates such as percentage cover would not be appropriate.

Sampling strategy can be **random** or **systematic** (see page 450).
A number of **quadrats** can be used, or a **line** or **belt transect** can be employed. A **point quadrat** may be the most preferable method of sampling in this case, since data records the number of hits for a particular species (see page 450). The larger the number of samples taken the more reliable the data collected, but there is obviously a limit to the amount of time and effort that can be employed. However, it is important that samples are representative of the whole area. This is because some species may be quite dominant over the grassland, whereas others that are very rare may be clumped together in one corner.

Maths skills

Simpson's Diversity Index (D)
A good measure of diversity takes into account the species richness and their abundance.
One commonly used method is **Simpson's Diversity Index (D)**.

$$D = \frac{N(N-1)}{\Sigma n(n-1)}$$

where N = total number of individuals in the sample
n = number of individuals for each species

The higher the index, the higher the species diversity.

Data from 200 randomly placed point quadrats on a lawn

Species	n	$n-1$	$n(n-1)$
grass species 1	80	79	6320
grass species 2	45	44	1980
clover	9	8	72
black medick	22	21	462
daisy	13	12	156
dandelion	3	2	6
germander speedwell	10	9	90
self-heal	14	13	182
moss	36	35	1260
	Total (N) = 232		Total $n(n-1)$ = 10528

$$D = \frac{N(N-1)}{\Sigma n(n-1)}$$

$$\text{So, } D = \frac{232 \times 231}{10528} = 5.09$$

A community where one species dominates over the others will tend to have a lower species diversity than one where the species numbers are more evenly spread.
For example, the two communities below each have three species present, with a total of 100 individuals.

a)

Species	abundance	$n(n-1)$
A	90	8010
B	5	20
C	5	20
total	100	8050

$$D = \frac{(100 \times 99)}{8050} = 1.23$$

b)

Species	abundance	$n(n-1)$
A	34	1122
B	33	1056
C	33	1056
total	100	3234

$$D = \frac{(100 \times 99)}{3234} = 3.06$$

The community with the higher diversity is b). The dominant species in a) causes the Diversity Index to decrease.

Loss of species diversity

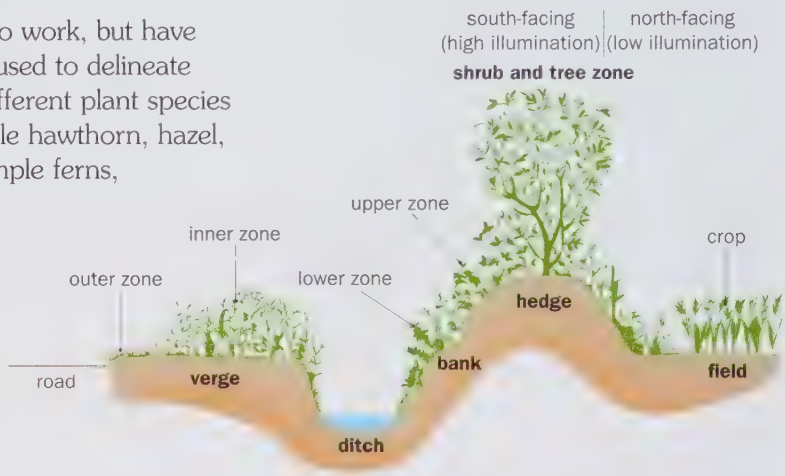
The extinction of species is currently taking place at an alarming rate. If natural selection operates, then the less well adapted species will be threatened with extinction.
This has always been the case, but what is different now is the accelerating pace of species extinctions and habitat losses as a result of human activities. These activities include agricultural practices, deforestation, pollution, hunting, habitat destruction and climate change.

Combine harvester at work on a monoculture

The effect of agriculture

Over the last few hundred years there has been huge increases in the human population. In part this has been due to the increased ability of the world's farmers to produce more food as a result of modern technology. These advances include:

- Arable farms use large **agricultural machines** to work very large fields. Examples are tractors and ploughs for preparing land for sowing seeds, and combine harvesters for harvesting crops.
- These large fields make the land easier and cheaper to work, but have resulted in the destruction of **hedgerows** previously used to delineate field boundaries. Hedgerows are made up of many different plant species at different zones, some at the shrub layer, for example hawthorn, hazel, elder and holly, and others at the bank layer, for example ferns, celandines, primroses and bluebells. These plants provide habitats and food for nesting birds, and hundreds of species of insects, which in turn provide small mammals with food. Traditionally hedgerows have acted as wildlife corridors allowing animals safe passage between woodlands.



Section of a hedgerow bordering a road on one side and a field on the other

- **Monocultures** are grown on these large fields. A monoculture is the same crop grown on the same land year after year. The main reason for growing a monoculture is to increase the productivity of farmland. The increased use of machinery and decreased labour costs mean that continuous cropping of one crop brings greater economic returns per unit area of land. Monocultures inevitably reduce both species diversity and genetic diversity of plants, but they also reduce the species diversity of animals because there are far fewer niches available.

Hedgerow being mechanically grubbed out in Oxfordshire

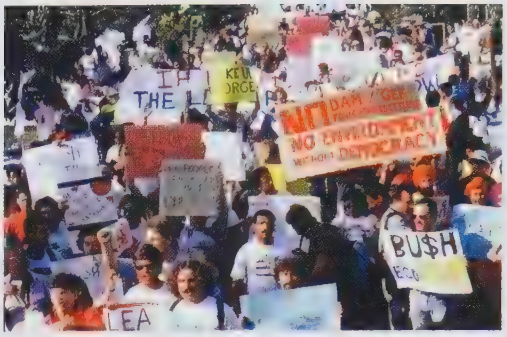
- **Agrochemicals** are used to improve crop productivity.
  Chemical fertilisers do not improve the structure of the soil, unlike organic fertilisers which will rot down and provide the soil with humus as well as nutrients. Chemical fertilisers containing nitrogen and phosphorus can leach out into nearby waterways causing eutrophication (see page 466).
  Pesticides are sprayed on crops to prevent damage from pests like insects and nematodes. But many pesticides are 'broad-spectrum', not just killing the target pests but also a wide range of other invertebrates so reducing animal species diversity (see page 469).
  Herbicides kill weeds that compete with crops for water, light and nutrients.
- **Selective breeding** has increased yield and made crops more resistant to drought and diseases. But as we have already seen it can result in a reduction in genetic diversity within species.

For the effects of **deforestation** on species diversity see page 460.

Conservation

Habitats are destroyed due to increased land-use for building, quarrying, dumping waste and agriculture. Human populations are making ever greater demands upon food resources and energy reserves, and producing more waste. It is important that we conserve the environment for the benefit of future generations. We have a duty of care to maintain not only biodiversity of species and habitats, but also the genetic diversity of species. We should conserve ecosystems, habitats and species for the following reasons:

- Ecosystems provide us with services such as providing food and fuels, and giving us areas for recreation. They provide us with useful substances such as medicines.
- Ecosystems help to maintain the balance of life on the planet, for example nutrient cycles.
- Habitats support a wide variety of organisms that interact in ways that we do not fully understand. This is often to the benefit of life on this planet, for example by keeping pests and diseases in check.
- Other species have as much right to live on this planet as we do. We have a role as guardians of the planet.



Demonstration at the UN Conference on Sustainable Development Rio+20, 2012

Endangered species

A species can be classed as being endangered if

- it is threatened with extinction,
- its numbers are reduced to a critical level,
- its population is so low that its reproduction is affected.

Many plants and animals have become extinct: the giant otter, wood bison, Parma wallaby and Tasmanian wolf to name a few. Their fate has been sealed due to the destruction of their habitats and their over-exploitation for commercial use. However, the American bison and the Saiga antelope in Russia have been brought back from the brink of extinction.

American bison

Deforestation has meant the extinction of thousands of rainforest plants that could have provided us with new medicines.
Illegal ivory poaching resulted in a loss of half the elephants in Kenya and 90% in Uganda in the 1970s.
As a result, the Commonwealth and International Trade in Endangered Species (CITES) imposed a worldwide ban on the ivory trade in 1989.
This move led to a significant increase in the elephant population.

The trade in furs and other animal skins seems senseless when there are plenty of imitation furs available. And the shooting and trapping has taken its toll on the wild species. The demand for these products comes mainly from the affluent countries of Europe and the USA. The trade in exotic birds takes 10 million birds from the wild each year. About half of these die even before they reach their destination. Commercial fishing requires legislation if it is to be sustainable. Improved fishing technology means that more fish are able to be caught. However, sustainable quotas should be agreed on how many fish can be caught, fishing should be avoided during the breeding season, and nets should have a minimum net mesh size to allow small, young fish to escape and survive to breed
(see page 234).

Confiscated pile of 27 tonnes of poached ivory and rhino horns being burned

Conservation in action

No species lives in isolation, so we have a duty to conserve ecosystems and habitats. Here are some of the ways in which this is being done.

Zoos and captive breeding programmes

Breeding animals in captivity, building up their numbers and eventually releasing them back into the wild has become increasingly common in many of the world's zoos. The Arabian oryx has been bred in captivity at Phoenix Zoo, Arizona, and herds have been released in areas where the species was previously extinct, such as Oman. Other species saved by captive breeding include the European bison and Pere David's deer. Many species of *Partula* snails became extinct on the Pacific Islands during the 20th century. Some are now kept in captivity and are being prepared for release back into their habitats.

Arabian oryx with young

Botanic gardens and seed banks

Botanic gardens are public gardens that keep collections of plants for conservation, research and education. We have seen the threat that deforestation, land development and agricultural expansion can bring to plant species diversity. There are now about 1600 botanic gardens worldwide, between them growing tens of thousands of plant species. Many of these are endangered species and by reintroducing them back into the wild the natural vegetation can be conserved. Seed banks are cold stores of seeds that originally concentrated on commercial crops, such as cereals and potatoes. But they also conserve seed stocks of endangered or valuable species. In southern England, the £18 million Millennium Seed Bank was constructed at Wakehurst Place. This building stores seeds from 10% of the world's estimated 250 000 wild flowering plants. The project stemmed from Britain's signing of the Convention on Biological Diversity in Rio, 1992. All the methods detailed above are examples of *ex situ* conservation.

The Svalgard Global Seed Vault in Norway is carved into the Arctic permafrost

National parks

These are large areas of land that have been set aside for wildlife. They may be occupied by people as well, and are patrolled by wardens, for example the game parks of east Africa such as the Masai Mara in Kenya and the Serengeti in Tanzania. Marine parks are protected areas of the sea where damage by fishing and pollution is prevented, for example Goat Island Marine Reserve in New Zealand. Ecosystems where land has been degraded can be re-established, for example the creation of the Guanacaste National Park in Costa Rica in 1989. These are examples of *in situ* conservation.

Sustainable management

In the UK the need to import so much timber from Scandinavia and North America can be reduced by recycling, and using wood that has come from sustainably managed forests. This means replacement planting as soon as trees are felled and establishing new forests on surplus agricultural land. It also involves the planting of more native trees in order to maintain and enhance biodiversity. As mentioned, if commercial fishing is to be made sustainable then effective legislation needs to be introduced. Many species are now ***overfished***. International agreements so far have failed to control the amount of fishing. Sustainable fishing and sustainable forestry often involve conflicting interests. Planning and cooperation is required at local, national and international levels if true sustainability is to be achieved (see pages 234 and 436).

A large catch of fish in a trawl being processed on a freezer trawler

Biology at work: Biodiversity in the Pantanal

One hectare of land in a tropical forest in the Amazon can have 650 tree species – more than in all of North America. This has left biologists baffled for decades. Successful species survive and reproduce, which depends on how readily they obtain resources. So if two species are too similar in their use of resources, they will compete with each other for a given **niche**. In any environment, niches are limited. That is why the diversity in a tropical forest cannot be explained by the exploitation of niches alone.

Although not as well-known as the Amazon rainforest, the Pantanal to the south is the world's largest wetland, covering 210 000 km^2 extending across Brazil, Bolivia and Paraguay. It is home to a staggering variety of plants and animals and is more biodiverse than the Amazon.




Seasonal flooding is the most important ecological feature of the Pantanal. Every year many parts of the **biome** change from terrestrial into aquatic habitats and vice-versa.
The Pantanal is characterised by a high density of various species of large vertebrates, many of them endangered, such as the Brazilian giant otter, the greater hyacinth macaw and the jaguar. There are densities of populations that are not observed in any other biome in Brazil, with averages per km^2 of 4.3 for alligators, 1.8 for capybaras (a giant guinea-pig) and 0.3 for marsh deer. It is for this reason that the Pantanal is considered one of the major wilderness areas remaining on Earth.

Species type	Numbers of species
plants	3500
birds	656
fish	325
mammals	159
reptiles	98
amphibians	53

Abundance of various animal and plant species in the Pantanal (Source: WWF)

Research of the jaguar population since 2009 at the Taiama Ecological Station on the Paraguay River in the Pantanal has shown that a population of 51 individuals exist in an area 300 km^2. A surprising number considering that the territory of these solitary big cats can be 70 km^2. It is thought that the high incidence of sandy beaches along the river that are used by large numbers of the jaguar's prey, such as capybaras, are responsible for this high number.

Jaguars on a sandy beach

The competition for niches is shaped by species' interactions with the environment, which includes both **abiotic** elements (climate, water, soil, etc.) and **biotic** elements (inter-specific interactions). Tropical forests have stable abiotic environments. Recent research has suggested it must be the biotic interactions that explain the extraordinary diversity in these tropical forests and wetlands. The research argues that an ***arms race*** between plants and plant-eaters is what drives evolutionary changes. When a plant-eater finds a new way to attack a plant, the plant must evolve to ***fight*** the plant-eater. Through many generations these adaptive changes cause the formation of new species, leading to the observed tropical diversity. This explanation is known as the 'Red Queen hypothesis'.

The lifespan of a plant can be hundreds of times longer than the average leaf-eater, which is usually a small insect. That is why a single tropical tree may have hundreds of distinct chemical compounds in its defence arsenal against herbivores.
Chemical analyses across forests in the Amazon show that neighbouring plants mostly have different defences than would be expected if it were a random process. Although Alice may not like it, the Red Queen seems to be in action.

Alice and the Red Queen

Biology at work: Sustainable fishing

Data for 2013 showed that worldwide 77 billion kilograms of fish were removed from the sea. Scientists fear that this rate of consumption may lead to the collapse of many of the world's fisheries.
It is generally accepted that if fish are to remain an important food source, sustainable fishing practices need to be widely adopted.

Plaice is one of the species under pressure from unsustainable fishing practices

Which species are under particular pressure?

Greenpeace has compiled a list of the major species sold in the UK that are under the most pressure, or are caught using the most wasteful or destructive fishing methods. Among these species are familiar names such as Atlantic cod, plaice, haddock and Atlantic salmon. Seafood is said to be sustainable if it comes from a fishery with practices that can be maintained indefinitely without reducing the target species' ability to maintain its population.

What determines whether a fishery is sustainable?

Apart from the health of the population the main determinant of a sustainable fishery is the method used to catch the fish.
Some techniques are very destructive such as **bottom trawling**, which effectively ploughs up the seabed, and others are indiscriminate such as **pair trawling**, which will catch non-target organisms (**bycatch**), such as dolphins. Other indiscriminate techniques are also associated with the efficient removal of hundreds or thousands of fish in one catch. **Purse seining** is an example of this type of fishing, which uses a net that herds fish together in large numbers.

The purse seine net encloses and removes thousands of fish in one catch

Overfishing

Techniques such as purse seining and **long lining** (a fishing line up to 100km in length with thousands of hooks) can result in overfishing, which basically means taking fish faster than their population can reproduce.
A good example of an overfished catch is the Bluefin tuna. This fish is in high demand, which has encouraged fishing using both purse seine nets and long lines. The result of these techniques has been a fall in the spawning population of Bluefin tuna by 70–80% since 1970.

Examples of sustainable fisheries

In the UK the best examples of sustainable fisheries are line caught mackerel and sea bass.
Line catching is a more selective technique and is often associated with small-scale fisheries. As a result the dangers of stock depletion and bycatch are greatly reduced.
Tuna and herring caught by rod and line are also good examples of sustainable fisheries.

Buying sustainable fish

A lack of clear information means that it is difficult for shoppers in the UK to be sure of buying sustainably-caught fish. However, the Marine Stewardship Council (MSC) does run a labelling scheme that certifies fisheries that are sustainable or making the effort to be so. Such a label informs shoppers that the fish they are buying is from a sustainable fishery.

Marine Stewardship Council (MSC) logo

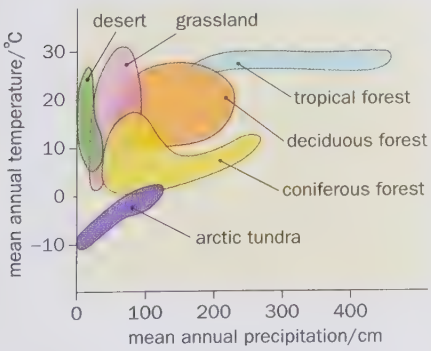
Summary

- Genetic diversity refers to the variety of alleles within a particular species.
- Species diversity refers to the number of different species and the number of individuals of each species found within a habitat.
- Ecosystem diversity refers to the variety of different habitats found within an ecosystem.
- Continuous variation occurs when characteristics show a continuous range of values, for example height.
- Discontinuous variation has characters that can be divided into distinct categories, for example blood groups.
- Loss of genetic diversity can occur due to the founder effect, genetic bottlenecks and selective breeding.
- The founder effect occurs when only a few individuals from a population colonise a new region and start a new isolated population.
- Genetic bottlenecks may be due to a chance event which results in a much smaller variety of alleles existing within a new population when it is established.
- Selective breeding means that certain alleles are selected and others are rejected, so reducing the genetic diversity of the resulting animals and plants.
- Stable ecosystems such as tropical rainforests have high species diversity.
- Hostile environments, such as deserts, are hard to colonise and survive in so have low species diversity.
- Measuring species diversity involves some method of sampling and the use of a diversity index.
- Loss of species diversity is mainly the result of human activities such as agriculture, deforestation, pollution, hunting, habitat destruction and climate change.
- Conservation aims to conserve the biodiversity of ecosystems, habitats and species.
- A species is considered endangered if it is threatened with extinction, has its numbers reduced to a critical level, or its population is so low that its reproduction is affected.
- Zoos and captive breeding programmes, botanic gardens, seed banks, national parks and sustainable management all contribute to conservation programmes.

Questions

1 Explain how each of the following act to reduce genetic diversity and give an example:
a) the founder effect,
b) genetic bottlenecks,
c) selective breeding.

2 The diagram shows the ranges of mean annual temperatures and precipitation (water falling as rain or snow) for six types of ecosystem. The Arctic tundra is considered to be an extreme, hostile environment whereas tropical forests are physically less hostile to living organisms.
a) Explain how the information in the diagram supports this view.
b) Describe and explain the relative effect of abiotic factors on the species diversity in the tundra and the tropical forest.



3 A tropical rainforest is considered to be a stable ecosystem. Explain this in terms of the following:
a) primary productivity,
b) species diversity,
c) number of micro-habitats,
d) number of food chains,
e) organic content of soil.

4 The table shows the total numbers of plants for each species found when sampling a grassland.

Species	A	B	C	D	E	F	G	H	I	J
Number	7	98	14	57	12	73	24	4	9	16

Calculate the diversity index (D) using the following formula:

$$D = \frac{N(N-1)}{\Sigma n(n-1)}$$

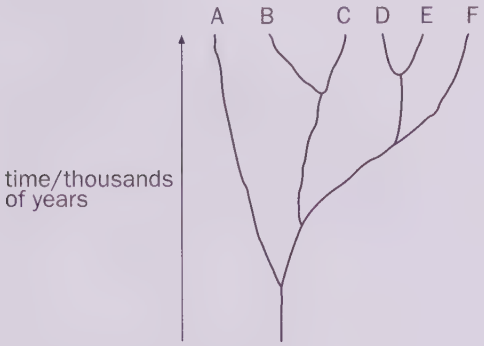
Where N = Total number of organisms of all species,
n = Number of individuals per species

Show your working.

5 Explain how each of the following agricultural practices contribute to a loss of species diversity:
a) the destruction of hedgerows,
b) monocultures,
c) the use of chemical fertilisers,
d) the use of pesticides.

Evolution and classification

1 The diagram below shows the relationship between six different species (A, B, C, D, E and F), which have evolved from a common ancestor.



a) i) Give the letters of the *two* species which are most closely related. [1]

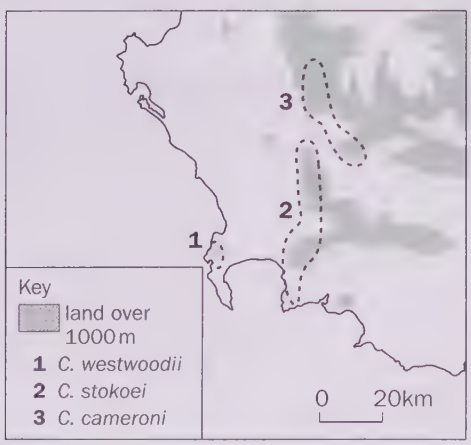
ii) Species A and species B are *less* closely related to each other than B and C. Use the information in the diagram to explain why. [1]

b) Living specimens of species A, B and C are available. Give *one* method scientists could use to determine which two of these species are most closely related. [1]

WJEC **[3]**

2 Beetles belonging to the genus *Colophon* are unable to fly and are found on hilltops in South Africa. The dotted lines on the map on the following page show the distribution of three species of this beetle. Suggest an evolutionary explanation for each of the following statements.

a) All of these beetles are of very similar general appearance. [1]



b) There are slight differences between the species of *Colophon* found in the three areas. [2]

c) The fact that beetles of the genus *Colophon* are unable to fly has been important in the evolution of twelve different species of the genus in a small area of South Africa. [2]

AQA (formerly AEB) **[5]**

3 *Testudo ephippium* is one of the species of giant tortoise found on the Galapagos Islands. Complete the table below to show its classification.

Kingdom	Animalia
	Chordata
	Reptilia
	Chelonia
Family	Testudinidae
Genus	

AQA (formerly NEAB) **[3]**

Biodiversity

4 Grevy's zebra *Equus grevyi* is one species of zebra found in East Africa.

a) What is a species? [1]

Unlike the plentiful plains zebra, the Grevy's zebra is endangered. In 1977, there were approximately 15 200 Grevy's zebras. Today there are fewer than 2000 remaining. Grevy's zebras are being crowded out of their grazing habitat by domestic livestock, and in the past have been hunted for their skins. The map shows the distribution of the remaining Grevy's zebra.

b) i) Use this information and your knowledge to explain what is meant by a genetic bottleneck. [2]

ii) A small introduced population survives in and around Tsavo East National Park in Kenya. The founder effect will influence the genetic diversity of Grevy's zebra in Tsavo National Park. Explain how. [2]

[5]

5 In 1800, only the red squirrel, *Sciurus vulgaris*, was found in Great Britain.
In 1879, a few individuals of *Sciurus carolinensis*, the grey squirrel, were introduced to southern England from the USA. Since then the range and number of grey squirrels have increased, and the range and number of red squirrels has decreased. Grey squirrels are larger, spend more time on the ground, and are less timid near people than red squirrels.
Northumberland is one of the few areas of England with a large population of red squirrels.
In an attempt to preserve the population of red squirrels, and the biodiversity of the UK, the government has funded a cull (trapping and killing) of grey squirrels in Northumberland.

a) i) Define the term biodiversity. [2]

ii) Suggest *two* reasons why the government feels it is important to conserve red squirrels in a particular area, such as Northumberland. [2]

iii) Some residents have objected to the culling of grey squirrels. Suggest *one* reason why people might object to the cull. [1]

Environmental groups have asked members of the public to report sightings of grey and red squirrels. In parts of Northumberland, the reported number of sightings of grey squirrels is higher than that of red squirrels.

b) Suggest *two* reasons why the actual number of grey squirrels might not be higher than the actual number of red squirrels in these areas. [2]

In 2010, a company applied for permission to build a wind farm in rural Northumberland. Before permission was granted for the development an Environmental Impact Assessment (EIA) was carried out by the local authority.

c) State *three* criteria that would have been considered when the EIA was carried out. [3]

OCR **[10]**

6 A group of students investigated the effect of outflow from a drain on the species diversity in a river. They sampled invertebrates from the river bed at two sites. One upstream of the drain, and one downstream of the drain. The table below shows their results.

	Mean number of organisms per m^2 of river bed	
Invertebrate species	**Upstream site**	**Downstream site**
flatworm	115	6
leech	4	32
snail	11	0
midge larva	10	2
black fly larva	74	0
mayfly nymphs	55	0
water hog louse	0	5
Gammarus	136	0
index of diversity	**4.08**	

The index of diversity can be calculated from the formula

$$D = \frac{N(N-1)}{\Sigma n(n-1)}$$

where D = index of diversity
N = total number of organisms of all species
n = total number of organisms of each species.

a) Use this formula to calculate the index of diversity for the invertebrates in the river at the downstream site. Show your working. [2]

b) It may be more useful to calculate the index of diversity than to record the number of species present. Explain why. [2]

c) The students could use the index of diversity to monitor levels of pollution in the river over a year. Explain how. [3]

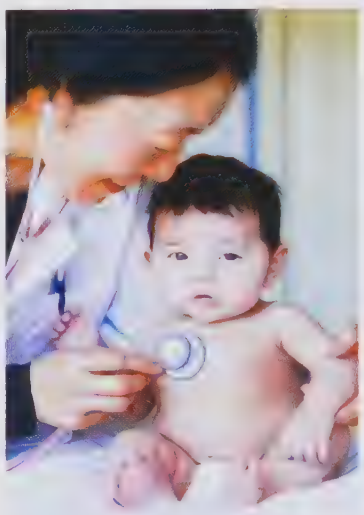
OCR **[7]**

14 The nature of disease

What do we mean by the word **health**?
Health has been described as
'the state of complete physical, mental and social well-being'.
Being healthy, then, means that you feel good physically, you have a positive outlook and are able to cope with the social and mental pressures that people experience in everyday life, and you do this without any great difficulty.
Being healthy is far more than just being free from disease.

To sustain a healthy lifestyle, a person needs to have a balanced and varied diet, should take exercise, have proper shelter and enough sleep. In addition, good hygiene will reduce the likelihood of infection.

What is disease?



To most of us the word 'disease' means that something is wrong with our body and that we feel unwell.
A disease is usually due to a malfunction of the body.
A doctor is able to diagnose what a disease is by looking at the **symptoms**. These may be physical, mental or both.

Diseases such as influenza are described as **acute**, because their effects come on suddenly and affect the body quickly.
The symptoms often disappear as quickly as they appeared.
Other diseases are more long-term, with the symptoms lasting for months or years.
Such diseases are referred to as **chronic**; their symptoms persist for a much longer time.
The table shows the major categories of disease and their causes.

Category of disease	Cause of disease	Examples
physical	temporary or permanent damage to part of the body	bone fractures, leprosy
communicable	invasion of the body by other organisms	rabies, malaria, influenza
deficiency	inadequate diet	kwashiorkor, scurvy, rickets
inherited	defective genes passed on from parents	cystic fibrosis, haemophilia, sickle cell anaemia
degenerative	organs and tissues 'wear' and do not work so well with age	arthritis, poor sight and hearing defects
mental	a wide range of disorders from psychological to those resulting from brain damage	depression, paranoia, schizophrenia
social	social interactions with family, friends, strangers	drug dependence, agoraphobia, alcoholism
self-inflicted	damage to the body as a result of the person's own actions	sun-related skin cancer

Communicable diseases

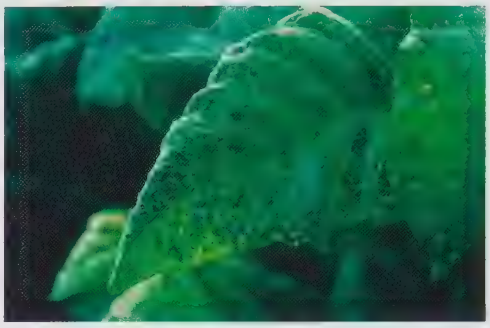
Organisms that cause communicable disease are called **pathogens**. Bacteria and viruses are probably the best known pathogens but many fungi, protoctists and parasites can also cause diseases in animals and plants. Diseases are said to be **communicable** or **infectious** if these pathogens can be passed from one individual to another.

The different types of pathogens include:

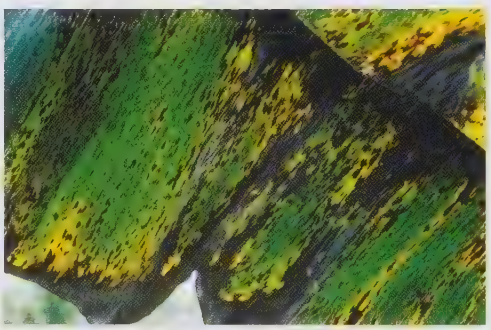
- **Bacteria** – cause diseases such as tuberculosis (see page 278) and bacterial meningitis (see page 294) which are spread by airborne droplets when an infected person coughs or sneezes.
  Ring rot occurs in potatoes and tomatoes and is caused by the bacterium *Clavibacter michiganensis*, which causes wilted foliage and rotting of potato tubers. It is spread when infected seed potatoes come into direct contact with healthy tubers.
- **Viruses** – cause infectious diseases such as HIV/AIDS (see page 280), influenza (see page 279) and in plants, tobacco mosaic virus (TMV), which has been at the centre of virus research since its discovery over 100 years ago. TMV consists of an RNA core and a protein coat. Once inside the cells of the tobacco plant, the virus releases its RNA which replicates to produce more viral particles (see page 75). The infection causes characteristic mosaic mottling and discolouration of the leaves.
- **Protoctists** – some pathogens and parasites are transmitted between hosts by **vectors**. For instance, mosquitoes act as vectors in the spread of malaria (see page 283). When the mosquito takes a blood meal, the malarial parasite *Plasmodium* may be injected into the person's blood along with the insect's saliva.
- **Fungi** – black sigatoka (*Mycosphaerella fijiensis*) is a fungus that is capable of destroying banana plantations. As its scientific name suggests it was first identified in Fiji in 1912. Over the next 40 years it spread to all banana-producing countries. It causes a rapid destruction of the leaf tissue, and up to a 50% reduction in bunch weight, so the crop becomes unsellable.
  Potato blight (*Phytophthora infestans*) is a well-known fungus which is notorious for causing the Irish potato famine of the 1840s. It affects the foliage of potato plants and eventually causes rotting of the tubers.
  Ringworm is a communicable skin disease in cattle caused by *Trichophyton verrucosum*, a spore-forming fungus. It is spread by direct contact causing exudates to ooze from the damaged skin and form scabs. Although unsightly, it causes little permanent damage or economic loss and cattle will recover without treatment.
  Athlete's foot is a common fungal infection of the skin causing flaking, inflammation and itching between the toes. It is transmitted in moist communal areas where people walk barefoot, for example in showers or by using an infected towel.
  It is estimated to affect 15% of the world's population.

Contaminated food or water can carry infectious diseases such as salmonella, cholera and typhoid.
Sexually transmitted infections (STIs) include gonorrhea and syphilis.
Any break in the skin surface reduces the barrier to infection and can allow pathogens such as tetanus and gangrene to enter the body through the skin's surface.

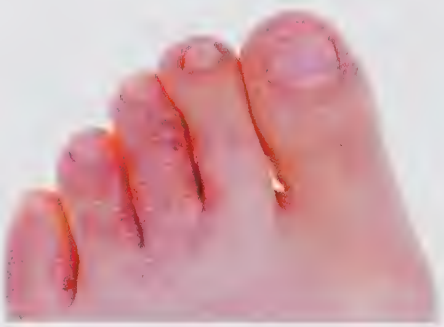
Slum conditions in Kibera, Kenya, encourage the spread of communicable diseases



Tobacco mosaic virus (TMV)

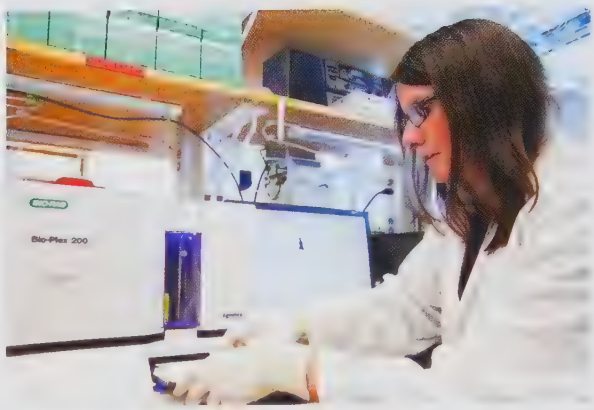


Black sigatoka disease is a fungus that attacks bananas



Athlete's foot is caused by a fungal infection

Patterns of disease



An epidemiologist processing data

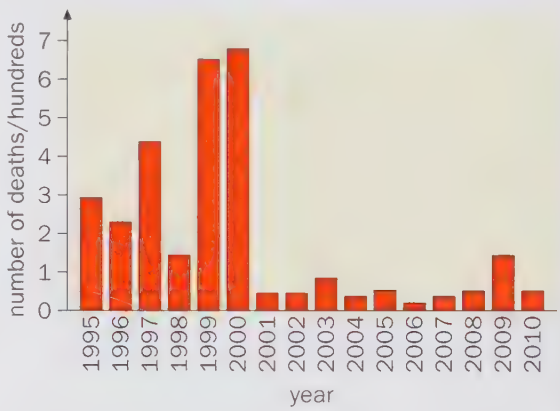
Epidemiology is the study of patterns of disease and the ways in which diseases spread through human populations.
Information about the distribution of a particular disease can enable us to identify its cause.
Such information can also be used to identify the ways in which communicable diseases are transmitted.
In the case of non-infectious diseases, the data collected may help to establish a link with a possible cause. An example of this was establishing the link between smoking and lung cancer in the 1950s.

Epidemiologists collect data from a particular target population. For instance, the numbers of deaths from lung cancer per 100 000 population aged between 18 and 65 in London.
Data expressed in this way allows fair comparisons to be made over time.
The type of data collected on the spread of disease includes:

- **incidence** – the number of new cases occurring over a particular time,
- **prevalence** – the number of people who have the disease over a particular time,
- **mortality** – the number of deaths from a certain disease over a particular time.

Such data can be used to assess the nature of a disease, which may be:

- **endemic** if a communicable disease is always present in a population; diseases such as measles, mumps and TB are endemic in the UK,
- **epidemic** if a communicable disease spreads rapidly through a population, such as the epidemics of new strains of influenza that can occur,
- **pandemic** if the disease spreads over a wide area, such as a continent or the whole world. TB and AIDS are pandemic diseases.



Deaths from influenza in the UK 1995–2010 (Source: WHO Mortality Database, March 2012)

Worldwide disease

In developing countries in Africa and Asia, about 40% of people die from communicable diseases.
Each year more than 14 million children under the age of 5 years die from diseases associated with poor medical aid, poor housing and malnutrition. These diseases include diarrhoea, dysentery and measles.

Contrast this situation with the developed countries (for example Europe, Japan, and the USA).
Here there is better medical care and few people die from communicable diseases. Instead, illnesses in these countries tend to be linked to affluence.
Overnutrition (leading to obesity) and smoking are among the unhealthy lifestyles that can lead to the onset of diseases such as cancers and **coronary heart disease** (**CHD**).

In developed countries, the low level of communicable disease means that a very high percentage of infants survive to become adults and that people live to an old age.

By contrast, in developing countries, life expectancy is shorter and infant mortality rates are higher, and this is linked to the incidence of communicable disease and a relatively poor diet.

Despite this gap, progress has been made and the World Health Organisation (WHO) reported a global average life expectancy of 70 in 2012, and an infant mortality rate of 34 deaths per 1000 live births for the year 2013. Improved education, good medical services, improved living conditions and the relief of poverty are essential if these goals are to be achieved.

Contaminated water supply

A balanced diet

A **balanced diet** is one that provides an adequate intake of the nutrients and energy needed to sustain the body and ensure health and growth.

A **nutrient** is a substance in food that provides a benefit to the body.

Each type of nutrient carries out one or more of three basic functions.

- ***To provide energy*** – this is mainly the role of carbohydrates and fats (proteins are only used as respiratory substrates if carbohydrates and fats are in short supply).
- ***To allow growth and repair*** of body cells and tissues. Proteins in the diet provide a source of amino acids with which cells can make their own proteins.
- ***To regulate the body's metabolism.*** These nutrients include vitamins and minerals that are needed in very small amounts in our diet. These are called **micronutrients**.

Carbohydrates, proteins and fats have to be supplied in large quantities in the diet every day, so these are called **macronutrients**.

Nutrients and their role in the body

Carbohydrates

Carbohydrates are composed of carbon, hydrogen and oxygen.
As you saw in Chapter 1, there are three main groups of carbohydrates:

- **monosaccharides** – single sugars, for example glucose, fructose and galactose,
- **disaccharides** – double sugars, for example maltose, sucrose and lactose,
- **polysaccharides** – chains of sugars, for example starch, glycogen and cellulose.

The major functions of carbohydrates in the body are as energy sources, energy stores or structural substrates.

Some foods rich in carbohydrates

Lipids

Lipids are composed of carbon, hydrogen and oxygen, but with less oxygen than carbohydrates.
Triglycerides are made up of three **fatty acid** molecules and one molecule of **glycerol**. Their main function is as an energy store.

Phospholipids make up a major constituent of the cell-surface membrane.

Proteins

Proteins are composed of carbon, hydrogen, oxygen and nitrogen (some also contain sulfur).
They are made up of **amino acids**, which form chains, helices and folded structures, giving each protein its particular property. (Remember, there are 20 different amino acids.)

Many proteins have a structural function but **enzymes** and **hormones** which control metabolism are globular proteins.

Some protein-rich foods

Differences in energy needs

There are a number of reasons why different people have different energy requirements.

The most important factors determining the energy requirements of an individual are metabolic rate and physical activity.

- **Basal metabolic rate (BMR)** is the rate at which energy is used up when the body is at rest.
  It is the rate of respiration that the body needs to keep 'ticking over' during periods of inactivity.
- **Diet-induced thermogenesis (DIT)** is the increase in the body's heat production by cellular respiration after food is eaten.
  This increase is greater if pure protein is consumed rather than carbohydrates and fats, but of course most diets are mixed.
- **Physical activity** is the factor that varies most in determining a person's overall energy requirements.
  The effect of physical activity varies according to body size and the duration and intensity of the activity.

The energy needs of people also vary according to age, gender, activity, pregnancy and lactation.

- **Age**

Children have a greater energy requirement than adults.
They have a larger surface area to volume ratio than an adult and therefore a greater heat loss.
A young child will weigh less than an adult but has a higher BMR because the child is still growing.

- **Gender**

Women have a relatively higher body fat content than men.
Fat tissue has a lower metabolic rate than muscle.
So women generally have a lower energy requirement than men.

- **Activity**

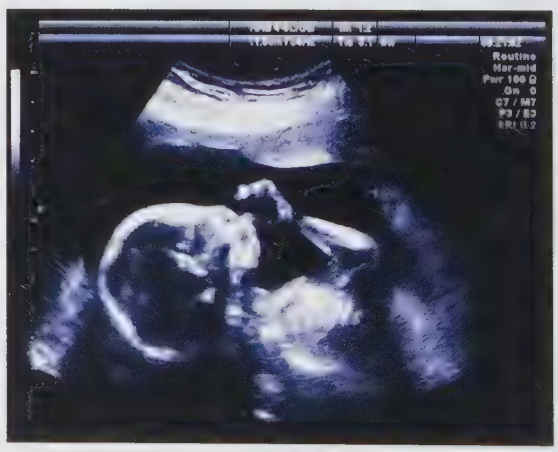
As we have said, physical activity uses up energy.
The more active you are, the more energy you need.
The type of activity and its duration will affect the amount of energy needed.

- **Pregnancy**

During pregnancy, the growth of the fetus uses up extra energy.
A woman in the later stages of pregnancy may require about 0.8 MJ more than the 9 MJ per day recommended for a non-pregnant woman.

- **Lactation**

During breast-feeding, the production of milk is a drain upon the energy reserves of the mother.
Some energy will have been stored by the mother as fat during pregnancy.
But she will also need to eat more each day to get the extra energy that she needs.



Ultrasound scan of fetus in womb

Differences in nutrient needs

Many of the factors that affect energy requirements also affect the body's need for other nutrients.

- **Age**

Infants and young children require more protein per unit of body mass than adults because they are still growing and need protein to make new cells.
Each tissue and organ has its own **critical period** when growth is rapid.
For instance, the critical period for the brain is during fetal development and the first year after birth.
There are two critical periods for muscle development – infancy and adolescence.

Elderly people sometimes pay too little attention to their diets.
Lack of fresh fruit and vegetables makes vitamin C deficiency quite common in this age group.

Osteoporosis or decalcification of the bones can also be a problem in old age.
Minor falls can easily result in bone fractures.
The condition is more prevalent in women (after the menopause) than in men.
It is caused by low vitamin D intake, although vitamin D levels can be raised by exposure of the skin to sunlight, which enables the body to make its own vitamin D.

Why do you think that small doses of vitamin D (5–10 mg per day) are recommended for housebound people who spend a lot of their time indoors?

X-ray of an elderly woman's hip showing a fractured femur due to osteoporosis

- **Gender**

A poor diet can lead to anaemia, which can be a problem in adolescent girls.
Anaemia is due to iron deficiency, which may be caused by the onset of menstruation.
A dietary supplement of iron tablets corrects the condition.

- **Activity**

Physical exercise can increase the body's protein requirement, for example activities such as body-building where greater development of muscle occurs.

- **Pregnancy**

There is a general need for increased nutrients during pregnancy.
This is especially true for vitamins A and C and for calcium which is needed for bone formation.
However, care should be taken not to exceed recommended doses of supplementary nutrients such as vitamins A and D as they can be toxic.

- **Lactation**

The demand for extra nutrients is greater during lactation than it is during pregnancy.
This is particularly true of protein, vitamin D and iron.
Since the milk-producing cells of the mother's breasts use nutrients derived from her diet, whatever she eats affects the composition of her milk.
So mothers should have a varied and balanced diet to produce healthy nutritious milk.

Dietary reference values and nutritional requirements

Each nutrient has a particular function in the body.
Some nutrients are needed in greater quantities than others.
For example, we need about 75 grams of protein per day but only a few milligrams of vitamin C.
Each person's nutrient and energy requirements are related to their age, gender, level of physical activity and state of health.
Some people absorb nutrients more efficiently than others and so have lower than average nutritional requirements.

In the UK, the estimated requirements for particular groups of the population are based on advice given by the Scientific Advisory Committee on Nutrition (SACN).
The SACN panel reviews scientific evidence and then makes proposals that are used by the government to formulate policy on nutrition and related health issues. In 2011, the SACN published its *Dietary Reference Values for Energy* report. The scientific evidence was examined by various groups within the UK population.

Dietary reference values

The SACN report details the **dietary reference values (DRVs)** for energy. This attempted to avoid the idea that everyone should be eating the ***same*** quantities of nutrients irrespective of age, gender, fitness, and so on.

DRVs	The nutrient and energy requirements for a particular group with reference to age, gender, fitness, etc.
EAR	An estimate of the average energy or nutrient requirements needed by 50% of the population
RNI	The amount of nutrients required to meet the needs of 97.5% of the population
LRNI	The amount of nutrients that meet the needs of 2.5% of the population

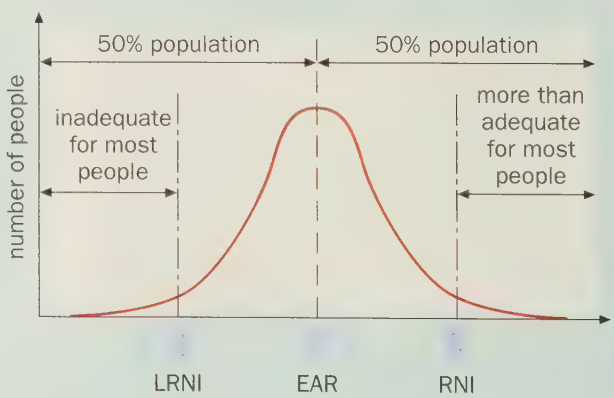
There are two groups of DRVs. The first refers to SACN's recommendations on energy and nutritional requirements and the second to vitamins and minerals. The graph here shows you how DRVs are used.

Maths skills

Estimated average requirement (EAR) is an estimate of the average requirement of energy or nutrients.
50% of the population will need more than the EAR for energy and nutrients and 50% of the population will need less.

Reference nutrient intake (RNI) is the amount of a particular nutrient that ensures that the needs of nearly all the population (97.5%) are met.

Lower reference nutrient intake (LRNI) is the amount of a nutrient that is enough for only a small number of people (2.5%). Most people will need more than this.
Note that RNI and LRNI apply ***only*** to nutrients.
The only DRV that refers to energy is EAR.



Energy or nutrient in diet every day

It is important to realise that these are not recommended intakes for an ***individual***, but a reference standard against which a comparison can be made for a ***population*** or ***group*** of people.

Safe intakes

The second group of DRVs refers mainly to vitamins and minerals.
The ***safe intake*** is the amount judged to be sufficient for everyone.

Levels of nutrient intake ***below*** the safe intake could risk deficiency.
But there is no evidence to suggest that intake above this level will give any further benefits.
Indeed some micronutrients are toxic in large quantities. Consequently, safe intakes are set well below levels that would be ***unsafe***.

How should DRVs be used?

As well as varying between different age groups, the requirements for energy and nutrients vary with gender, and, for females, during pregnancy and lactation.

The RNI should be used when assessing the dietary intake of a group. The nearer the average intake of the group to the RNI, the less likely it is that any individual will have a deficient intake.

The nearer the average to the LRNI, the greater the probability that some individuals are not getting enough. When planning a diet for a particular group, the aim should be to provide the RNI.

Age	Protein/ g day^{-1}	Calcium/ mg day^{-1}	Iron/ mg day^{-1}	Zinc/ mg day^{-1}	Vitamin A/µg day^{-1}	Folic acid/ µg day^{-1}	Vitamin C/mg day^{-1}
0–3 months*	12.5	525	1.7	4.0	350	50	25
4–6 months	12.7	525	4.3	4.0	350	50	25
7–9 months	13.7	525	7.8	5.0	350	50	25
10–12 months	14.9	525	7.8	5.0	350	50	25
1–3 years	14.5	350	6.9	5.0	400	70	30
4–6 years	19.7	450	6.1	6.5	500	100	30
7–10 years	28.3	550	8.7	7.0	500	150	30
males:							
11–14 years	42.1	1000	11.3	9.0	600	200	35
15–18 years	55.2	1000	11.3	9.5	700	200	40
19–50 years	55.5	700	8.7	9.5	700	200	40
50+ years	53.3	700	8.7	9.5	700	200	40
females:							
11–14 years	41.2	800	14.8	9.0	600	200	35
15–18 years	45.0	800	14.8	7.0	600	200	40
19–50 years	45.0	700	14.8	7.0	600	200	40

*Formulated
All the values assume a well-balanced diet in which DRVs for energy and all other nutrients are met. (µg = microgram 1000 µg = 1 mg)

RNIs for protein and six micronutrients

Maths skills

Calculating EARs for energy

EARs for energy are based upon the present lifestyles and activity levels of the UK population.
As with nutrient requirements, the energy requirements are related to age, gender, body size and levels of activity.

EARs are calculated by multiplying the BMR by the person's current **physical activity level (PAL)**.

$$EAR = BMR \times PAL$$

This overall ratio of energy used to BMR is determined by the lifestyle of a person.
A PAL factor of 1.4 reflects the lifestyle of most adults in the UK. This factor is suitable for adults who do little physical activity at work or in their leisure time.

For more active people, larger PAL values are used.
For example, a PAL of 1.9 is appropriate for very active adults.

Age	EAR/MJ day^{-1}	
	males	females
0–3 months (formulated)	2.28	2.16
4–6 months	2.89	2.69
7–9 months	3.44	3.20
10–12 months	3.85	3.61
1–3 years	5.15	4.86
4–6 years	7.16	6.46
7–10 years	8.24	7.28
11–14 years	9.27	7.92
15–18 years	11.51	8.83
19–49 years	10.60	8.10
50–59 years	10.60	8.00
60–64 years	9.93	7.99

The EARs for adults (over 19 years) given here are based on low activity levels (PAL of 1.4).

EARs for energy for different age groups and sexes

Calculate the EARs for:

a) a 16-year-old male with a BMR of 8.15 MJ day^{-1} at the following PALs: 1.1, 1.4 and 1.9,

b) a 19-year-old female with a BMR of 5.78 MJ day^{-1} at the following PALs: 1.2, 1.4 and 1.8.

Compare each of these values with the figures for EARs given in the table for PALs at 1.4.

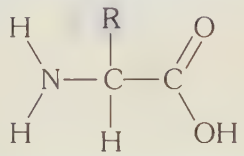
Essential amino acids

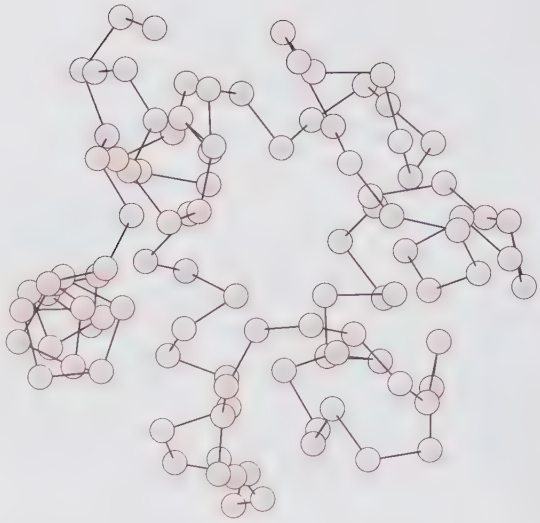
Proteins have a number of functions in the human body:

- they make up body protein such as muscle,
- all enzymes are proteins and so they control metabolic pathways,
- many hormones, for example insulin, are proteins.

All these proteins are made inside cells from a pool of amino acids.

There are 20 different amino acids. As you have seen in Chapter 1, they all have the same basic structure, but each one has a different R group.





The tertiary structure of the protein cytochrome C

Amino acids can be divided into two main groups.

- **Non-essential amino acids**, which the body is able to make itself. There are eight of these, which we can synthesise from simpler compounds.
  For instance, the amino acid alanine can be synthesised from the compound pyruvate.
- **Essential amino acids** (**EAAs**), which can only be obtained in our food.
  There are 12 amino acids that cannot be synthesised from existing organic compounds in the body.
  These EAAs must be supplied in the diet as we need them to make particular proteins.

Some EAAs are used to synthesise non-essential amino acids.
This takes place by the process of **transamination**.
For instance, the EAA phenylalanine can be converted to the non-essential amino acid tyrosine.
This means that if we are short of phenylalanine, then we may also be short of tyrosine unless it is present in the diet.

As we have said, cells assemble proteins from a '***pool***' of available amino acids in the cell. This pool is stocked with amino acids derived from protein that we have eaten.

As a general rule, any animal protein that we eat contains all the EAAs. But proteins in plant material contain only ***some*** of the essential amino acids. So if you are a vegetarian, you may need to supplement your diet to ensure that you have the full range of amino acids.

A vegetarian meal

Your cells are continually drawing on their pool of amino acids to make new structural proteins, enzymes and hormones.

If you are short of protein in your diet, the amino acid pool may be topped up with amino acids from the breakdown of body protein such as muscle.

Essential fatty acids

Lipids in the diet consist of fats, oils and waxes.
Most of our lipid intake (about 95%) consists of **triglycerides**.

You should remember from Chapter 1 that a triglyceride is made up of one molecule of glycerol and three molecules of fatty acids.

The other 5% of our lipid intake consists of **phospholipids**, which form an important part of our cell-surface membranes, and **cholesterol**.

Animal products generally have a high lipid content, since fats act as the main energy stores in the bodies of many animals.
Animal products with high lipid contents include dairy products, fatty meats and fish such as herring and mackerel.
The lipid contents of many plants are quite low, except for seeds, nuts and olives.
This is because their main energy store is carbohydrate, such as starch.

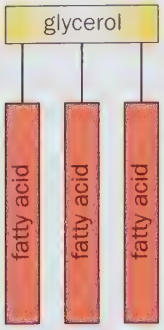
The body can synthesise many of the fatty acids it needs itself.
The few that it cannot make are called **essential fatty acids (EFAs)** and they must be supplied in our diet.
Examples include linoleic acid and linolenic acid.
They are vital for the formation of the phospholipids that make up the cell-surface membrane of plant and animal cells.

The liver has an important role in the conversion of EFAs such as linolenic acid into a form that can be used to make hormones such as prostaglandins.
These compounds are important in triggering the actions of the immune, renal and circulatory systems.

We need only very small amounts of these EFAs in our diet.
In fact, people with a healthy diet probably have a year's supply of EFAs in their fat stores.

However, if there is a deficiency of EFAs in the diet, it can reduce growth in infants, affect the healing of wounds, and cause hair loss and scaly skin in adults.

Remember that, where possible, your diet should contain unsaturated fats, as these carry far fewer health risks.



a simple diagram of a triglyceride

The structure of a triglyceride

Many animal products have a high content of saturated fatty acids

A fast food outlet in Kuwait

Vitamins

Vitamins are a group of unrelated organic compounds.
They cannot be synthesised by the body and therefore they are ***essential*** to the diet.
They are needed only in small amounts but if they are lacking in the diet they can result in **deficiency diseases**.
This is because many vitamins are needed by the body to make **coenzymes**, such as NAD, FAD and NADP.

A coenzyme is a non-protein that needs to be present if an enzyme is to work.

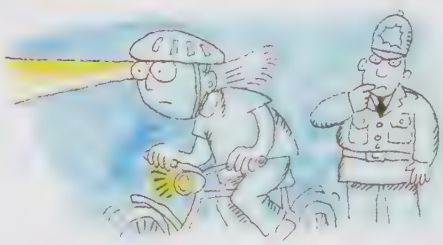
So, without coenzymes, enzymes fail to function and biochemical pathways become blocked.
For instance, the vitamin **niacin** is needed for the formation of NAD and without it many of the energy-releasing reactions of the cell cannot take place.

Maintaining vitamin and mineral intake

Vitamin A

Vitamin A is also called **retinol** and is found in only a few foods.
It is present in milk, eggs, liver and fish liver oils.
It is also present in fruits such as mangoes and papaya.
Retinol can be synthesised from carotene, a photosynthetic pigment found in vegetables such as carrots, spinach and cabbage.

Children deficient in vitamin A often develop dry skin and hair.
They become prone to infections of the ear, urinary and digestive systems.
Most noticeable, however, is an inflammation of the eyes leading to a drying and ulceration of the cornea called **xerophthalmia**.
The ability to see in dim light is diminished, a condition known as **night blindness**.
Light-sensitive cells in the retina called **rods** are able to detect light of low intensity.
They convert vitamin A into the light-absorbing visual pigment **rhodopsin**.
A lack of retinol in the diet means that not enough rhodopsin is synthesised and rod cells are unable to function (see page 355).



'He must be fond of carrots'

Vitamin D

Vitamin D has been called 'the sunshine vitamin' because if the skin receives enough sunlight, the body is able to synthesise enough vitamin D without it being needed in the diet.

However, given our climate, we rely upon a dietary intake of vitamin D.
Dark-skinned people living in Britain produce little vitamin D themselves.
So they need to ensure that their diet includes sufficient amounts.

Sources of vitamin D in the diet include fish liver oils, egg yolk and milk.
Vitamin D is necessary for the small intestine to absorb calcium.
It is also needed to regulate the deposition of calcium in bone cells.
So deficiency symptoms include the lack of calcium in bones, causing **rickets** in children.
In adults, deficiency gives rise to the condition known as **osteomalacia**, which leads to a softening of the bones and an increased chance of fractures.

Rickets causes bowing of the legs, and wide elbow and knee joints

Malnutrition

Malnutrition is nutrition that deviates from the normal.
When these deviations are large, then clinical symptoms appear.

Undernutrition is often associated with underdeveloped nations.
We are accustomed to seeing news programmes on the television showing starving people in famine-hit countries.
Yet it is ironic that one of the most serious health problems, CHD, is most common in affluent, industrialised societies.
CHD is associated with **overnutrition** and **obesity**.

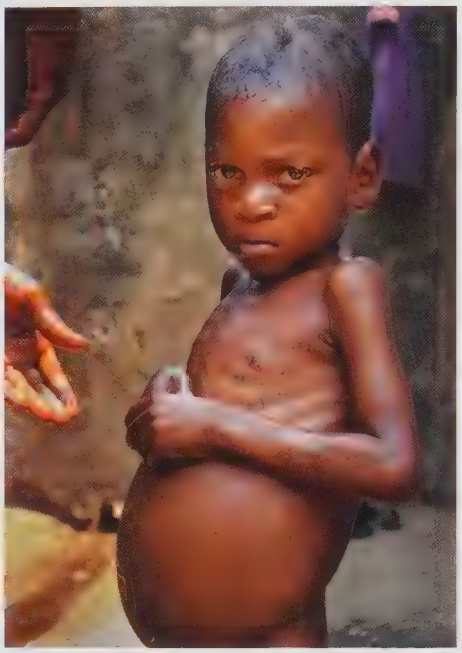
No more food, Berdale, during the Somali famine

Undernutrition

Undernutrition can be general (starvation) or specific, as in the case of the deficiency of a particular vitamin, mineral or food type.
One of the more common deficiency diseases in the world is xerophthalmia, caused by a deficiency of vitamin A.

During starvation, the basal metabolic rate (BMR) is reduced.
BMR is the rate at which energy is used when the body is at rest.
It is the rate of respiration that the body needs to keep 'ticking over' during periods of inactivity.
People can survive for many weeks without food, provided they have access to water, because during periods of starvation, the body draws upon its stores of carbohydrate, fat and protein for energy.

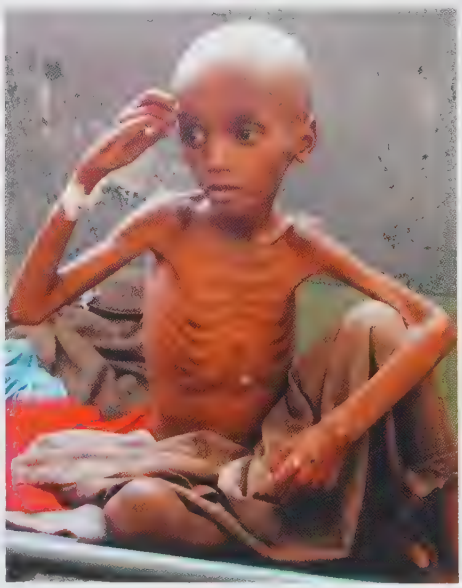
Worldwide, the most common form of undernutrition is **protein energy malnutrition** (**PEM**).
As the term suggests, this is caused by a lack of dietary energy and protein.
In its extreme forms it can lead to **kwashiorkor** and **marasmus**.
In both conditions the child is underweight, though this is more extreme in a child with marasmus.



A child showing the symptoms of kwashiorkor

The clinical signs of kwashiorkor are **oedema** (a swelling of the legs), sparse, dry hair, a flaky appearance to the skin and a bloated 'moon face' appearance.
The 'moon-faced' child is apathetic, showing little interest in its surroundings.
There is an accumulation of fat in the liver which can lead to **cirrhosis** (permanent damage due to the replacement of healthy liver cells with scar tissue).

A child with marasmus has a very low weight for its age and thin arms and legs, with little muscle or fat.
The face has a wizened appearance, referred to as an 'old man's face'.
There are fewer biochemical changes and in this respect the child with marasmus has adapted to its poor diet better than one with kwashiorkor.



A child suffering from marasmus

In either case, the child is in danger and any feeding should involve the frequent serving of very small amounts of food.
This is because PEM causes pancreatic and intestinal cells to die.
So fewer digestive enzymes are secreted and the surface area for the absorption of digested food is much reduced.

Starvation

Dietary survey data for poor communities in developing nations has shown energy intake to be much lower than the DRV. The significance of this is that protein that should be used for growth and repair of cells has to be used as an energy source. So children showing the symptoms of protein deficiency, as in the case of kwashiorkor, are really suffering from insufficient energy intake.

A food drop during an Ethiopian famine

Most aid programmes that supply food to famine-stricken areas aim to increase the energy intake of the population. Staple foods such as wheat and rice will satisfy energy intake and also provide enough protein. However, tropical crops such as cassava (a root crop) and sweet potato, whilst having a high energy content, are low in protein, so they need to be supplemented with other protein foods.

The body adapts to progressive starvation by

- quickly using up ***glycogen*** stored in the liver,
- drawing upon the body's ***fat*** stores (this may last for between 4 and 6 weeks depending on the amount of fat stored),
- using ***protein*** as an energy source, which results in a wasting of the muscles and other tissues.

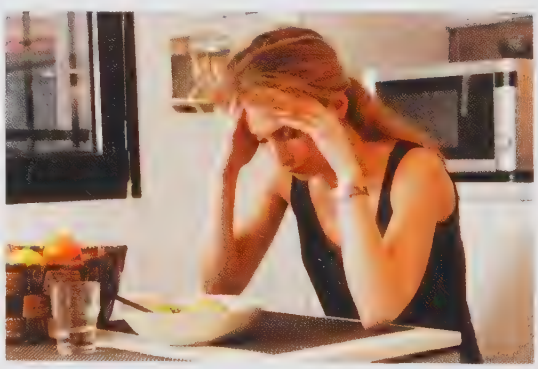
Anorexia nervosa

Anorexia nervosa is a wasting disease and its symptoms are very similar to that of marasmus. In contrast though, it is usually found in developed countries and can be brought on by psychological distress. Anorexia is most common in teenage girls from middle to high income families.

A teenager with obvious symptoms of anorexia

Sufferers of anorexia nervosa lose their appetite, eat little food and become dangerously thin. The symptoms include:

- wasting, as muscle tissue is used as a source of energy once the body's fat reserves have been used up,
- a decrease in body temperature, metabolism and heart rate,
- slowing of growth and sexual development (the normal menstrual cycle stops),
- a greater susceptibility to infections,
- depression, so that the affected person's thoughts are dominated by food and eating less and less.



The causes of anorexia are complex, but tend to involve low self-esteem and anxiety about growing up and sexuality. It often develops from a desire to diet, but anorexics who have lost a great deal of weight still see themselves as being too fat.

People with anorexia often fail to see that they are starving themselves. Treatment focuses on building up their self-esteem and if this is achieved, then normal eating patterns and weight gain usually follow.

Obesity

In Britain and other affluent countries, more people suffer from overnutrition than from undernutrition.
If a person takes in more energy in food than required, then the surplus respiratory substrates are converted to storage fat. This particular form of malnutrition is caused by a combination of factors:

- high intake of fatty foods and refined foods containing a lot of added sugar,
- too little exercise,
- social and emotional stress, leading to 'comfort eating',
- physiological problems such as an underactive thyroid gland, although these tend to be rare.

Maths skills

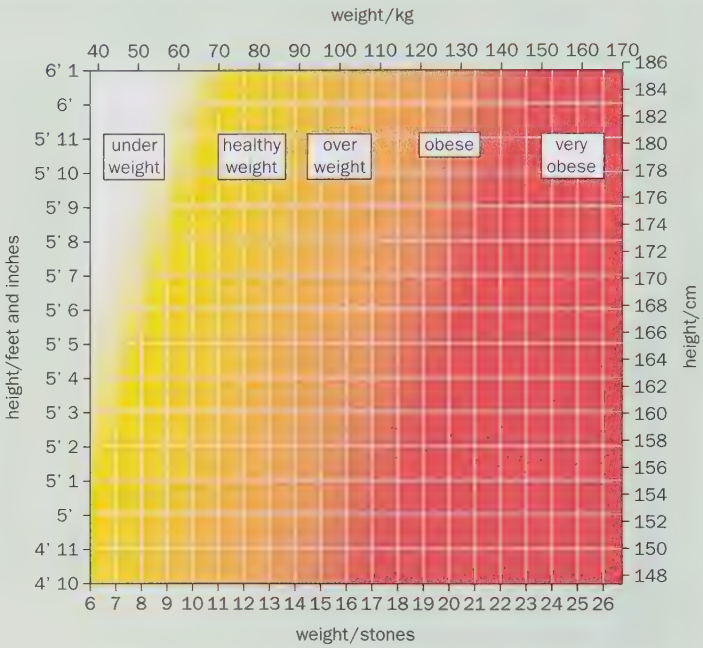
There are two ways in which people can identify being obese:

- being 20% above the recommended weight for his or her height,
- having a **body mass index (BMI)** greater than 30.

$$\text{BMI} = \frac{\text{body mass (in kg)}}{\text{height (in metres)}^2}$$

A person with a BMI of less than 20 is underweight, between 25 and 30 is overweight, and more than 30 is obese.
If you are 20–24, then you are just right!

Waistband measurements are easier than calculating the BMI.
Scientists have shown that women with waistband measurements of over 80 cm (31.4 inches) and men with waistband measurements greater than 94 cm (36.9 inches) are twice as likely to develop cardiovascular diseases.



Average height to weight ratios

Obesity can cause or lead to an increased incidence of

- CHD,
- high blood pressure (hypertension),
- angina (blood flow to the heart muscle is sufficient, but the coronary arteries cannot deliver the additional oxygen needed for exercise, so any physical effort results in chest pains),
- varicose veins (the walls of the veins become stretched due to the accumulation of blood from poor circulation),
- diabetes, gall bladder disease, osteoarthritis and some cancers.

Which of the following actions would be best for an obese person to lose weight?

- cut down on all carbohydrates,
- cut down on fat,
- cut down on starchy and fibrous foods.

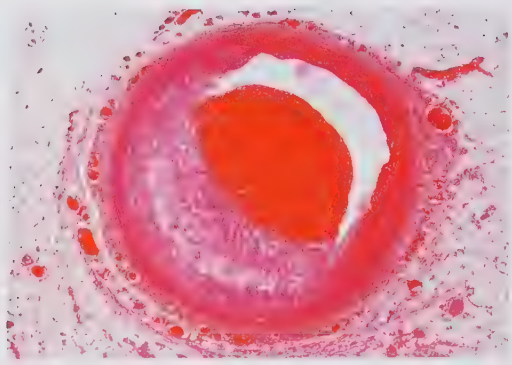
Which of these would be the least helpful? Give reasons for your answers.

The 'fattest man in the world' from the film 'Monty Python's The Meaning of Life'

Coronary heart disease

CHD is one of the main causes of death in Britain and many other developed countries.
A blockage or constriction of the coronary arteries results in heart failure.

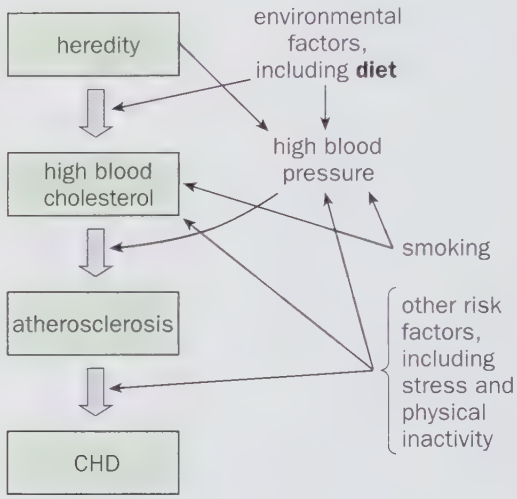
The smooth lining of healthy blood vessels allows blood to flow through them easily.
But the lining can be damaged by the build-up of a fatty deposit called **atheroma**.



Atheroma building up inside an artery

This narrowing of the arteries is gradual.
It cuts down the flow of blood and the first signs are often noticed during exercise.
Because of the restriction of blood flow, blood pressure increases and the heart has to beat faster than usual to deliver blood to the tissues.
This can cause pains in the chest and arms known as **angina**.
Someone with angina is more likely to suffer a heart attack.

A heart attack, or **myocardial infarction**, occurs when there is a sudden and severe blockage of the coronary artery.
This cuts off the blood supply to the heart muscle.
The affected part of the heart muscle becomes damaged due to lack of oxygen and the heart may stop beating altogether.
A victim of cardiac arrest will die unless the heart starts to beat again within a few minutes.



How CHD can develop

Risk factors

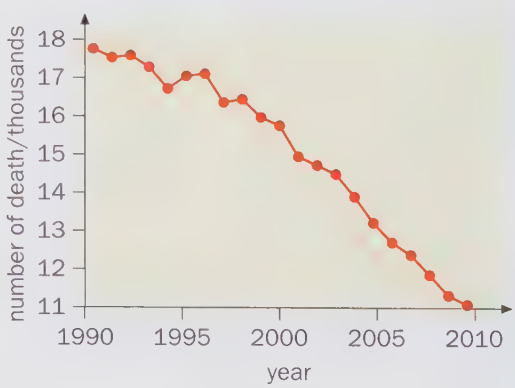
CHD is not caused by any one particular factor but by a combination of things.
Age, gender, weight, amount of exercise, blood pressure, diet, smoking and inherited genes are all risk factors.
High levels of blood cholesterol have been shown to increase the risk of CHD.

- A diet high in saturated fat results in high cholesterol levels.
- Our bodies need some cholesterol (see page 17) although the body cells are able to manufacture some of this themselves.
- The intake of polyunsaturated fats results in lower blood cholesterol so these are recommended in the diet instead of saturated fats.

Regular exercise increases fitness, reduces body fat, lowers blood pressure and reduces the risk of heart disease.
But diet, as you have seen, is also important in the reduction of the incidence of CHD.

Use the information about diet in this chapter and in Chapter 1 to explain how the following dietary recommendations act to decrease CHD:

- eating less fat,
- eating less sugar,
- eating more fibre,
- eating less salt.



Deaths from heart disease in the UK 1990–2010 (Source: WHO Mortality Database, March 2012)

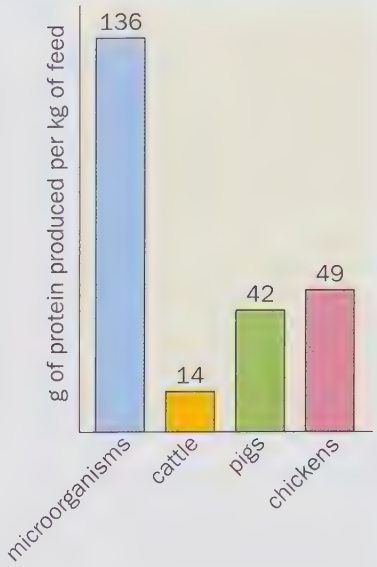
Biology at work: Biotechnology and food production

You have already seen how fermentation on an industrial scale can produce useful products such as penicillin. Microorganisms can also play a significant role in food production.

Microorganisms are very efficient producers of protein, and in the 1960s the food producers Rank Hovis McDougall (RHM) began a research programme that led to the production of **mycoprotein**.

This is a high protein material produced by a filamentous fungus called *Fusarium venetatum*. RHM found this fungus growing in a field in Buckinghamshire, England, and realised that it had great potential as a food material. It is fibrous, has a neutral taste and, most importantly, has a high protein content (48% of its dry mass).

Mycoprotein is produced on an industrial scale in fermenters by a process known as **continuous culture**. You will read about continuous culture fermenters on page 403. The mycoprotein fermenters are 40 m high and run continuously for 5 weeks at a time.
This involves the continuous addition of growth medium and the removal of the protein product at the same time.



Protein production by microorganisms outstrips most other organisms

At the end of the cycle, the fungus and culture medium are removed from the fermenter for **downstream processing**.
This involves filtration to separate the growth medium from the brown, fibrous 'filter cake'.
The filter cake is frozen quickly to prevent it from becoming a fertile growth medium for other microbes. The freezing also produces ice crystals which help to push the fibres together; this creates bundles that help to give mycoprotein its meat-like texture.
The 'cake' can then be used as the base material for a number of food products.


Fusarium growing on a cereal

Downstream processing can actually increase the percentage of protein by some 40–45%.
This occurs when the material and some of its original mass is lost due to the evaporation of water.

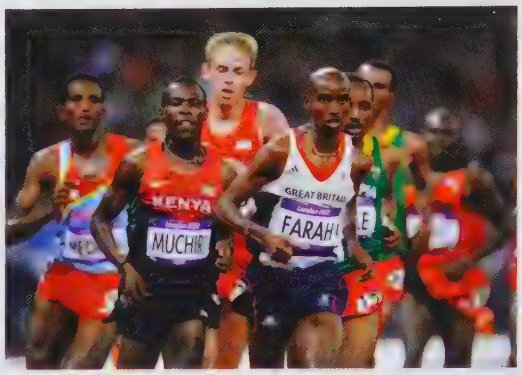
Due to its fibrous nature and high protein content, mycoprotein makes an ideal meat substitute and since the mid-1980s it has been actively used in this way. Marketed under the name 'Quorn', it has appeared in a variety of meat dishes such as savoury pies and curries. Within 20 years there were over 90 products on the market containing Quorn.


Some food products derived from mycoprotein

The main attraction of mycoprotein is that it is a healthy, low-fat alternative to meat. However, the mission of its developers was always to provide the world with a nutritious, abundant, environmentally-friendly protein. This aim remains valid, especially with concerns over land availability for rearing livestock, and in theory mycoprotein offers the potential for famine relief if it can be produced cheaply enough and in sufficient quantities.

Biology at work: Carbo-loading and performance

Athletes have always tried to find ways to improve their performance. With the exception of illegal drug use, this has mainly been through the adoption of specialised diets and training regimes. Improved knowledge and understanding of sports physiology has led to the development of many commercial products. These are often endorsed by successful athletes and claim to enhance performance, particularly for endurance races. They claim to work by 'super-charging' glycogen stored in muscle fibres. Are these claims mere sales hype, or do the products actually work?



At the time Mo Farah won gold at the London 2012 Olympics he endorsed Lucozade sports drink

Carbohydrates and fats are both used to supply the glycogen necessary for all forms of physical exercise. However, it is the type of muscular activity that determines which nutrient is used for fuel:

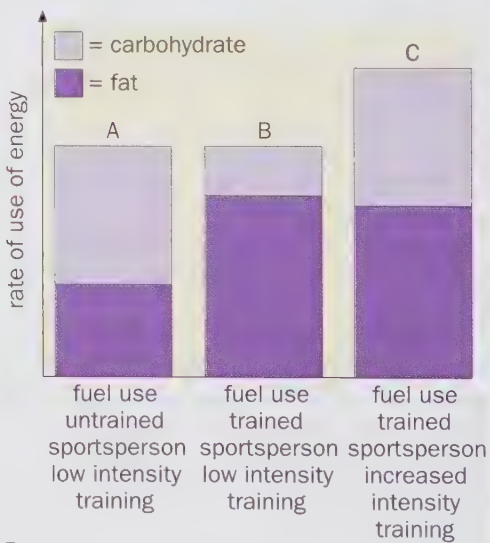
- **exercise duration** – intermittent or prolonged,
- **exercise intensity** – light or heavy.

With increasing duration and intensity of exercise, there is a reduction in the dependence on carbohydrate as a fuel source and an increase in the reliance on fat. The concept of a glycogen-loaded diet, or **carbo-loading**, was first devised in the 1960s. It is achieved by the following steps.

- 7 days prior to a major event, an intensive training run depletes muscle glycogen levels.
- For the next 3 days the athlete eats mainly fats and proteins to deprive the muscles of carbohydrate and increase the activity of the enzyme **glycogen synthase**, which is necessary for glycogen production.
- Training is reduced during this period to prevent total glycogen depletion and possible injury. This is known as the taper.
- The final 3–4 day period utilises a carbohydrate-rich diet, restricted intake of fats and proteins, and high fluid intake, together with low intensity training.

Fuel and exercise

Exercise intensity	Exercise duration	Fuel used
maximal sprint	short	carbohydrate
low to moderate	moderate – up to 2 hours, e.g. jogging	carbohydrate and fat equally
severe	prolonged, e.g. cycling	less carbohydrate, more fat



Increased carbohydrate together with the increased glycogen synthase levels results in increased muscle glycogen storage with the overall effect of improved performance. There are some disadvantages with this regime:

- increased body weight due to increased intake of water,
- weakness, depression and irritability during the depletion phase.

A recent modification to carbo-loading advises a normal diet with light training until the day before the race. On the day before the race the athlete consumes small amounts of carbohydrate and on the day itself performs a very short, extremely high intensity workout.
This technique claims comparable or better results than traditional carbo-loading as a result of increased liver glycogen storage.
It is this effect which most commercial products seek to boost, not the longer-term build-up of glycogen stores, which takes several days to effect through carbo-loading.

Summary

- Health can be described as the state of complete physical, mental and social wellbeing.
- Disease is usually due to a malfunction of the body and can be diagnosed by symptoms.
- Communicable (or infectious) diseases are caused by pathogens, which may be passed from one person to another.
- Epidemiology is the study of the patterns of diseases and how they are spread.
- A balanced diet has an adequate intake of nutrients and energy needed to sustain the body and ensure health and growth.
- Different people have different energy requirements and different nutrient needs.
- Dietary reference values outline the nutrient and energy requirements for a particular group of people with reference to age, gender and fitness.
- Essential amino acids can only be obtained from food and cannot be synthesised from existing organic compounds in the body.
- Similarly, there are some essential fatty acids that need to be present in the diet.
- Vitamins are a group of organic compounds that are essential to our diet and, if lacking, can cause deficiency diseases.
- Undernutrition due to lack of dietary energy and protein can result in kwashiorkor and marasmus.
- Obesity can occur if a person takes in more energy in food than is required.
- Coronary heart disease can bring about a blockage of the coronary arteries, resulting in heart failure.

Questions

1 a) Describe the likely long-term effects that an excessive fat intake can have upon the blood vessels.
b) Why do some nutritionists consider that it is more healthy to eat plant fats rather than animal fats?

2 Describe and explain the different energy needs and nutrient requirements of each of the following groups:
a) a very active man,
b) a breastfeeding woman,
c) a girl aged 12–14,
d) a pregnant woman,
e) a child aged 6,
f) a child aged 1.

3 Define each of the following terms and explain their use in dietary health:
a) dietary reference values (DRVs),
b) estimated average requirement (EAR),
c) reference nutrient intake (RNI),
d) lower reference nutrient intake (LRNI).

4 a) Describe and explain the likely effect on health of changing to a lower fat and higher fibre diet.
b) Explain why the amount of salt eaten should be reduced.
c) Suggest why medical opinion favours a combination of exercise and diet, rather than diet alone in order to lose weight.

5 Diet and lifestyle are factors that affect the likelihood of a person developing cardiovascular disease. Explain how each of these factors can influence the development of cardiovascular disease.

6 The body mass index (BMI) is one measure of obesity:

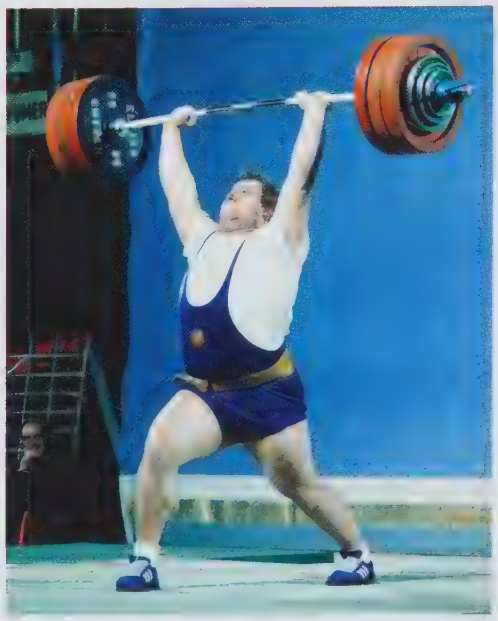
$$\text{BMI} = \frac{\text{body mass in kg}}{\text{height in m}^2}$$

The normal range for the BMI is 20 to 25.
A person with a BMI of 30 is considered to be obese.
a) Calculate the BMI for a man with a mass of 85 kg and who is 180 cm tall.
b) What advice would you give this man about his weight. Give reasons for your advice.
c) Explain how the balance between energy intake and energy expenditure affects obesity.
d) Regular exercise increases the basal metabolic rate (BMR).
i) What is meant by the BMR?
ii) Explain how an increase in BMR could decrease the chance of heart disease.

15 Exercise and health

Endurance is the ability of the body to carry out exercise.
Weightlifters need to have good muscular endurance to be able to lift very heavy weights and support them for a short time.
Other sports that require high muscular endurance, but relatively low cardiovascular endurance are boxing, wrestling and sprinting.

In contrast, high levels of cardiovascular and respiratory endurance are needed to run a marathon.
This sort of exercise takes place over a longer period of time and requires far less muscular endurance.
Sports requiring high levels of cardiovascular and respiratory endurance include distance running, swimming and cycling.

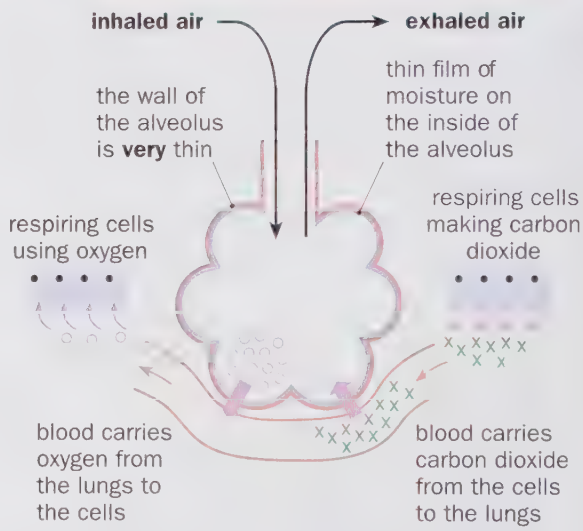


Breathing and exercise

Gas exchange is considered in Chapter 8.
You should remember that we breathe in more oxygen than we breathe out and this maintains the concentration gradient that allows oxygen to diffuse from the alveoli into the blood.
Similarly, we need to breathe out carbon dioxide to maintain the concentration gradient that allows carbon dioxide to diffuse out of the blood capillaries and into the alveoli.

Air is exchanged between the lungs and the atmosphere when we breathe. The volume of air exchanged depends upon the depth of each breath and the breathing rate.
At rest, only about 0.35 dm^3 of air is exchanged with each breath.
This represents only about one-seventh of the alveolar air, so the changes in gas composition are relatively small.

We cannot empty our lungs completely.
With forced breathing we can exhale about 3 dm^3 of air, although about 1 dm^3, known as the **residual volume**, remains in the lungs and cannot be breathed out.
At rest, our residual volume is much larger, with about 2.5 dm^3 of air remaining in the lungs.
With strenuous exercise, the ***depth*** of breathing increases and breathing ***rate*** increases. This enables us to cope with the greater demands that exercise places on our bodies.



Gas exchange at the alveolus

Maths skills

Ventilation rate is the total volume of air taken into the lungs in 1 minute (expressed in $dm^3\ min^{-1}$).
It enables us to measure the effect that exercise has on our breathing.

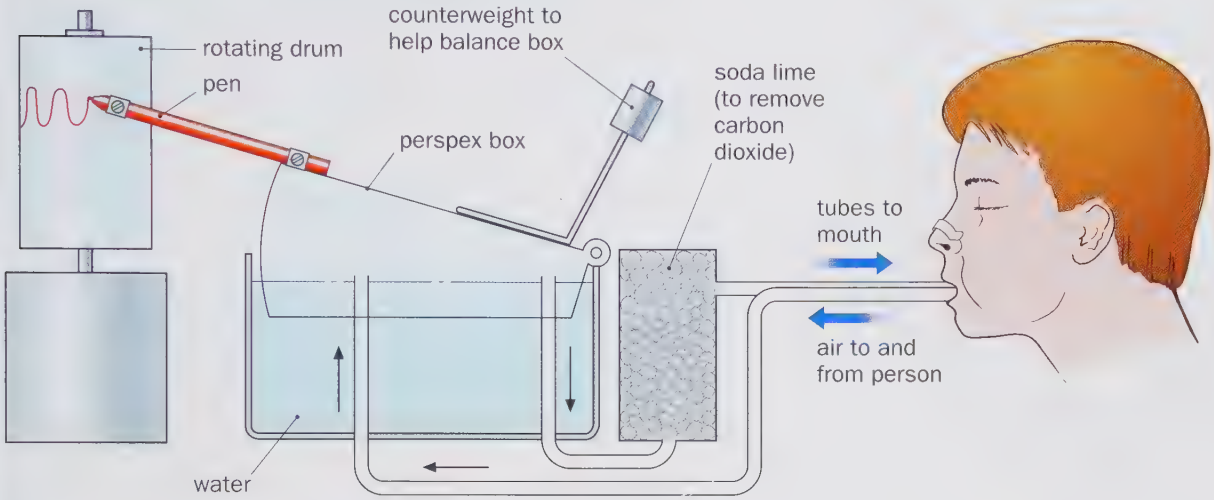
ventilation rate = breathing rate × tidal volume
(Tidal volume is the volume of air moved in and out of the lungs in a single breath.)

Athletes at the peak of their fitness are able to increase their ventilation rate by increasing their tidal volume.
There is only a small increase in their rate of breathing.

Measuring human lung volume

A **spirometer** is used to measure the volume of air that moves in and out of the lungs.
It is basically a clear, plastic box, filled at the bottom with water.
The space inside the spirometer contains oxygen.

When you breathe out the box moves up, and when you breathe in the box moves down.
These changes can be recorded as a trace on a revolving drum, called a **kymograph**.
With most spirometers the exhaled air passes through a container of soda lime, a chemical that absorbs carbon dioxide, although this is not always the case.



In the first experiment, there was no soda lime in the container.
A person was asked to breathe normally through the mouthpiece.
The first trace records the person's **tidal volume**.

Tidal volume is the volume of air breathed in and out during a single breath.

Look at the trace and read the tidal volume.

The person was then asked to breathe in as hard as possible and then breathe out as hard as possible to give the **vital capacity**.

Vital capacity is the maximum volume of air that can be breathed in or breathed out of the lungs.

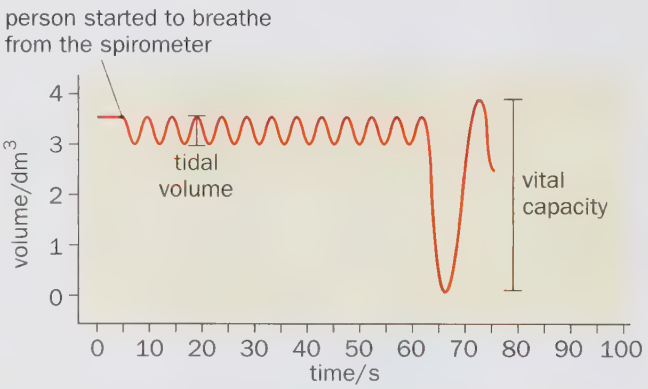
Now try to read the vital capacity from the trace.

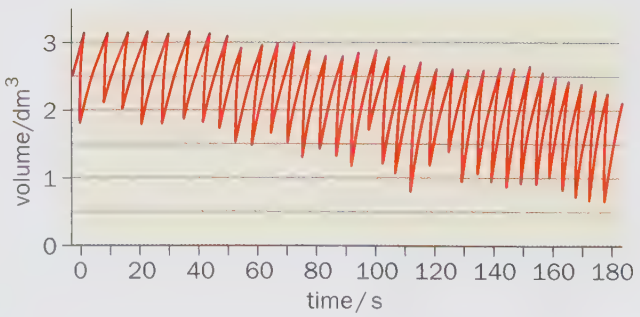
In a second experiment, soda lime was place in the container.
The person was asked to breathe normally and the second trace was obtained.

Why do you think the trace falls with time?

What was the breathing rate of the person in breaths min^{-1}?

What was the total amount of air breathed in and out of the lungs in 1 minute?





A trace from a spirometer containing soda lime

Pulse rate

As you saw in Chapter 10, each time the heart contracts it forces blood out into the aorta and pulmonary arteries.

The volume of blood pumped out with each heartbeat is called the stroke volume.

This surge of blood from each heartbeat causes a bulge in the walls of the arteries, which travels along the arteries.
Where arteries are near the body surface, you can feel (or even see) each bulge travelling as a **pulse**.
One pulse is equivalent to one heartbeat.

Taking a person's pulse

You have probably taken someone's pulse by feeling an artery at the wrist or at the neck.

The **pulse rate** is the same as the heart rate.
Pulse rate is usually counted as the number of pulses recorded in 30 seconds, which is then doubled to give the pulse rate in beats per minute.

The **resting pulse rate** gives a good indication of a person's fitness.
The fitter you are, the lower your resting pulse rate.
The same volume of blood is being passed out of the heart per minute, but with fewer heartbeats.

Pulse rate	Level of fitness
less than 50	outstanding
50–59	excellent
60–69	good
70–79	fair
80 and over	poor

General relationship between resting pulse rate and levels of fitness

Physically fit people tend to have a larger stroke volume as a result of their increased heart size, particularly the left ventricle.
As much as 5 dm^3 of blood can be pumped out of the heart every minute.
As their stroke volume is greater, their hearts do not have to beat as quickly during exercise.
When the exercise is over, their pulse rate returns to normal far more quickly than for unfit people.
Athletes who compete in endurance events such as the pentathalon tend to have a low pulse rate and a large heart.

The resting pulse rate for a person can range between 60 and 100 beats per minute.
The pulse rate tends to fall with increasing age.
The average pulse rate for a healthy, fit young adult is about 70.

Pulse rate is affected by certain risk factors.
Some of these are the result of a person's lifestyle.
For instance, higher pulse rates would be recorded for people who

- smoke,
- take little exercise,
- have a diet high in saturated fats.

An accident waiting to happen

Blood pressure

As you saw in Chapter 10, the cardiac cycle describes the sequence of events that take place during one heartbeat.
There are alternate contractions (**systole**) and relaxations (**diastole**) of the heart.

During ventricular systole, both ventricles contract at the same time to force blood out of the heart.
At this point the ***maximum*** arterial pressure is achieved.

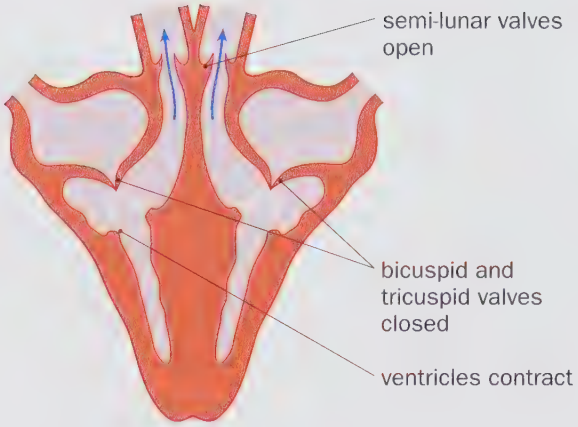
Systolic pressure is the pressure at which blood leaves the heart through the aorta from the left ventricle.

After ventricular systole, the ventricles relax and the pressure in the ventricles drops.
The semi-lunar valves in the aorta close when the pressure of the blood in the aorta is greater than the pressure of the blood in the left ventricle.
A steady flow of blood through the arteries is then achieved by the pulse.

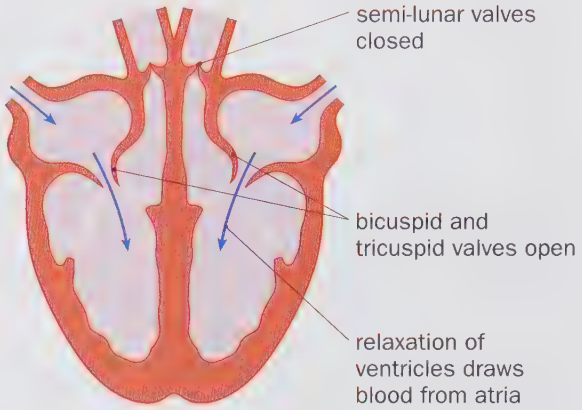
Diastole is when the ventricles relax.
At this time the pressure inside the left ventricle drops below that in the arteries.

Diastolic pressure is the minimum blood pressure in the aorta.

Diastolic pressure gives an indication of the ***resistance*** of the arteries to blood flow.
If resistance to blood flow is low, then the diastolic pressure will be low.
If resistance to blood flow is high, then the diastolic pressure will be high.
Atherosclerosis, or 'hardening of the arteries', occurs when fatty deposits line the artery wall, increasing the resistance to blood flow.
The heart then has to work harder to force oxygenated blood through to the tissues.



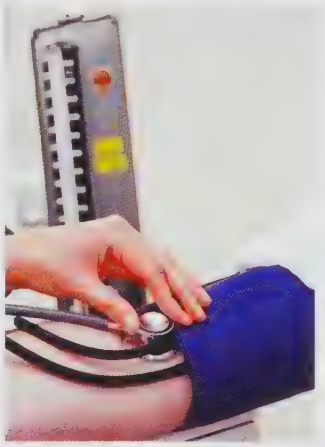
Ventricular systole



Diastole

Measuring blood pressure

Blood pressure can be measured with a **sphygmomanometer**, which gives readings for both systolic and diastolic blood pressure.
The first figure (systolic pressure) is put over the second figure (diastolic pressure) to give a fraction.
A healthy young adult's blood pressure is about 110/70 mmHg.


Measuring blood pressure with a sphygmomanometer

Hypertension

Hypertension is the harmful condition of having a constantly high blood pressure.
The causes and treatment of hypertension are explained on page 178.
A person can lower their blood pressure, reducing hypertension, by

- taking regular exercise,
- reducing their intake of alcohol,
- reducing the amount of salt in their diet,
- losing weight,
- not smoking.

Category	Blood pressure/mmHg	
	systolic	diastolic
below normal	< 90	< 60
normal	90–120	60–80
borderline	120–140	80–90
hypertension	> 140	> 90

The World Health Organisation classification of adult blood pressures

Energy and exercise

Active people make more use of their muscles.
In order to function, muscles need a source of energy called **adenosine triphosphate (ATP)**.
ATP is made in the cells during respiration.

All muscular contractions require ATP, but exercise puts increased demands on the body to synthesise more of this important compound.
Only a limited amount of ATP is present in muscle cells, enough in fact to allow you to run as fast as you can for only a few seconds.
After this time you rely on the re-synthesis of ATP.

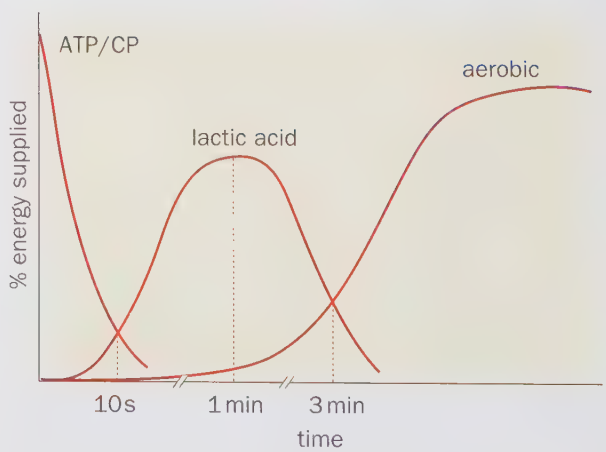
There are three basic pathways for this replacement.
Which pathway operates at any given time depends on how intense the activity is, how immediate the energy requirement is and whether or not sufficient oxygen is present.

The **ATP/CP** (**phosphocreatine**) pathway provides an almost instant replenishment of ATP.
The phosphocreatine is broken down and the energy released is used to add a phosphate to ADP to re-form ATP.
This system is especially useful during the initial stages of very intense activity such as sprinting.
However, phosphocreatine supplies (like ATP) are limited, and after around 10 seconds ATP re-synthesis by this pathway will fail.

The body then depends on glycogen (its store of carbohydrate) for the re-synthesis of ATP.
Glycogen is broken down into glucose, which is then converted into pyruvate.
In the absence of oxygen, the glucose is converted into **lactic acid** (or **lactate**).
This process is technically known as **anaerobic glycolysis** or the **lactic acid system**.
It only releases about 5% of the energy in a molecule of glycogen.
However, it is released quickly.
Therefore it is useful for short-term, high intensity exercise, such as a 100 m swim.
It is most effective in events lasting around 1 minute.

The remaining 95% of the energy available in the glycogen molecule is released via the **aerobic system**.
This is the route that involves the Krebs cycle and the electron transport chain.
It can take up to 3 minutes to complete this final stage of respiration but the advantage is that this has the potential to release the relatively vast amount of energy that is available. As a result, this system is most important for endurance events, such as 10 000 m runs and cycle races.

These athletes will be using the ATP/phosphocreatine and anaerobic glycolysis system

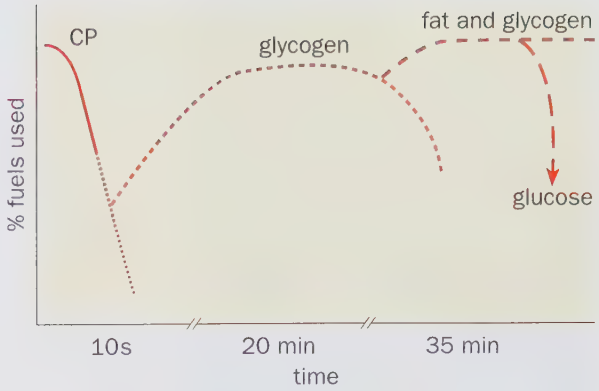


The three pathways that provide energy in the muscles

Fuels for the re-synthesis of ATP

The most abundant fuel stored in the body is fat.
However, glycogen stored in the liver and muscle will be the first energy source used during exercise.
Contrary to popular belief, it is these glycogen stores that are of most importance for muscle contraction and not blood glucose, which is used mainly to supply the brain and nervous system.

Glycogen is used in preference to fats partly because it requires less oxygen for its breakdown. However, once again glycogen is in limited supply, and in endurance events the body will need to use some fat in order to conserve glycogen. When athletes talk of 'hitting the wall' in endurance events, this is the point at which all the glycogen reserves are gone. Training programmes for endurance athletes help the body to become increasingly reliant on fat reserves, although the body can never rely on fat alone.



The relationship between the duration of exercise and the type of fuel used

Glycogen loading

Glycogen loading or **supercompensation** is a dietary technique often practised by endurance athletes. The idea is to increase the athlete's muscle glycogen stores to such an extent that by the time of a race they may be at twice the normal levels.

So how does this technique work? Seven days prior to a race, athletes will deplete their glycogen stores with the aid of endurance training. For the next 3 days, carbohydrates are omitted from the diet. In the remaining days leading up to the race, lots of high carbohydrate meals, for example pasta dishes, are consumed to boost the glycogen stores.

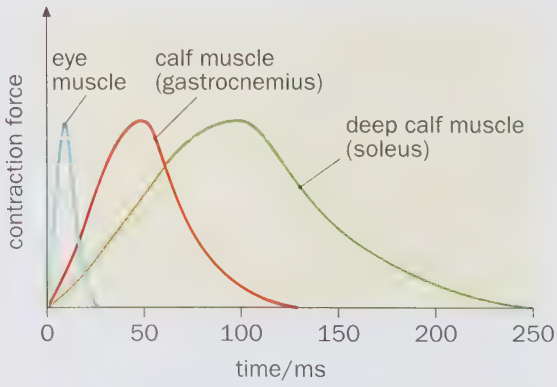
This technique has been shown to be effective in maximising aerobic energy production, although research has also shown that with trained athletes the initial glycogen depletion is usually not necessary.

The start of the London marathon

Slow- and fast-twitch muscle fibres

Skeletal muscle is composed of two types of muscle fibres: slow- and fast-twitch fibres, depending upon their type of respiration and speed of contraction.

Slow-twitch fibres	Fast-twitch fibres
adapted for aerobic respiration	adapted for ATP/CP pathway
able to contract for long periods	adapted for sustained short bursts of activity
slow contraction speed limited by rate of oxygen supply	fast contraction speed, not limited by oxygen supply
not susceptible to fatigue as no lactate produced	muscle fatigue due to lactate production
large numbers of mitochondria	few mitochondria present
high myoglobin content helps to provide oxygen for aerobic respiration, this gives fibres a red colour	lack of myoglobin, so cells appear white
associated with endurance activities, such as jogging, cycling and endurance swimming	associated with explosive type activities, such as sprinting and weightlifting



Graph shows speed of contraction of different muscles over time. Which muscle contains a) mostly fast-twitch fibres, b) mostly slow-twitch fibres, c) both types?

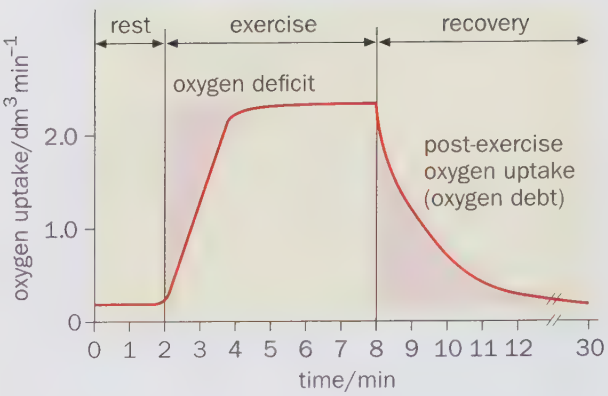
The oxygen debt

As you have seen, during short-term, high intensity exercise, such as sprinting, the muscles revert to anaerobic respiration to re-synthesise ATP.
This causes an accumulation of lactate in the muscles.

During the recovery period just after vigorous exercise, breathing continues faster and deeper.
Extra oxygen is needed to break down the lactate and restore oxygen levels in the body.
This is called the **oxygen debt**.

Sprinters often hold their breath during a 100 m race and quickly build-up lactate in their muscles.
Afterwards they need up to 7 dm^3 of oxygen to get rid of it, and have to breathe in deeply to pay back their oxygen debt.

Distance runners could not stand such a build-up of lactate. They run at a much slower speed and although they build-up some lactate in the early stages of a race, they are able to get rid of this while they are running.
You can find out more about lactate formation in muscle on page 308.



Oxygen uptake before, during and after strenuous exercise. The post-exercise oxygen uptake (oxygen debt) is that amount over and above the resting uptake (unshaded area) during recovery.

Aerobic exercise

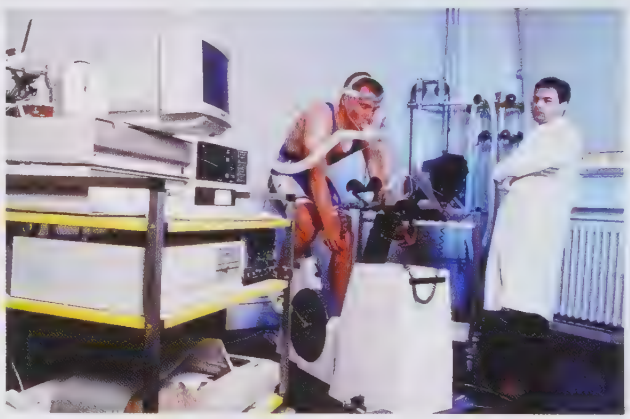
Your body adapts to regular exercise and becomes more efficient at carrying it out.

Aerobic exercise makes full use of both your gaseous exchange and cardiovascular systems.
The type of exercise can vary from marathon running, swimming or team sports to jogging or just a brisk walk.

Aerobic exercise improves ventilation of the lungs and makes the circulatory system more efficient at delivering oxygenated blood to the tissues.

Regular aerobic exercise has been found to

- increase heart muscle (particularly the left ventricle),
- increase the stroke volume (the volume of blood pumped out with each heartbeat),
- decrease the resting heartbeat rate,
- lower systolic and diastolic blood pressures, so reducing hypertension,
- increase both tidal volume and vital capacity of the lungs,
- increase the utilisation of fat, so reducing a person's weight,
- increase muscle size and enhance the amount of glycogen and fat stored in muscle,
- decrease blood cholesterol levels,
- improve a person's resistance to disease.



An athlete undergoes a fitness test at the British Olympic Medical Centre

Tobacco smoke

Cigarette smoke contains thousands of harmful chemicals that pose a threat to human health.
The three most hazardous chemicals are tar, nicotine and carbon monoxide.

- **Tar** collects in the lungs as the tobacco smoke cools.
  It is a mixture of many toxic chemicals.
  Some of these are **carcinogens** (substances that cause cancer).
  These carcinogens affect the DNA in the cells of the alveoli, resulting in mutations.
  Normally genes control cell division, so that it stops when enough cells have been produced for growth and repair of lung tissue.
  But the gene mutations form **oncogenes**, and normal cell division goes out of control.
  This can eventually lead to the formation of a malignant tumour.

Tumour suppressor genes normally inhibit cell division.
Carcinogens in tobacco tar can also cause them to mutate, so that they become inactive and this can also lead to cell division running out of control.

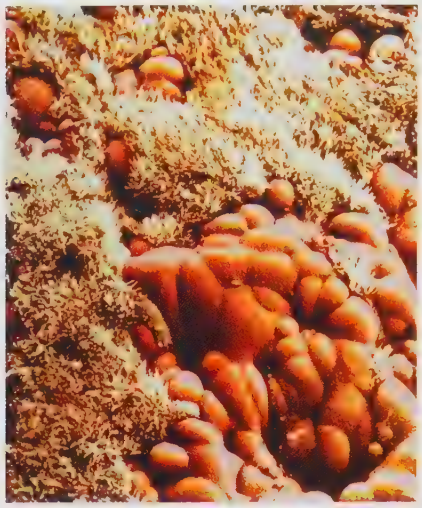
Oncogenes and mutated tumour suppressor genes can both lead to lung cancer.
The developing tumour cells can find their way through the epithelial cells and into the lymph capillaries of the lung.
From here they may circulate around the body forming **metastases** (secondary tumours) in other organs such as the liver and the brain.

- **Nicotine** is one of the most powerful poisons known.
  It is the substance that makes tobacco addictive and its absence results in the withdrawal symptoms that people experience when they try to give up smoking.

Nicotine stimulates the release of the hormone **adrenaline** into the bloodstream.
Adrenaline has many effects on the body, but significantly it causes an increase in heart rate and raises blood pressure.
Many long-term smokers develop raised blood pressure and this can lead to other problems with the cardiovascular system, including atherosclerosis, coronary heart disease (CHD) and stroke.

- **Carbon monoxide** is a gas that combines more readily than oxygen with haemoglobin in the red blood cells.
  This causes a reduction in the amount of oxygen in the blood and so the heart has to work harder to supply the body with the oxygen it needs.

Smokers inevitably have raised carbon monoxide levels and this affects the oxygenation of the tissues.
In the short term, this means that the smoker is unable to participate in physical activity.
However, in the long term, high levels of carbon monoxide can lead to a hardening of the arteries, especially the coronary arteries supplying oxygen to the heart muscle.



SEM of a cancer in a bronchus

Cause	Total
heart disease	40 000
lung cancer	38 000
chronic bronchitis emphysema	26 000
total smoking related deaths	104 000

Estimated numbers of smoking-related deaths in the UK in 1990

A nicotine patch

Even knowing the risks, people still smoke

Diseases of the lungs

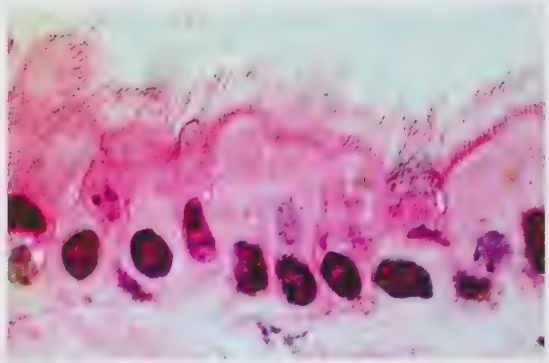
Chronic bronchitis

The tar in tobacco smoke produces two reactions in the lungs.

- The goblet cells in the epithelium of the air passages are stimulated to secrete more mucus.
- The ciliated cells of the epithelium which waft the mucus out of the air passages do not work and may even be destroyed.

Both these actions result in an accumulation of mucus in the bronchial passages.
Therefore the mucus remains stuck in the lungs.
Bacteria and viruses accumulate and breed in the mucus.

The result is **chronic bronchitis** and 'smoker's cough'.
Large amounts of **phlegm** (a mixture of mucus, bacteria and some white blood cells) are produced, which the sufferer attempts to cough up.



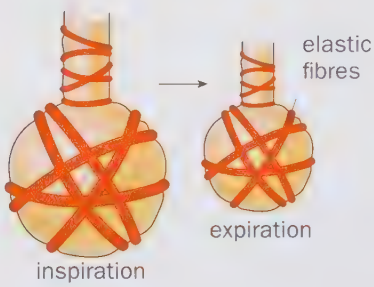
Ciliated cells lining the air passages

Emphysema

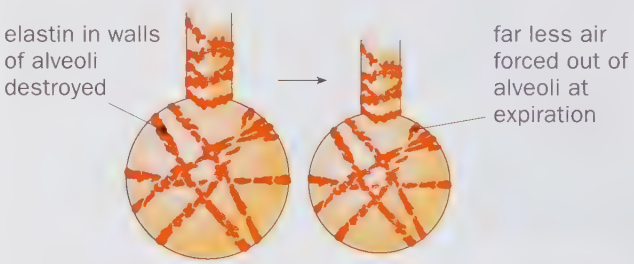
This disease often develops from bronchitis in smokers.
Substances in tobacco smoke stimulate **mast cells** to secrete protein-digesting enzymes.
These enzymes destroy the elastin in the walls of the alveoli, which means they do not stretch and recoil as the lungs inflate and deflate.
As a result the bronchioles can collapse, and air trapped in the alveoli can cause them to burst.
So the surface area of the lungs available for gas exchange is much reduced.
The loss of elastin also makes it more difficult to force air out of the lungs when breathing out.
A person with healthy lungs should be able to expire $4\,dm^3$ of air after a deep breath, whereas a person with emphysema may only be able to force out about $1.2\,dm^3$ of air.

This means that a person suffering from emphysema exchanges far less gas with the atmosphere.
As a result their blood is not as well oxygenated and they have to breathe more rapidly.
Breathlessness becomes more frequent as the disease progresses.
Eventually the sufferer may become bed-bound and have to resort to a face mask in order to get a sufficient supply of oxygen.

A smoker may breathe in only 15% of the smoke produced from a cigarette, while the remaining 85% is released into the room.
Evidence suggests that non-smokers suffer from the effects of this smoke, a condition known as **passive smoking**.
Because of this, smoking has been banned from more and more public places in various parts of the world.



Normal alveoli stretch and recoil as lungs inflate and deflate



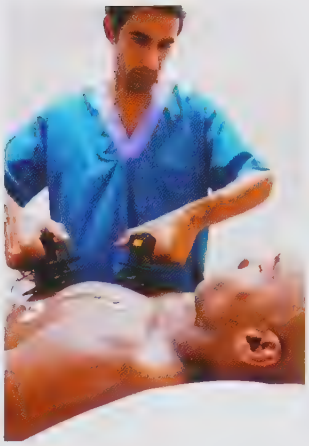
The development of emphysema

Passive smoking: somebody else's problem

Smoking and heart disease

The correlation between smoking and lung cancer was established as long ago as the 1950s.
It was some time later that smoking was identified as a risk factor for CHD and other cardiovascular disorders.

People of all ages who smoke are at a greater risk of dying from CHD than non-smokers of the same age.
There is also a link between the number of cigarettes that a person smokes per day and their chances of dying from CHD.
In the UK about 110000 people die prematurely each year due to the effects of cigarette smoking.
About half of preventable, early deaths are due to diseases of the heart and blood vessels.

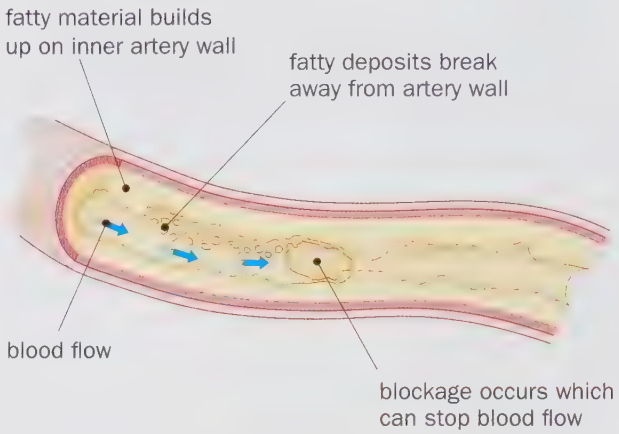


Emergency cardiac treatment

Atherosclerosis

This is the result of a build-up of fatty deposits (plaque) on the inner walls of arteries, forming atheroma.
The arteries become thicker and less flexible.
This leads to a narrowing of the vessels, which inevitably reduces the flow of blood.
As a result, the heart has to pump harder to get the blood through.

Initially this may result in **angina**, with the symptoms of severe chest pains brought on by physical exertion.
The pain is caused by a shortage of oxygenated blood to the heart muscle, but the muscle tissue does not die.



Heart attack (myocardial infarction)

Atherosclerosis in one of the two coronary arteries that supply the heart muscle can be potentially fatal.
The build-up of fatty tissue may result in an artery becoming completely blocked by a blood clot (**thrombosis**).
The heart muscle becomes starved of oxygen and dies.
This is called a **coronary heart attack** or **myocardial infarction**.
If the damage to the heart tissue is extensive, then the person may die.
However, many people survive heart attacks with treatment, followed by adjustment of their lifestyles.
This means reducing risk factors by giving up smoking, taking exercise and eating a more appropriate diet.

Aneurysm

Atheromas are also linked to an increased risk of **aneurysm**.
An aneurysm is a bulge in a blood vessel caused by a weakness in the vessel wall. The commonest places where they can occur are in the brain and the aorta. If the blood vessel bursts, this haemorrhaging can cause extensive brain damage, or in the case of an aortic aneurysm prove fatal.

Again, atheromas may form in an artery supplying the brain. Part of the brain becomes starved of oxygen and dies, and the person is said to have had a **stroke**. This can result in loss of function or sensation, associated with the part of the brain that is affected.
Smoking causes increased blood pressure and so makes the chance of a stroke occurring more likely.

Stroke victim 'learning' to walk again

Links between smoking and disease

Linking smoking and lung cancer

Cigarette smoking became really popular amongst men during the First World War.

It became fashionable with women later in the 1940s. Epidemiologists soon found a correlation between smoking cigarettes and the incidence of lung cancer.

Not surprisingly, when doctors first saw the evidence, many of them gave up smoking.
As a result, the number of deaths from lung cancer amongst doctors decreased dramatically.

From the graph above, you can see the sort of data that helped scientists establish the link between smoking and lung cancer.
In the early part of the 20th century, deaths from lung cancer were low but these increased dramatically as the habit of cigarette smoking became more widespread.
In contrast, deaths from other lung diseases such as tuberculosis were falling, as a result of improved medical care and better housing.

Statistics also demonstrated a link between the number of cigarettes that a person smokes and the risk of dying from lung cancer.
The second graph clearly shows that the more cigarettes smoked, the greater the risk of a premature death from lung cancer.

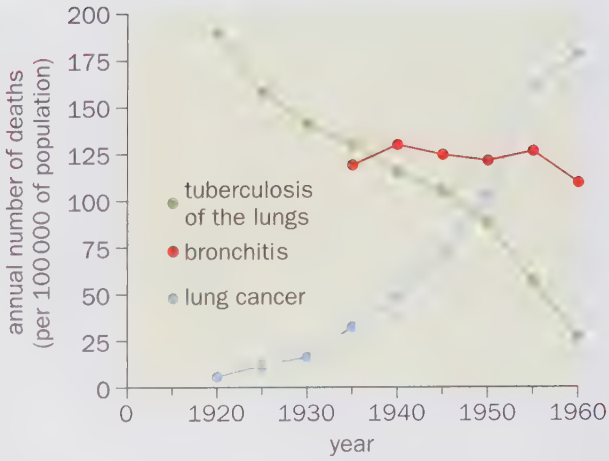
Direct evidence linking smoking and lung cancer have come from two different sources.

- **Experimental animals** were used in the 1960s to investigate the effects of cigarette smoke on the lungs. Tumours similar to those that occurred in humans were found in the lungs of dogs exposed to cigarette smoke.
- **Chemical analysis** was carried out on the tar extracted from cigarette smoke.
  A number of carcinogenic substances were found in the tar.

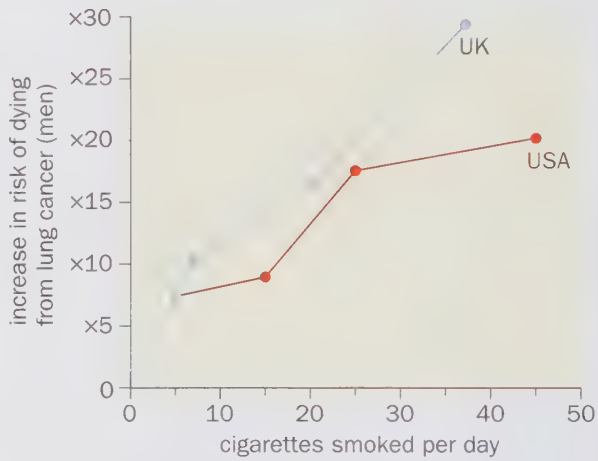
Evidence such as this clearly establishes the link between the smoking of cigarettes and the incidence of lung cancer.

Linking smoking and heart disease

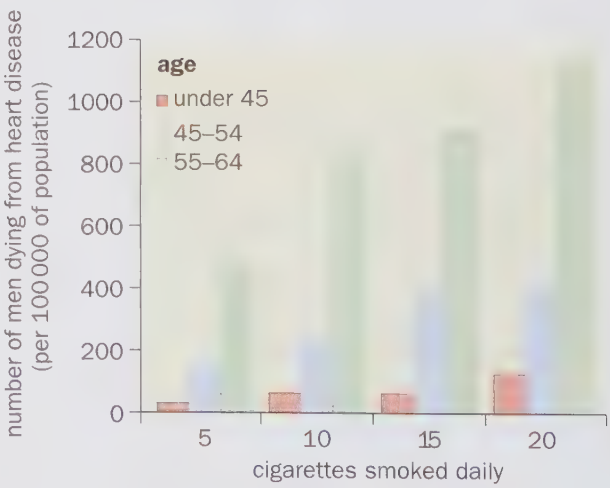
We have already looked at the effects of smoking on the cardiovascular system.
Atherosclerosis makes it harder for the heart to pump oxygenated blood to the tissues.
This can result in angina, a coronary heart attack or a stroke.
Again epidemiological evidence, such as this graph from the Royal College of Physicians, UK, shows a clear link between the number of cigarettes smoked each day and the risk of dying from heart disease.



Deaths from lung disease in England and Wales 1920–60



The relationship between male deaths from lung cancer and the number of cigarettes smoked daily



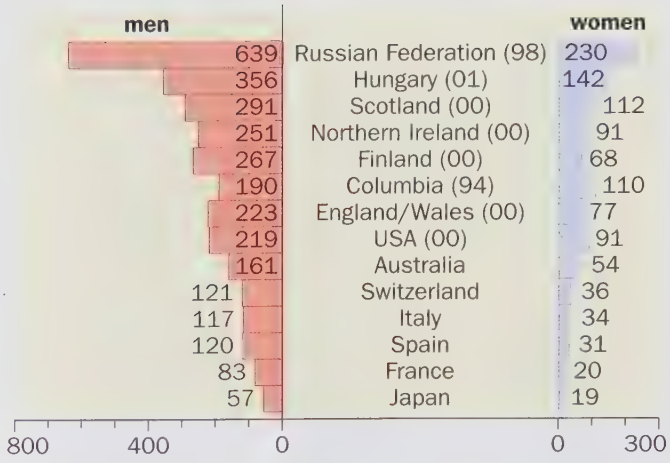
Smoking and the risk of death from CHD amongst men

Global distribution of CHD

Epidemiological evidence has shown that there are differences in the death rates from CHD across the world.
As you can see from the data, death rates from CHD are highest in northern Europe and lowest in Japan.

In Britain, the incidence of CHD is higher in Scotland, Northern Ireland, northern England and north-west England than it is in other parts of the country.
It is also greater amongst men than women, particularly amongst manual workers.
The incidence of CHD also varies between different ethnic groups, being higher in people of south Asian origin.

This sort of data can help in the identification of the **risk factors** that contribute to CHD.



Number of deaths from CHD of people aged 35–74 per 100000 population. Unless stated otherwise, the figures are for 1999

- **Smoking**

As you have seen, smoking accelerates the development of atherosclerosis and increases the risk of CHD.

- **Heredity**

People who inherit a tendency for high blood cholesterol levels suffer increased incidence of CHD.

- **High blood lipid levels**

A diet rich in saturated fat can lead to high levels of low density lipoproteins and cholesterol, which can be deposited as plaque on the lining of arteries.

- **Obesity**

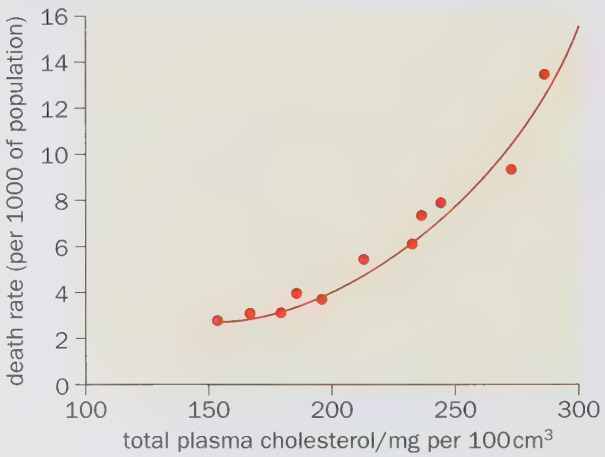
Obesity is often linked to CHD, but this could be due to other risk factors such as high cholesterol intake.

- **Diabetes**

Diabetics are more likely to develop CHD and at an earlier age.

- **Exercise**

Lack of exercise makes the heart less healthy, increases atherosclerosis and increases blood pressure.



The relationship between blood cholesterol levels and risk of death from CHD

Additional risk factors contributing to CHD appear to be ageing, being male, and lifestyle.
Obviously we have no control over some of these risk factors, as is the case with a person who inherits high blood cholesterol or diabetes.
But some risk factors are ***preventable***.
We can reduce the risk of CHD by leading a healthier lifestyle. Eating a diet low in saturated fat, giving up smoking and taking more exercise can all significantly reduce the risk of a person getting CHD.

Biology at work: Coronary artery bypass graft

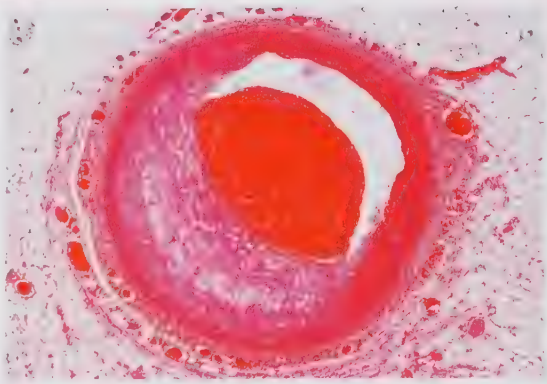
The purpose of the **coronary artery bypass graft (CABG)** is to relieve patients of the symptoms of CHD.
In the western world CHD is responsible for around one-third of all deaths in individuals over the age of 35.

CHD results from the narrowing and possible blockage of the coronary arteries that supply the heart muscle with oxygen and nutrients.
The arteries become narrowed by cholesterol-rich deposits called **atheroma**, and if blood clots form on the rough surface of these deposits then complete blockage may occur.
The build-up of these deposits is known as **atherosclerosis**.

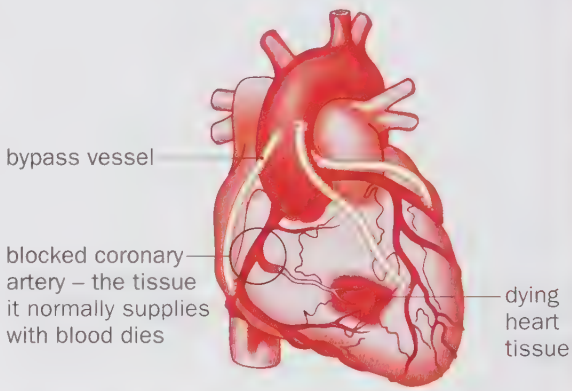
A common symptom of CHD is angina.
This is a pain (often brought on by physical exertion) that starts in the chest and often spreads up the neck and down the left arm.

Complete blockage of an artery will result in the death of part of the heart muscle and this causes what is commonly called a heart attack. If a large area of the heart muscle is affected then the attack can be fatal.

A CABG is carried out when the symptoms of heart disease have not been reduced by drugs and lifestyle changes, such as stopping smoking or adopting a more sensible diet.



A microscopic section of a blocked artery



How a bypass operation works

Identifying the blockage

The site of the blockage is identified by a type of X-ray called an **angiogram**.
Blood vessels don't show up clearly on ordinary X-rays so a special dye is injected.
This dye highlights the blood vessels as it moves through them showing up clearly on the angiogram.
This makes it obvious where there is a narrowing or blockage of the vessel.

Angiogram of the coronary arteries

Carrying out the CABG

A blood vessel is taken from another part of the body, usually the chest, arm or leg, and is attached to the coronary artery above and below the narrowed or blocked area. The new blood vessel is known as a graft. Depending on the severity of the CHD a number of separate grafts may be needed. The operation typically takes 3–6 hours and is carried out under general anaesthetic. Usually the heart is stopped and the blood supply is re-routed through a heart-lung bypass machine which takes over the circulation and oxygenation of the blood.

After surgery

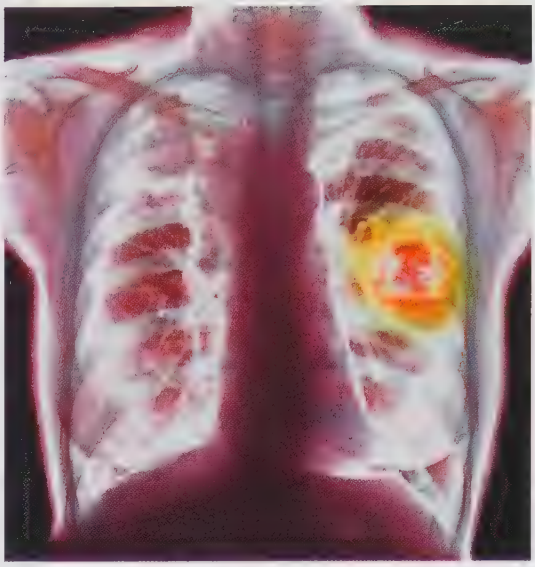
As a result of the CABG most patients experience a significant improvement in symptoms such as breathlessness and chest pain. However, the operation is not a cure for heart disease, and without suitable lifestyle changes it is quite possible for the grafted vessels to become narrowed and blocked.

Biology at work: X-rays and body scanning

X-rays were discovered as far back as 1895.
Ever since then they have been used in medicine as an important diagnostic tool.

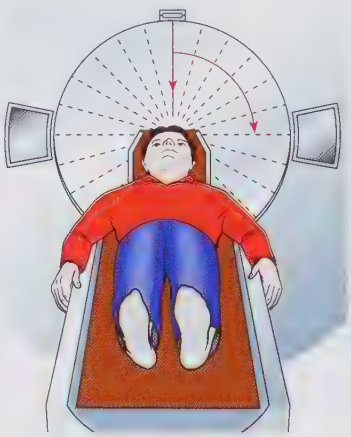
Low doses of X-rays are used to produce images of body tissues.
Dense tissues such as bone absorb X-rays more than soft tissues like skin and muscle.
As a result, when an X-ray of a leg, for example, is taken, it casts a shadow onto a photographic plate.
Damage such as a hairline fracture can be then easily seen.

Parts of the body comprised largely of soft tissue, such as the digestive system, can be studied using a special X-ray technique.
This involves the use of an opaque liquid that clearly shows up on an X-ray.
In the case of the digestive system this technique is commonly known as having a barium meal, because the opaque liquid contains a barium compound.



An X-ray showing the red/yellow tumour in a lung cancer victim

Computerised tomography (CT) scanning produces even clearer and more detailed images than X-rays.
CT scans are also sometimes known as CAT scans, which stands for computerised axial tomography.
Tomography is the technique of using X-rays to obtain images of sections through a part of the body.
These images are taken at various angles and then a computer is used to construct images of the tissues being studied.
The completed scan is then analysed by a radiologist (a specialist in interpreting images of the body).
CT scans can be used to study a variety of conditions, including brain tumours and injuries to internal organs.
They are also being used now to look at the heart.

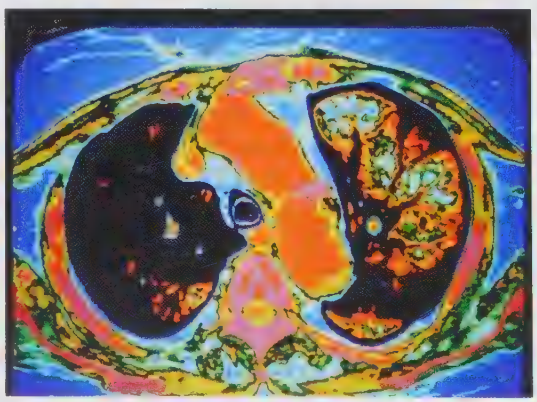


CT scanning set up

Magnetic resonance imaging (MRI) is an alternative form of scanning, which uses strong magnetic fields and radio waves.
The results of an MRI scan can be used to help diagnose conditions, plan treatments and assess the effectiveness of previous treatments.
As they do not use X-rays, MRI scans are safer for people who may be particularly vulnerable to the effects of radiation, such as pregnant women and babies.
However, due to the strong magnetic field, not everyone can have an MRI scan. For example, they are not always possible for people with implants, such as a pacemaker.

X-rays can increase the risk of mutations in cells and so the dosage of radiation used for medical purposes is very small.
As an additional precaution abdominal X-rays are not usually performed if there is a likelihood of pregnancy.

Radiographers and radiologists operate the machinery from behind a protective screen and wear a film badge to monitor their exposure to the X-rays.



CT scan through the thorax showing one lung (black) diseased with pulmonary emphysema

Biology at work: Exercise – putting life into your years

It was the US President Abraham Lincoln who famously said, "In the end, it's not the years in your life that count, it's the life in your years." Recent evidence of increasing physical activity levels would appear to support this quote. However, statistics still show that less than half of adults in the UK meet government recommendations for levels of activity, and this proportion is in decline. People in poor physical condition suffer a reduced quality of life and even loss of independence, increasing the risk of certain diseases linked to a sedentary lifestyle.
All of this may be preventable with sensible and realistic advice.
The promotion of physical exercise is based on a number of health benefits:

	All adults	16–24	25–34	35–44	45–54	55–64	65–74	75+
men								
*meeting recommendation	67	83	76	71	70	55	58	36
some activity	10	6	9	10	9	14	11	12
low activity	4	2	4	4	3	5	4	6
inactive	19	8	11	14	18	26	27	46
women								
*meeting recommendation	55	57	61	66	62	55	52	18
some activity	13	15	13	13	13	12	15	10
low activity	6	7	6	5	4	6	5	7
inactive	26	22	19	16	21	27	27	65

*Adults aged 19–64 years should aim to be active daily. Over a week, activity should add up to at least 2½ hours of moderate intensity activity in bouts of 10 minutes or more – one way to approach this is to do 30 minutes on at least 5 days a week

Physical activity levels (%) in adults by sex and age in England 2012. Adapted from Table 1.1, Physical activity statistics 2015, British Heart Foundation

- reduced risk of certain diseases such as cardiovascular disease,
- enhanced physical capacity,
- reduction in the decline associated with ageing,
- promotion of mental health, stress reduction and enhanced self-esteem.

Recent research has shown that even brisk walking for 30–45 minutes in those aged 60–80, three or four times a week, can stave off dementia by increasing the size of those parts of the brain associated with decision-making and memory.
The evidence for these benefits is assumed but the exact details are less clear. There are two complicating factors that account for this lack of clarity.

- **Human lifestyle** – genetics, diet, stress, smoking, the environment and socio-behavioural factors are all contributory factors to the causes of diseases.
- **Exercise** – is a very general term to describe a wide range of activities from aerobics to weight-training, all of which have varying effects.

'Take more exercise' is too simple a statement for a doctor to make. Exercise is something that requires gross muscular activity and is associated with an increase in metabolism. There are three broad categories.

Amateur runners in a 10k race

- **Aerobic** – involves a significant increase in cardiovascular activity using the major muscle groups in a sustained and rhythmic way. Exercise of this type is usually recommended for 30 minutes continuously, three times a week. Health benefits can be gained from much lower doses.
- **Muscle strengthening** – strengthening exercises require the muscles to work against a resistance, which can be provided by weights, exercise machines or a person's own body weight. Benefits from moderate exercise include improved posture, less fatigue, reduced musculo-skeletal problems.
- **Flexibility and mobility** – this kind of exercise helps maintain mobility in the joints through slow controlled actions and muscle stretching. It reduces the risk of poor posture and over-extending of muscles, strains and sprains.

For the individual, regular exercise of an appropriate intensity, frequency and duration can promote health. More realistic advice in the future, to effect more of a population change may be for exercise that is moderate in intensity, reduced in duration and of increased frequency.

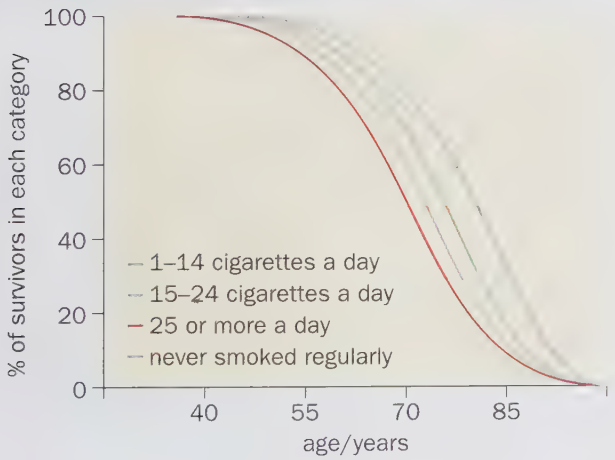
Summary

- Ventilation rate is the total volume of air taken into the lungs in 1 minute.
- Tidal volume is the volume of air breathed in and out during a single breath.
- Vital capacity is the maximum volume of air that can be breathed in or breathed out of the lungs.
- The volume of blood pumped out with each heartbeat is called the stroke volume.
- Pulse rate is the same as heart rate and resting pulse rate is a good indication of a person's fitness.
- Systolic pressure is the pressure at which blood leaves the heart through the aorta from the left ventricle.
- Diastolic pressure is the minimum blood pressure in the aorta.
- The ATP/phosphocreatine pathway provides an almost instant replenishment of ATP in muscle cells.
- After vigorous exercise, more oxygen is needed to break down lactate and repay the 'oxygen debt'.
- Tobacco smoke contains many harmful chemicals including tar, nicotine and carbon monoxide.
- Diseases of the lungs include chronic bronchitis, emphysema and lung cancer.
- Smoking is a high risk factor that can lead to coronary heart disease.

Questions

1 Fitness can be defined as the capacity to do physical work at a particular constant heart rate. Describe and explain how each of the following affects fitness:
a) emphysema, b) regular exercise.

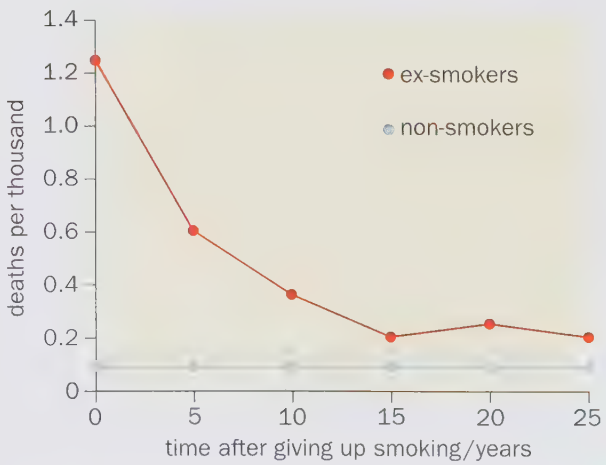
2 a) The graph shows the effect of smoking on life expectancy. Use information from the graph to calculate how the chances of living to 85-years-old is affected for someone smoking 15–24 cigarettes a day compared with someone who never smoked regularly.



b) The incidence of both bronchitis and emphysema is increased by smoking cigarettes.
i) Describe the symptoms of bronchitis.
ii) Explain why patients suffering from emphysema can often walk only a few metres before having to stop for a rest.

3 a) Give two differences that can be seen between healthy lung tissue and lung tissue from a person with emphysema.
b) Explain why people suffering from severe emphysema may find it difficult to climb stairs or walk up hills.
c) i) Emphysema is a common industrial disease of people who have worked in mining and quarrying industries. Suggest one feature that these industries have in common that could increase the risk of emphysema.
ii) Suggest one way in which workers in these industries could be protected from exposure to this factor.

4 The graph shows the death rates of non-smokers and ex-smokers from lung cancer.
a) Suggest one reason why the risk of cancer decreases only slowly for a number of years after a person has stopped smoking.
b) Suggest why people who smoke have a higher risk of lung cancer than non-smokers.
c) i) Describe how the effects of smoking increase the risk of one ***named*** respiratory disease other than cancer.
ii) Give one of the symptoms of the disease that you have named.
d) Describe one method by which respiratory diseases can be diagnosed.

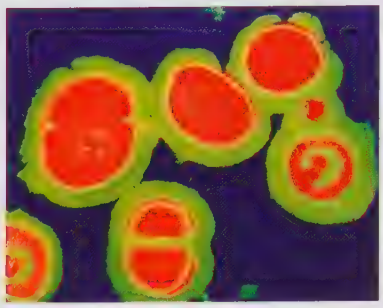


Millennium bugs

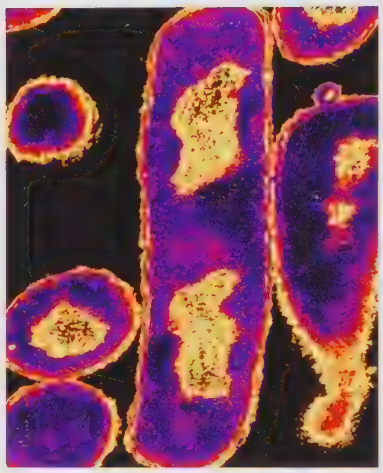
The genetic make-up of bacteria and viruses are constantly changing. The antibiotics and antiviral drugs used to treat diseases caused by bacteria and viruses are becoming increasingly ineffective. Resistance to antibiotics is growing as a result of their over-use.

MRSA (methicillin-resistant *Staphylococcus aureus*) is of great concern to experts in infectious diseases.
There is an increasing possibility that if you go into hospital for an operation, or if you develop an open wound, MRSA will be there, ready and waiting to cause infection and delay healing.
This bacterium was 95% controlled by penicillin in the 1940s, but now less than 10% responds to penicillin.

In Scotland in 1997, a new strain of *Escherichia coli 0157* caused an outbreak of food poisoning that affected 500 people and killed 20.
This food poisoning could have been avoided by good hygiene, but this new strain got into the human food chain and existing antibiotics proved useless.
The emerging pattern is that more and more strains are becoming resistant to an ever-growing list of antibiotics.



MRSA



TEM of E. coli bacterium (×20 000)

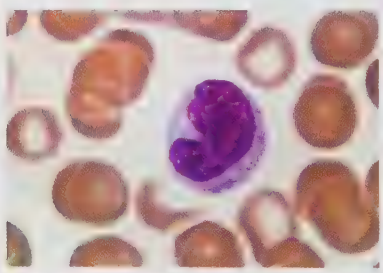
White blood cells and defence

As mentioned in Chapter 10, the function of white blood cells, or **leucocytes**, is to defend the body against **pathogens** (disease-causing organisms).
There are five kinds of white blood cells. They all originate in the bone marrow from the division of **stem cells** (which also make red blood cells and platelets).

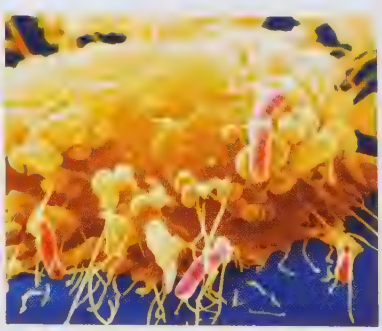
- **Neutrophils** have a characteristic lobed nucleus and are the largest. They are concerned with phagocytosis.
- **Lymphocytes** are relatively small with a large, round nucleus.

There are two types of lymphocytes, both of which play a major role in the **immune system**.
In the **humoral response**, B-lymphocytes produce antibodies to counter pathogens and their toxins.
T-lymphocytes are involved in the **cellular response** or **cell-mediated immunity**.
Both types of lymphocytes migrate from the bone marrow to the lymph nodes and spleen where they mature.
On their way, the T-lymphocytes pass to the **thymus** (a gland in the neck).
Here they become 'sensitised' and are able to recognise specific **antigens** (chemicals on the surface of microorganisms and foreign matter).
Together, neutrophils and lymphocytes account for 90% of the white blood cells in the body. The other 10% are made up of **monocytes**, **eosinophils** and **basophils**.

- Monocytes are large cells with a kidney-shaped nucleus. They develop into **macrophages,** which are phagocytic.
- Eosinophils stain red and are associated with allergies.
- Basophils stain with basic dyes, such as methylene blue, and are able to release chemicals such as histamines that cause inflammation.



White blood cell: a monocyte

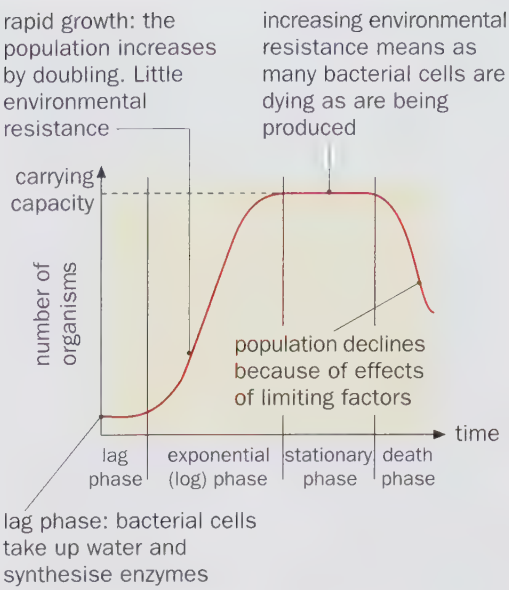


SEM of alveola macrophage attacking E. coli bacteria

Bacterial growth

Bacteria are a major group of pathogens against which white blood cells defend us. Basically there are four main phases of growth.

- The **lag phase** when the cells are active but there is little growth as they are taking up water and starting to produce enzymes.
- The **exponential** or **log phase** where the population increases rapidly. There is a doubling of the bacterial cells at each **generation time**. With optimum conditions, plenty of nutrients and ample space, subsequent generation times get shorter because there are no limiting factors. Eventually the **carrying capacity** is reached. This is the maximum population that an environment can support.
- The **stationary phase** sees bacterial cells dying more or less at the same rate as they are produced. The population encounters limiting factors in the form of nutrient depletion and a fall in pH as carbon dioxide and other wastes accumulate.
- The **death phase** occurs when more bacterial cells are dying than are being produced, so the population declines. Causes of death may be shortage of nutrients, lack of oxygen, or the accumulation of toxic waste products.



Factors affecting growth

Temperature

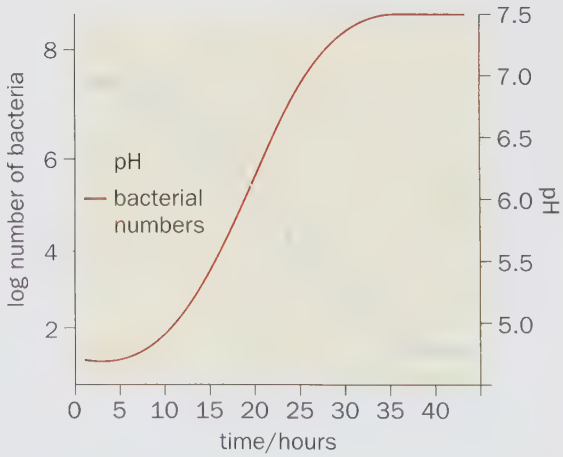
Bacteria can be classified into three groups according to the range of temperature at which they grow best.

- **Thermophiles** have an optimum temperature of above 40 °C. They grow in hot springs, compost heaps and hot water heaters. One species has been found growing in the hot water escaping from thermal vents on the ocean floor at a temperature of 250 °C!
- **Mesophiles** have an optimum temperature between 20 and 40 °C. They include most bacteria, including those pathogenic to humans.
- **Cryophiles** grow best at temperatures below 20 °C. These bacteria live in the Arctic and Antarctic Oceans, but also in fridges and freezers.

Thermophiles thrive in piles of manure

pH

Most bacteria have enzymes that ensure optimum growth at a neutral pH of 7, but can tolerate a range of pH from 6 to 8.
Very few bacteria can tolerate a pH of less than 4.
(Most bacteria are killed in the human stomach at a pH of 2.)
As you have seen, bacteria produce waste products that lower the pH of the medium and can lead to death of the bacterial population.



Relationship between bacterial numbers and pH with time

Oxygen

Oxygen is needed by aerobic bacteria for respiration to produce ATP for growth and reproduction, but is not needed by anaerobic bacteria.
The oxygen may come from the water in the culture medium.
Obligate anaerobes are killed if oxygen is present.

Nutrients

Nutrients are essential for growth and usually include carbon, hydrogen, oxygen, nitrogen, sulfur and phosphorus.
For instance, nitrogen is needed for amino acid and protein synthesis.
Lack of a particular nutrient can lead to decline and death.

Monitoring the growth of bacteria

An understanding of how microorganisms grow is important. Biotechnologists using microorganisms in fermenters need this information, as do environmental health officers testing food or water samples.
Therefore being able to count the microorganisms in a sample is an important skill.

There are a number of techniques used, one of which is the **haemocytometer**.

The haemocytometer is basically a modified microscope slide that was originally designed to count red blood cells.

The central section of the slide is 0.1 mm lower than the outer sections and has a ruled area engraved on it.

The middle area has a grid 1 mm square and is called the type A square.
This is further sub-divided into 25 type B squares, each with an area of $0.04\,mm^2$.

Finally, each of these squares is divided into 16 type C squares, each with an area of $0.0025\,mm^2$.

Using all these dimensions, we can calculate the volume of liquid in each square beneath a coverslip.
For example, the volume of the type C square will be $0.00025\,mm^3$.

The haemocytometer has a special coverslip.
If this is correctly positioned then a rainbow pattern (called Newton's rings) will be visible where it touches the surface of the haemocytometer.

If a sample of culture medium is placed on the haemocytometer and viewed under a microscope, the number of cells in any particular type of square can be counted.
Any cells touching the bottom or right hand side are ignored, but those touching the top and left hand side are included.

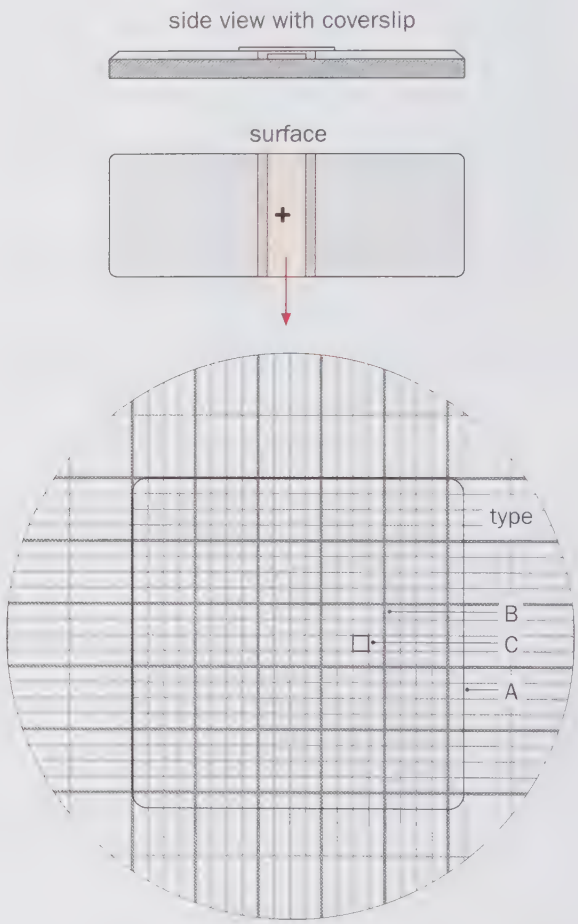
Given that we know the volume of a medium in a square and bearing in mind any dilution of the original sample, we can calculate the number of cells per mm^3.

This count can be carried out at specified time intervals using replicates for accuracy.

With practice, cell counts with the haemocytometer can be made very quickly.
However there are a number of disadvantages with this method:

- cell counts can be unreliable due to the small volume of sample on the slide.
- it is possible to count dead cells as well as living ones leading to inaccurate totals.
- living cells may be obscured by debris in the sample.

Collecting river water samples for microbiological examination



Use of a haemocytometer

Dilution plating and turbidimetry

The haemocytometer will give a **total cell count**, not distinguishing dead cells from those that are **viable**, that is, capable of growth and reproduction.

Dilution plating

A total viable cell count can be made using the **dilution plate** technique.

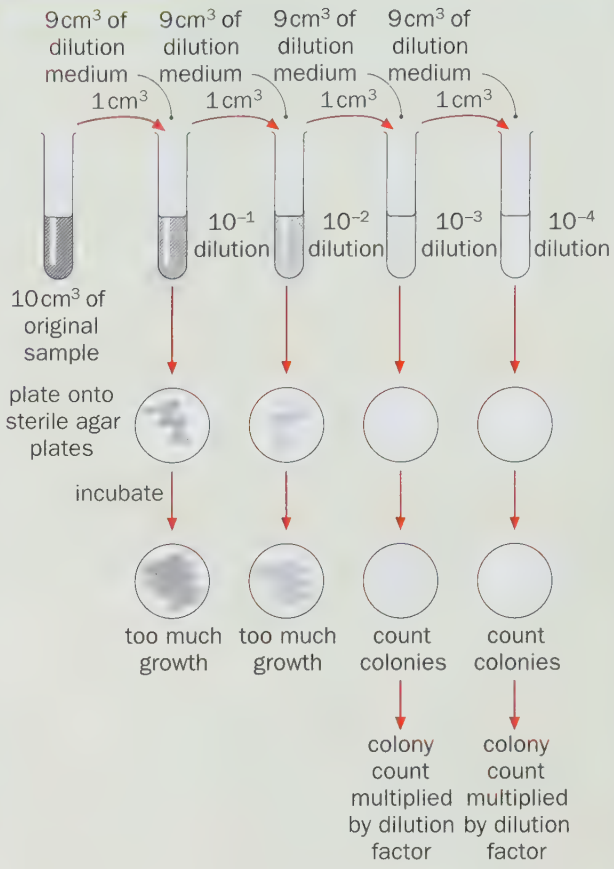
A culture medium is subjected to a series of dilutions. A small sample of each dilution is streaked onto a sterile agar plate.
The plates are then incubated at 25–30 °C for 2–5 days.

After this time, the plates are examined, the aim being to find a dilution at which colonies of bacteria can be easily seen and counted as separate and not overlapping.

The assumption is made that each colony arises from a single cell from the original medium.

Therefore to find the total viable cell count, the number of colonies is simply multiplied by the appropriate dilution factor.

Maths skills



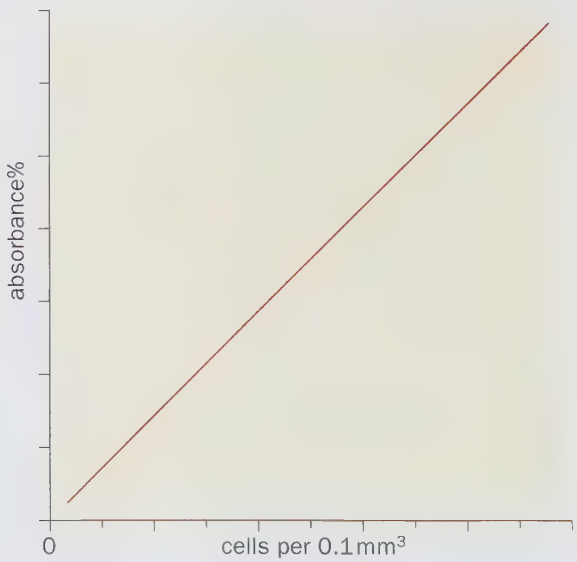
Dilution plating technique

Turbidimetry

A third technique, known as **turbidimetry**, involves using a **colorimeter** to measure the cloudiness (turbidity) of the culture medium.

A colorimeter shines a light beam through the sample and the amount of light absorbed is measured.
As cells grow and divide, more light is blocked.
The absorbance figures can be compared to a calibration curve.
This is a graph prepared by using known concentrations of cells and recording their absorbance.

This technique is clearly less time-consuming than the other two but it does depend upon the assumption that the turbidity is solely caused by the microorganisms.
The culture mixture must be continually stirred to prevent settling.



Relationship between % absorbance and bacterial cell numbers

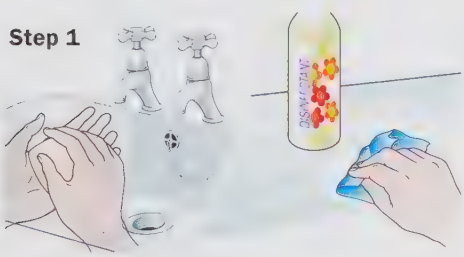
Culturing bacteria

All bacterial cultures should be regarded as potentially dangerous. Certain safety precautions should be taken and aseptic techniques used whenever you work with microorganisms.

- Any cuts should be covered with a clean, waterproof dressing before any work is attempted.
- Do not eat or drink during practical work and avoid sucking pens or pencils.
- Windows and doors should be closed to avoid the possibility of airborne contamination.
- Wash your hands with antibacterial soap before and after working with microorganisms.
- Wipe down the bench with disinfectant such as 10% sodium chlorate(I) before and after working.
- Report any spillages of cultures to your teacher immediately.
- Tape petri dishes securely after inoculation and label them.
- Never remove the lid of a sealed petri dish.
- Never incubate cultures of microorganisms above 30 °C.
- Sterilise all media and containers by autoclaving after use.
- Dispose of all cultures in plastic petri dishes after use by placing them in an autoclavable plastic bag and autoclaving them at 103 kPa (121 °C) for 20 minutes.

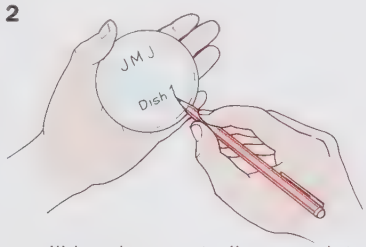
Agar is used as the medium for growth in the example above. Nutrient broth is also used as a medium. **Selective media** are used for the growth of selective microorganisms, for example if a microbe is resistant to a certain antibiotic, then the antibiotic can be added to the medium so as to prevent the growth of other cells that do not possess the resistance.

Step 1



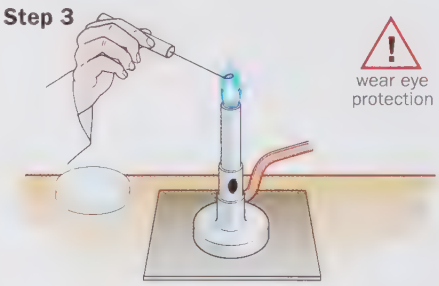
- Before you start to work with microbes:
  –wash your hands and
  –swab the bench with disinfectant.

Step 2



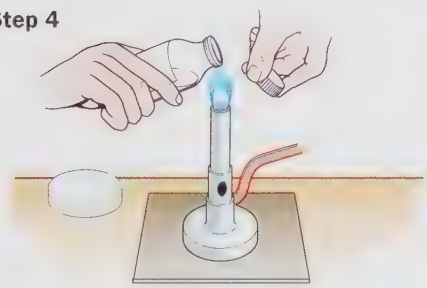
- You will be given a sterile agar plate. Keep the lid on the petri dish.
- Label the base with your name.

Step 3



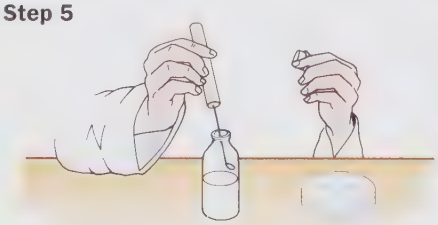
- Heat an inoculating loop in a Bunsen flame until it is red hot.

Step 4



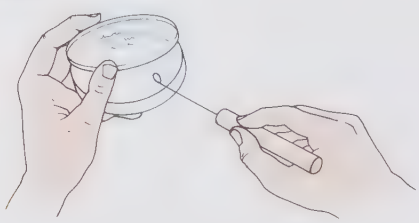
- Unscrew the bottle and hold the opening of it in a flame for ***2 seconds.***

Step 5



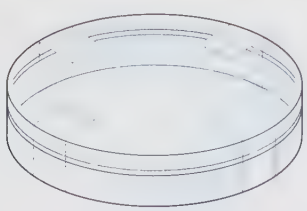
- Dip the sterile loop into the microbe sample and then replace the cap on the bottle.

Step 6



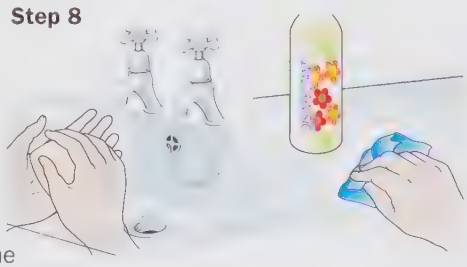
- Slightly lift the lid. Gently streak the loop over the surface of the agar. Replace the lid. Re-sterilise the loop in a Bunsen flame.

Step 7



- Seal the petri dish with sellotape. Put the dish upside down in an incubator at 25 °C for 2–3 days.
  Never open a sealed petri dish.

Step 8



- Swab the bench with disinfectant
- Wash your hands.

Bacterial disease

The ability of bacteria to produce disease is affected by a number of factors.

- **Pathogenicity** is simply how ill an infection can make you.

Many bacteria are not pathogenic at all. For instance, the millions of bacteria normally found in your large intestine do you no harm. In fact, many of them are beneficial to you.
On the other hand, bacteria such as those causing tuberculosis, bubonic plague and typhoid can lead to diseases that are fatal.

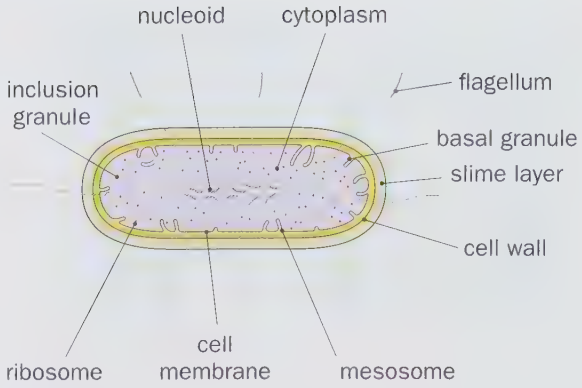
Pathogenicity is the result of toxins produced by the bacteria. Some of these are released as waste products and are known as **exotoxins**, for example tetanus, *Staphylococcus* spp.
Other toxins, called **endotoxins**, are part of the bacterial cell itself. When bacterial cells die, they break up and these endotoxins are released, for example *Salmonella* spp.

- **Infectivity** is the number of bacteria needed to cause infection.

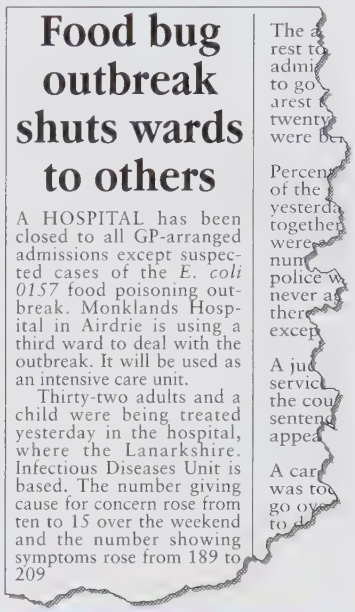
This varies from one disease to another, but most require large numbers of bacteria, as in the case of *Salmonella* food poisoning. In contrast, typhoid fever is far more infective and requires only a small number of bacteria to be present. The fewer the bacteria required to trigger illness, the more **virulent** the disease is.

- **Invasiveness** is the ability of bacteria to spread within the body of the host from the point of entry to other tissues where they multiply.

This is not easy for bacteria to do since they have to overcome the body's defence system and avoid phagocytosis.
The bacteria also have to penetrate the tough connective tissue and fibres found around tissues. They do this by secreting enzymes that break down the tissues. Fortunately, not many bacteria have evolved the ability to do this. Those that have, such as bubonic plague and anthrax, are highly invasive.



The basic structure of a bacterial cell


Food bug outbreak shuts wards to others

A HOSPITAL has been closed to all GP-arranged admissions except suspected cases of the *E. coli 0157* food poisoning outbreak. Monklands Hospital in Airdrie is using a third ward to deal with the outbreak. It will be used as an intensive care unit.

Thirty-two adults and a child were being treated yesterday in the hospital, where the Lanarkshire Infectious Diseases Unit is based. The number giving cause for concern rose from ten to 15 over the weekend and the number showing symptoms rose from 189 to 209

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Salmonella food poisoning

Food poisoning caused by *Salmonella* comes on suddenly as a result of the presence of large numbers of bacteria (it is not very virulent). The symptoms, which usually occur within 12–24 hours of eating contaminated food, include fever, vomiting, diarrhoea and abdominal pain. It can occasionally prove fatal.

Salmonella bacteria from an animal's gut may contaminate other parts of the animal's body during slaughter and processing.
If the meat is then chilled or frozen, little bacterial growth will occur until defrosting. Bacteria may be transmitted during defrosting if any water drips onto other foods and contaminates them. Similarly, the bacteria can be transmitted by handling raw chicken. Inadequate cooking can accelerate bacterial growth.
Salmonella may be passed on in eggs and unpasteurised milk.
A sufferer may also act as a ***carrier*** since the bacteria colonise the small intestine and may be egested in the faeces for up to 4 months.

Precautions include the adequate thawing of frozen food and thorough cooking. Raw and cooked foods should be stored separately in fridges, and the transfer of bacteria from raw to cooked foods via hands, utensils and work surfaces should be avoided.


TEM of Salmonella bacterium


Raw and cooked foods should not be stored together

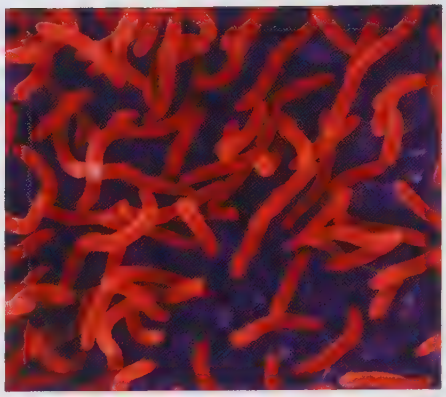
Cholera

Cholera is a water-borne disease caused by the bacterium *Vibrio cholerae*.
It tends to occur in areas where there is a lack of proper sanitation, an unclean water supply or contaminated food.
Water supplies become contaminated when infected people pass out large numbers of the bacteria in their faeces.
Many of these people may not actually suffer from the disease and are called ***symptomless carriers***.

Transmission can occur by drinking contaminated water or by infected people handling food or cooking utensils without washing their hands.
The bacterium multiplies in the small intestine and secretes a toxin which prevents the normal absorption of salts and water.
The symptoms are severe diarrhoea and loss of fluid.
Dehydration can cause death if the condition is not treated within 24 hours.

Cholera can be treated by giving the patient a solution of salts and glucose intravenously.
If the sufferer is able to drink, then **oral rehydration therapy** can be given.
Treatment is often effective since the glucose takes salts with it as it is absorbed into the blood in the small intestine.

Effective treatment of sewage and the chlorination of drinking water mean that cholera is virtually unknown in developed countries.
In many developing countries, overcrowding, poor sanitation and the lack of clean water encourages the spread of the disease.
These countries do not have the financial resources to solve the problems of poor housing, lack of proper sanitation and untreated water supplies.



SEM of Vibrio cholerae

Oral rehydration therapy given to Rwandan refugee during cholera epidemic

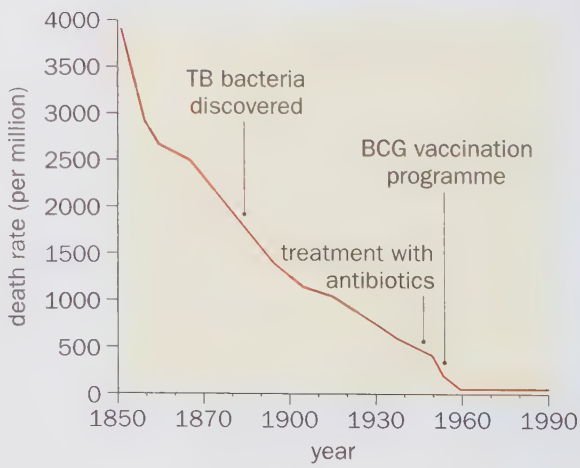
Tuberculosis

Tuberculosis (TB) is a bacterial disease that was once thought to be nearly eradicated.
However, in recent years it has shown a resurgence and is estimated to kill 2 million people a year worldwide.

Pulmonary TB is the most common form and is caused by the bacterium *Mycobacterium tuberculosis*.
It attacks the lungs and the lymph nodes, especially those in the neck.
Sufferers have a persistent cough and fever and lose weight through lack of appetite.
The bacterium is transmitted in airborne droplets and can spread rapidly where people live in overcrowded conditions.
The bacteria may remain in the lungs or the lymph system for years before becoming active.

The introduction of the antibiotic streptomycin along with improved housing conditions brought about a large decrease in the incidence of TB in the UK in the 1940s.
This was the case in many other developed countries too and it was hoped that the disease could be completely eradicated.
However, TB is now showing a worldwide increase due to some strains of the bacterium becoming resistant to drugs.
Poor housing and an increase in homeless people has also contributed to the increased incidence of TB in developed countries.

Overcrowded street in Jaipur, India



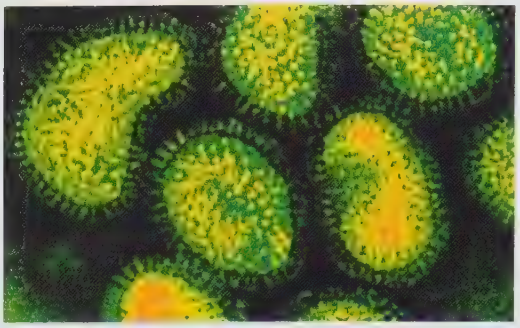
Decline in annual death rate from TB in England and Wales

Viral disease

We looked at the structure and reproduction of viruses on page 75. You should remember that they are extremely small (0.02–0.3 μm), and are made up of DNA or RNA surrounded by a protein coat.

Viruses are intracellular parasites which are only capable of reproducing inside living host cells.
They cause disease by

- damaging the host cells that they invade,
- producing toxins as they invade cells and reproduce.



TEM of influenza virus

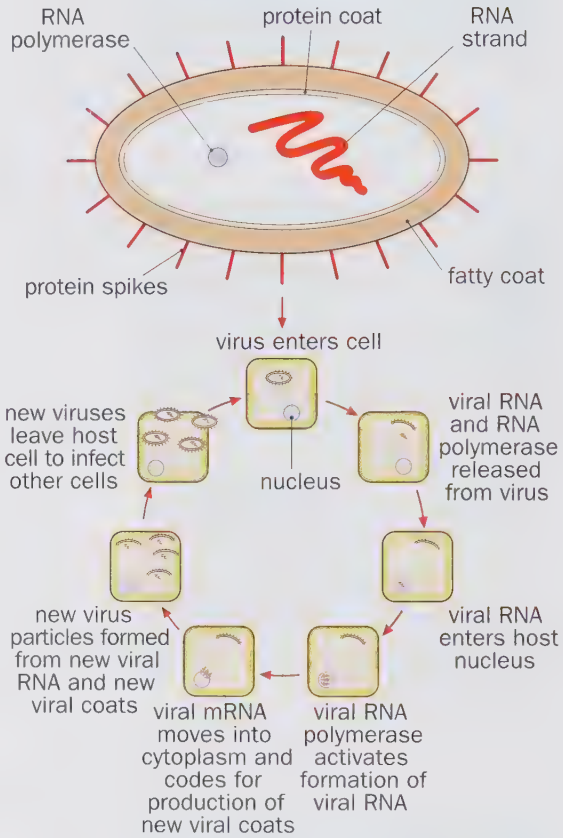
Influenza

Influenza is an RNA virus with a spiky protein coat consisting of two types of antigen which bind specifically with the host cell.

There are three main types of influenza virus: A, B and C.
Only influenza type A causes serious epidemics.
Deaths can occur due to secondary respiratory infections by bacteria causing bronchitis and pneumonia.
Type B usually causes milder, more local outbreaks. Type C produces minor cold-like symptoms and is not very common.

Transmission of influenza is by droplet infection (coughing and sneezing).
These droplets may be inhaled directly or may evaporate to give airborne virus particles that can survive dry conditions for some time.
Transmission is more likely in crowded and poorly ventilated places.

The virus affects the respiratory passages, especially the nose and throat.
The **incubation period** (the time between infection and the symptoms developing in a person) is about 2 days.
The symptoms may last up to 4 days and include headache, sore throat, backache and fever, with the body temperature rising to 40 °C with shivering and sweating.
The virus attacks the epithelial lining of the trachea, bronchi and bronchioles, and, as mentioned above, secondary infections by bacteria may also occur. A person rarely remains infective for more than 6 days.



How the influenza virus attacks cells

Treatment of viral infections is difficult because the virus is inside the cells of the host.
Drugs may not penetrate these cells and if they did they may even damage them further. Antibiotics are ineffective against viruses so for the main part we have to rely upon the body's own defence system.
Rest, aspirins or paracetamol to reduce temperature, and plenty of fluids help to relieve some of the symptoms of influenza.

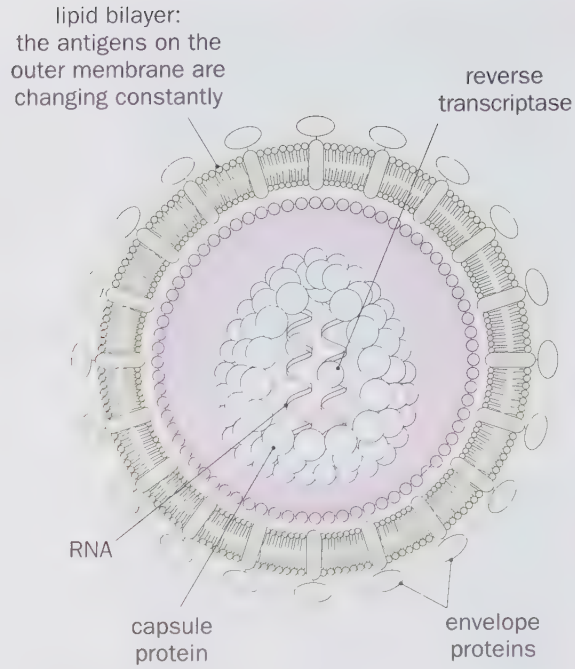
Vaccination helps to protect particularly susceptible individuals.
But protection is short-lived and mass vaccination is not recommended.
Use of tissues and handkerchiefs cuts down the spread of droplets by sneezing and coughing.
Better ventilation and less overcrowding in work places also helps.
The spread of influenza would be much restricted if affected people stayed isolated instead of going to work and transmitting it to others.

Acquired immune deficiency syndrome (AIDS)

AIDS is caused by the human immunodeficiency virus (HIV).
The virus attacks **helper T-cells** in the body's immune system.
HIV resembles the influenza virus in appearance with its outer lipid envelope with glycoprotein spikes.
The protein core contains two RNA molecules instead of one.

HIV is a **retrovirus**. This means that it uses the RNA to produce a single strand of DNA, called **copy DNA (cDNA)**, inside the host's cell.
The enzyme **reverse transcriptase** catalyses this reverse transcription of DNA from an RNA template.

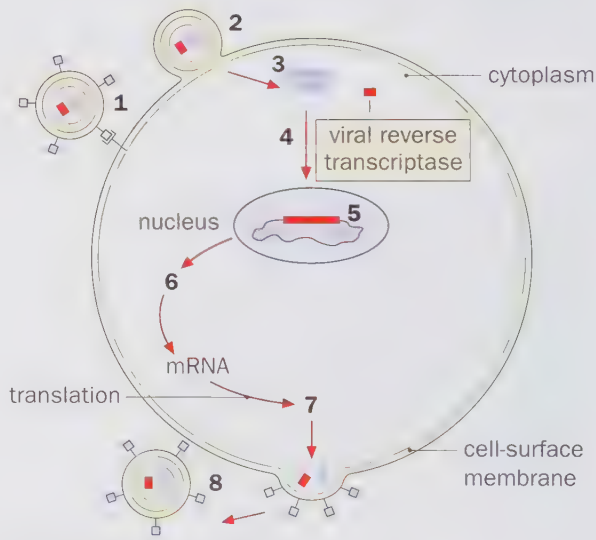
The virus can remain latent for many years before being activated, when it will start to replicate and destroy the host cell.
By reducing the number of helper T-cells, HIV weakens the body's ability to fight disease.
Eventually the person infected with HIV can succumb to any of a number of other infections because of their weakened immune system.
So AIDS is actually the name given to a collection of diseases brought on by the weakening of the body's immune system.



The structure of HIV

Replication of HIV

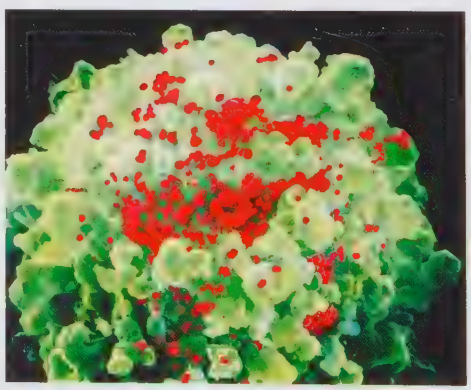
1 The HIV retrovirus attaches to a receptor site on the surface of the host cell.
2 The envelope of the virus fuses with the host cell-surface membrane.
3 The viral RNA, under the action of reverse transcriptase, acts as a template to make a single strand of DNA (cDNA).
4 It then forms a second complementary DNA strand.
5 The resulting double strand of DNA inserts itself in the chromosomal DNA of the host cell, forming a provirus.
6 The viral DNA is transcribed into mRNA.
7 The mRNA directs the synthesis of viral proteins.
8 New viruses are made from the protein and RNA and leave the host cell to infect other cells.



The replication of HIV

So eventually each infected helper T-cell bursts open to release thousands of new viruses which can infect other helper T-cells.
As the number of viruses increases, so the number of helper T-cells is reduced.
Eventually the immune system can become so weakened that the symptoms of AIDS begin to appear.

It is important to realise that HIV does not necessarily result in AIDS.
Some infected people remain symptomless and are therefore carriers.
They make antibodies against the virus, which can be detected in a blood test.


SEM of a T-lymphocyte infected with HIV virus (red) (×5000)

Signs, symptoms and transmission of AIDS

For every 100 people infected with HIV per year, about 12 develop the signs and symptoms of full-blown AIDS.
This can take up to 7–8 years to develop.

Initially after infection, short-lived symptoms of flu-like illness may appear.
These may include swollen lymph glands and a raised temperature.
This is followed by a period of months or even years when there are no signs of illness.
As the immune system weakens, infections that would normally be held in check start to take hold.

Symptoms of these **opportunistic infections** include a rare form of skin cancer called **Kaposi's sarcoma**.
Other types of cancer may appear and the sufferer may experience weight loss, fever, diarrhoea and deteriorating brain function leading to dementia.
The most common cause of death in AIDS patients is a rare type of pneumonia.

The red ribbon is a symbol of awareness and support for those living with HIV

Transmission

HIV is mainly transmitted in the blood or in semen.
The virus enters the body through slight abrasions that may occur during sexual contact or via hypodermic needles contaminated with infected blood.

Early in the history of the disease, the transfusion of infected blood placed haemophiliacs at risk since they required regular injections of factor VIII.
Developed countries soon introduced sterilisation and screening of blood and blood products prior to transfusion.

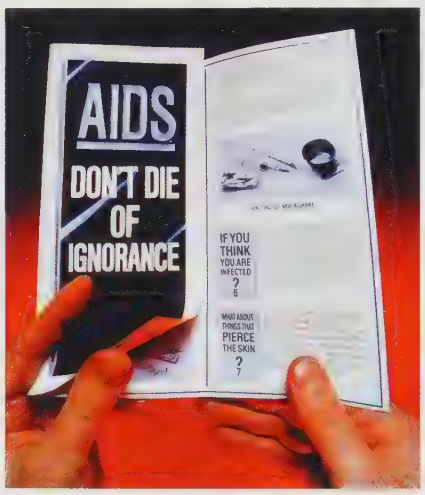
Sexual transmission of HIV can occur between male homosexual partners.
Damage to the lining of the rectum during anal intercourse can cause bleeding and infection by contaminated blood or semen.
Either partner may infect the other.

Heterosexual transmission of HIV is rare, since the walls of the vagina are much tougher and do not bleed as easily.
People who have a large number of sexual partners are more at risk.
Secretions from the vaginal wall may carry HIV to the male partner via his penis.
Alternatively, HIV from infected semen may get across the lining of the vagina, especially if any ulceration has occurred.

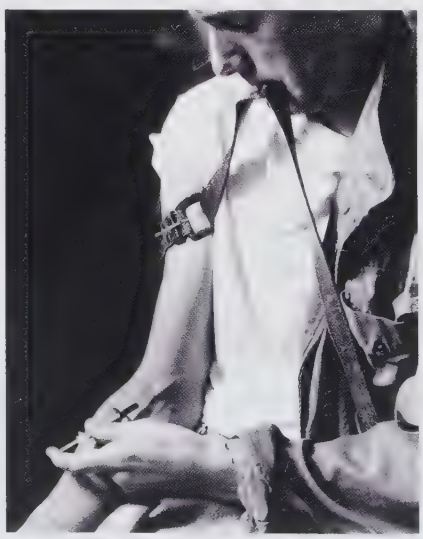
The second highest risk group is those drug users who inject intravenously.
In 1986, HIV spread alarmingly through drug addicts in Edinburgh who shared needles and syringes.

Needles were in short supply in Edinburgh after pharmacists stopped their sale in 1982 in a bid to curb drug mis-use.

Unborn babies are at high risk of infection from HIV if the pregnant mother carries the disease since it can pass across the placenta to the fetus.
A lactating mother can also pass the virus to her infant in breast milk.



This leaflet produced in 1997 was distributed to every household in the UK



A drug addict injecting

Treatment and prevention of HIV

The World Health Organisation has estimated that 30 million adults have become infected since the pandemic started. In 2012 there were 1.6 million AIDS-related deaths and 2.3 million new cases of HIV recorded.

Treatment

There is no cure for AIDS and as yet no vaccine for HIV.

- Scientists are trying to develop drugs that will inactivate HIV, but as we mentioned earlier, the problem is that the drugs may damage the host lymphocytes.

If drugs do reduce the number of T-lymphocytes in the blood, the patient is open to opportunistic infections.
The best known drug so far developed is **zidovudine (AZT)**. This stops HIV replicating by binding with reverse transcriptase and blocking its action.
But this can result in harmful side-effects, such as anaemia.

- The development of a vaccine offers the best hope of attacking the HIV virus and preventing AIDS.

However, the surface proteins of the virus can change so it is hard to produce a vaccine that can counter this.

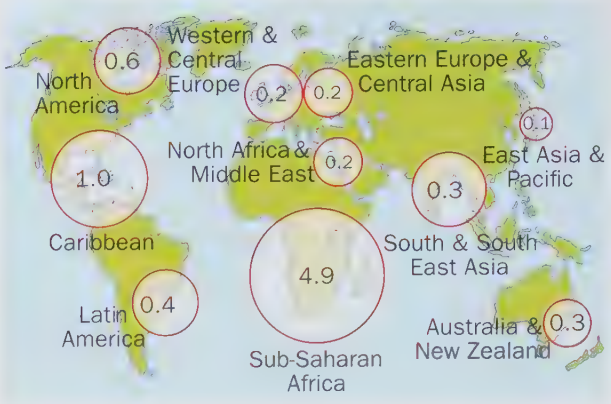
HIV is a retrovirus and retroviral genes can cause cancer. So any vaccine based on an attenuated (killed) whole virus could cause cancer.

Prevention

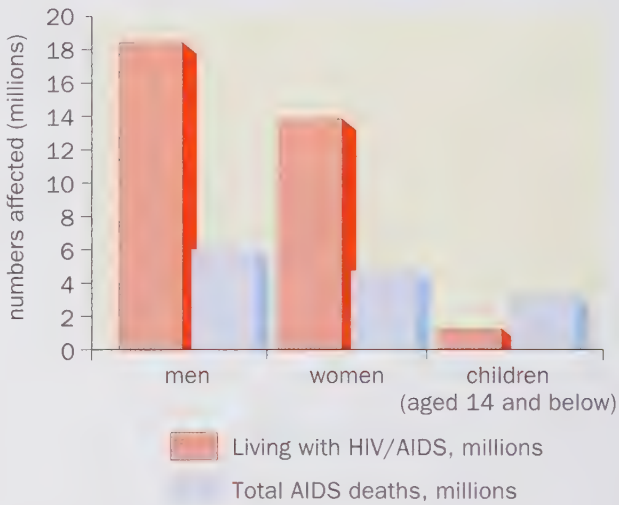
Precautions that can be effective in reducing the spread of HIV include the following:

- The use of condoms to provide a physical barrier to the transmission of the virus during sexual intercourse.
- Setting up free needle exchange schemes for those people who inject drugs. This reduces the risk of transmission from the use of shared needles and syringes.
- Screening donated blood for HIV antibodies and eliminating any contaminated blood being used for transfusion.
- Education programmes make people aware of the methods of transmission of the HIV virus and how they can be prevented.
- **Contact tracing** is important in the control of the spread of HIV in the UK. Any person diagnosed as HIV-positive is asked to identify people who they may have put at risk. These people are then offered an HIV test, which reveals the presence of HIV antibodies in their blood. However, these antibodies will only appear several weeks after the person has become infected.

Figures published in 2012 by UNAIDS report that since 2001 there has been a 33% decrease in new HIV infections.
Experts are hopeful that the AIDS epidemic will continue to slow down.



Percentage of adults (15–49 years) living with HIV/AIDS, 2011



Numbers affected by AIDS

Screening blood for contamination

Malaria

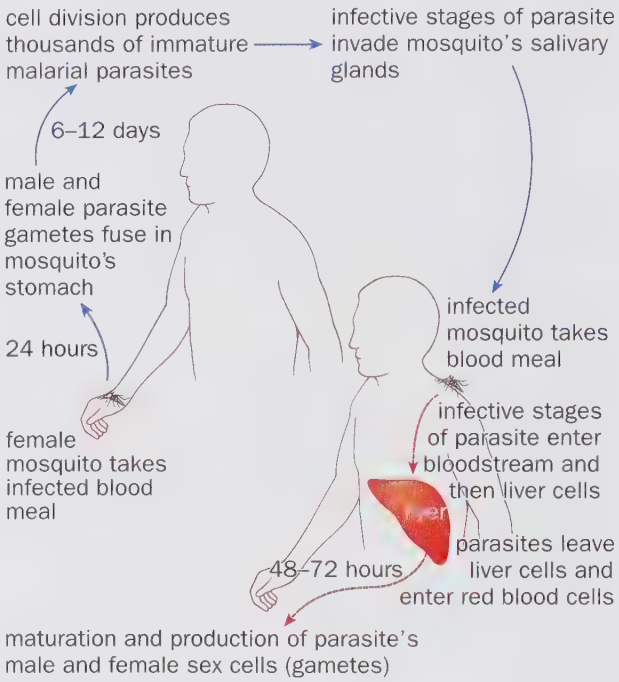
Malaria is not caused by a bacterium or a virus.
The microorganism responsible is the protoctist *Plasmodium*.
This malarial parasite infects liver cells and red blood cells.

Plasmodium is carried from one person to another by the female *Anopheles* mosquito.
The mosquito is called a **vector** because it transmits the disease.
The protoctist has a complex cycle of transmission which includes both sexual and asexual stages.
When the mosquito bites an infected person, it takes up red blood cells that contain the gametes of *Plasmodium*.
These gametes fuse in the gut of the mosquito, producing the infective form of the protist which then migrates to the insect's salivary glands.
When the mosquito bites an uninfected person, *Plasmodium* is transmitted into the blood together with an anticoagulant in the saliva.
Plasmodium then invades the red blood cells and liver cells of the newly-infected person where it multiplies asexually producing many more of the parasites.

The female Anopheles mosquito transmits malaria

The most dangerous species is *Plasmodium falciparum*.
P. falciparum can cause red blood cells to stick together, leading to the blockage of blood vessels to the brain, lungs and kidneys.
Symptoms of malaria include high fever, chills and heavy sweating, and if untreated the sufferer may die within a few days.

Malaria flourishes in some of the warmer regions of the world.
Poorer countries are at risk since they do not have the resources or expertise to combat the disease.
Mosquito control programmes have often been disrupted by civil wars.



The life cycle of the malaria parasite

Treatment and prevention

Most efforts to control malaria are directed at destroying the mosquito vector and so breaking the cycle of transmission.
Control measures have included:

- draining ponds and ditches where the mosquito lays its eggs and where the larvae develop but this is expensive and it is impossible to drain all breeding areas,
- stocking ponds and lakes with fish that eat mosquito larvae,
- spraying insecticides on the surfaces of ponds and lakes to kill the larvae and pupae,
- using nets treated with insecticide around people's beds to protect them from mosquitoes.

Treatment of malarial victims has included:

- antimalarial drugs such as quinine and chloroquinine to prevent the parasite spreading within the human body; however, the over-use of such drugs has resulted in resistant strains of *Plasmodium* appearing,
- development of vaccines to attack different stages of the parasite inside the body,
- insect repellents sprayed on the skin to prevent the mosquito from landing.

Healthworker fumigates homes to remove mosquitoes

You can see how the allele for sickle cell anaemia can give people protection from malaria on page 213.

Natural defence barriers

Most microorganisms find it difficult to get inside the body. If they did not experience resistance from the body's defences, then we would become constantly ill and would eventually die.

There are four main ways in which the body prevents potential pathogens from entering.

Physical defence

The skin provides a physical barrier to the entry of pathogens. The tough, outer layer of dead cells contains keratin and very little water, which microorganisms need for growth.
If we keep our skin healthy, it is rarely penetrated by microorganisms.
The skin also secretes various chemicals which inhibit the growth of bacteria such as

- tears – the lachrymal glands secrete tears which dilute and wash away microorganisms and irritant chemicals from the eyes,
- sebum – secreted by the sebaceous glands contains fatty acids that have an antimicrobial action,

Another physical barrier is provided by mucus – a sticky secretion produced by goblet cells that line the air passages. The mucus traps many airborne pathogens.

Mechanical defence

Nasal hairs filter the air that is drawn into the nasal passages. Bacteria and other particles trapped in the mucus are swept away from the lungs by **cilia**.
Cilia are tiny hairs that beat with a wave-like motion.

Chemical defence

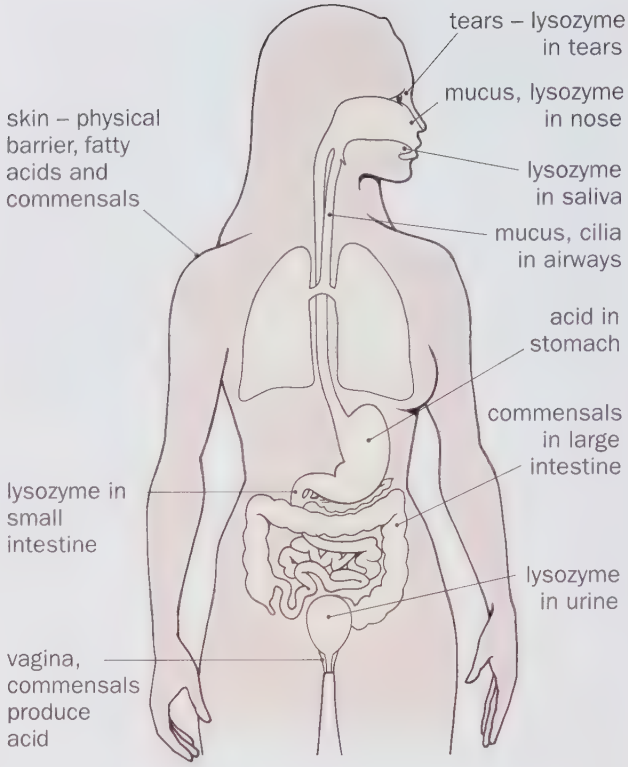
Tears, mucus, saliva and sweat all contain chemicals that inhibit the growth of microorganisms.
Lysozyme is an enzyme found in many of these secretions. It catalyses the hydrolysis of molecules in the cell walls of bacteria.
In addition to lysozyme, sweat contains lactate, which also slows bacterial growth.

Hydrochloric acid present in gastric juice kills most microorganisms that get as far as the stomach.

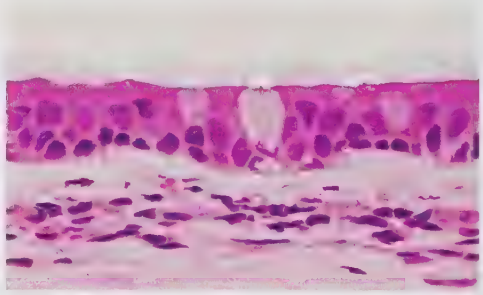
The vagina contains harmless bacteria that convert carbohydrate to lactate, which kills off pathogenic bacteria.

Biological defence

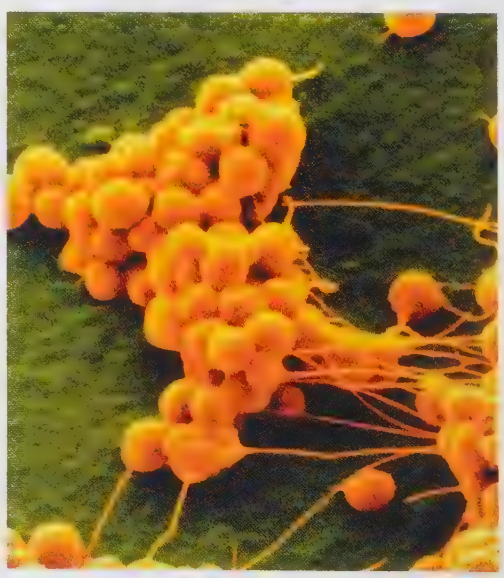
There are natural populations of harmless bacteria living on the skin and mucous membranes that inhibit the growth of many pathogenic microbes.
They protect us by competing with pathogenic bacteria for nutrients.
Wide-spectrum antibiotics can destroy these useful bacteria and so remove some of the body's defences.



Barriers to infection



Cilated cells of the respiratory pathway waft mucus along



SEM of symbiotic bacteria on the skin surface (×2000)

Immune response

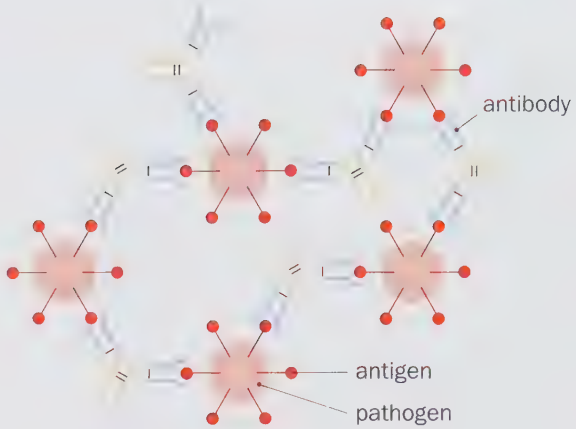
An immune response is the way in which the body responds to invasion by a specific pathogen or antigen.
This response involves the production of cells and chemicals designed to defend the body against the pathogen.

Antigens are substances that can produce an immune response.
Antigens trigger the production of **antibodies** by the immune system.
Each type of antibody is specific to a particular antigen and reacts with it to make it harmless.

Self antigens are part of the cell-surface membrane of body cells.
They do not activate an immune response, except in the case of auto-immune diseases, because the body recognises them as being part of us.
Non-self antigens are found on the cell walls and cell-surface membranes of bacteria, viruses, fungi, animal parasites, pollen and incompatible blood cells.
Toxins released by some bacteria and viruses may also act as antigens.
These do produce an immune response as the body recognises them as being foreign.

Antigens are large complex molecules that take the form of a protein, polysaccharide or glycoprotein.
They have two distinct characteristics:

- they stimulate the formation of specific antibodies,
- they react specifically with these antibodies.

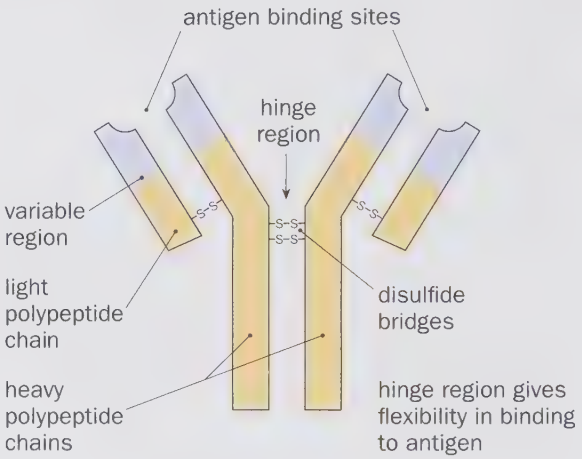


Pathogens immobilised by the formation of an antigen–antibody complex

The nature of antibodies

Antibodies are glycoproteins which belong to a special group of blood proteins known as **immunoglobulins.**

The basic structure of an antibody consists of two pairs of polypeptide chains.
Two of the chains are long and are referred to as **heavy (H) chains**.
The other two shorter chains are referred to as **light (L) chains**.
The chains are held together by disulfide (S-S) bridges.



The basic structure of an antibody

Each antibody molecule has two identical antigen binding sites.
These are different for each kind of antibody, which allows the antibody to recognise and attach ***specifically*** to a particular antigen.
It is the sequence of amino acids at the antigen binding sites that makes the three-dimensional shape that fits with the specific antigen.
It's like the lock and key mechanism in which an enzyme binds to its specific substrate.
In this case, an **antibody–antigen complex** is formed.

The antigen attachment site is known as the ***variable*** region and is specific to each antigen.
The rest of each polypeptide chain is termed the ***constant*** region, as it is common to other antibodies too.

Some antibodies act by immobilising the antigens so that they can be destroyed by phagocytosis.
Others, known as **antitoxins**, are able to neutralise the toxins released by bacteria such as those that cause cholera, diphtheria and tetanus.

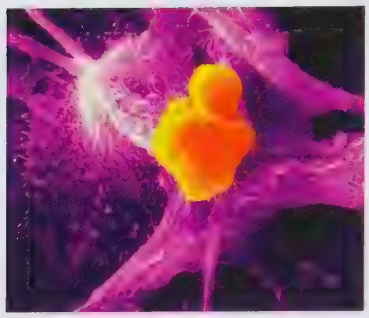
Computer graphic representing two antibodies bound to an antigen

Cellular response

There are many different types of white blood cells involved in immunity. Cellular response, also known as cell-mediated immunity, involves **T-lymphocytes** and **macrophages.**

Macrophages

Macrophages are phagocytic (see page 170).
Although macrophages are non-specific in their response to pathogens – they engulf and digest all types of foreign cells and viruses – some of the antigen molecules become embedded in the macrophage cell-surface membrane.
This can alert T- and B-lymphocyte cells to the fact that the body has been invaded by a particular pathogen.



Macrophage attacking cancer cell

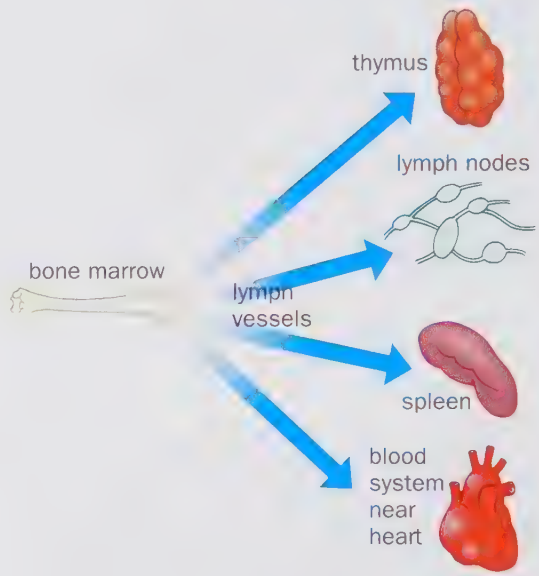
Lymphocytes

Lymphocytes are white blood cells that recognise and react with antigens. There are two main types: **T-lymphocytes** and **B-lymphocytes**. These develop from **stem cells** in the bone marrow. They eventually migrate to the **spleen** and **lymph nodes** where they mature.

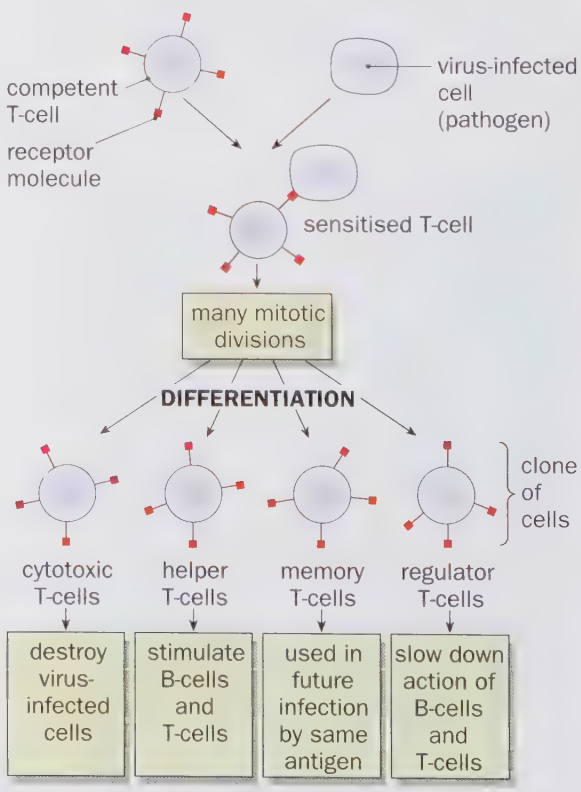
T-lymphocytes initially pass to the **thymus** (a lymph gland in the neck) where they are activated.
These activated, or **competent**, T-lymphocytes then also pass to the lymph nodes and spleen.
There are millions of types of T-lymphocytes, each recognising and attacking a particular type of antigen. This is known as **cell-mediated immunity**. It takes place like this:

- Macrophages engulf the pathogens bearing the antigens.
- Binding sites on the surface of particular T-lymphocytes recognise and fit with the molecular structure of the antigen.
- The T-lymphocytes become activated and start to multiply rapidly. They produce a large clone of identical cells, all of which can recognise the antigen as being foreign.
- This clone differentiates (sub-divides) into the following cell types:
  - i) **cytotoxic T-cells** (killer cells) destroy the antigen directly by attaching to them and releasing the chemical **perforin** to kill them,
  - ii) **helper T-cells** attract and stimulate macrophages and promote the activity of other T- and B-cells to increase antibody production,
  - iii) **memory T-cells** have no action but multiply very fast if a second invasion of the antigen occurs, producing an even bigger clone of T-lymphocytes and resulting in rapid destruction of the antigen,
  - iv) **regulator T-cells** slow down the vigorous response of the T-cytotoxic cells and the T-helper cells, so slowing down and stopping the immune response.

Cytokines are a group of small proteins important in cell signalling and include **interleukins**.
Cytokines are produced by the immune cells.
They act through receptors on the cell-surface membrane of these cells and regulate the balance between cell-mediated and humoral immune responses.



The origin and circulation of lymphocytes



Antibody-mediated immunity

Sometimes known as the **humoral response**, **antibody-mediated immunity** involves the production of B-lymphocytes, which are activated by antigens attached to the macrophage membrane. B-lymphocyte activity is also stimulated by helper T-cells.

The B-lymphocytes attack and destroy the antigen on the surface of microorganisms and other foreign (non-self) material by producing antibodies that circulate in the blood, lymph and tissue fluid.

There are ***three*** different types of B-lymphocytes.

- **Plasma B-cells** which secrete antibodies into the circulation.
  Each antibody is specific to the pathogenic antigen.
  Plasma cells produce antibodies very quickly (as many as 2000 per second for each cell).
  An active plasma cell can live for 4–5 days.
- **Memory B-cells** live for a long time in the blood.
  They do not produce antibodies, but are programmed to remember a specific antigen and respond very rapidly to any subsequent infection.
- **Dividing B-cells** produce more B-lymphocyte cells.

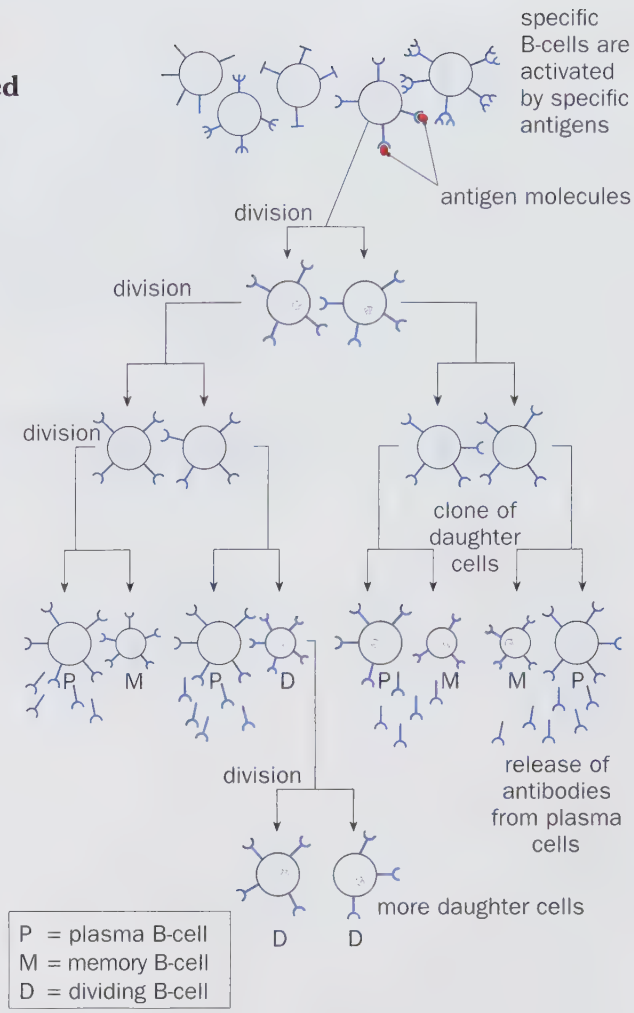
Stages in antibody-mediated immunity

- Macrophages engulf the foreign material of the pathogen.
- Antigens from the pathogen embedded in the macrophage cell-surface membrane are recognised by B-lymphocytes.
- B-lymphocytes are activated when specific binding sites on their cell-surface membrane attach to the antigens.
- The activated cells either enlarge to form plasma cells, which produce antibodies, or multiply to form a clone of memory B-cells. Dividing B-cells continue multiplication to produce a large clone.
- The antibodies circulate and bind with any material with the specific antigen and destroy it.
- Any further invasion by a pathogen with the same antigen will trigger the same events to happen, since there is a large clone of memory cells specific to that antigen.
  These can be activated to produce plasma B-cells very quickly and so produce vast quantities of a specific antibody.

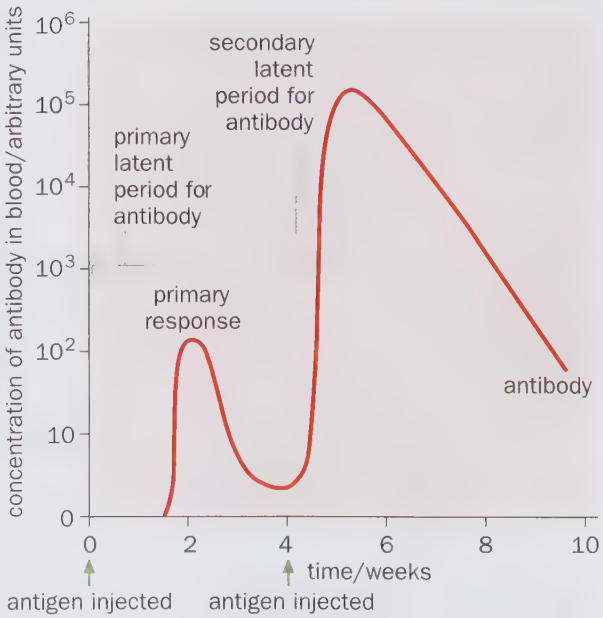
What do you think is meant by the **primary** and **secondary responses** of antibody production?

Compare
a) the length of the latent period and
b) the amount of antibody produced in the primary and secondary responses.

From what you have learnt about the action of B-lymphocytes, explain:
a) the differences in the rate of response,
b) the increasing production of antibodies in both responses.



Antibody-mediated immunity



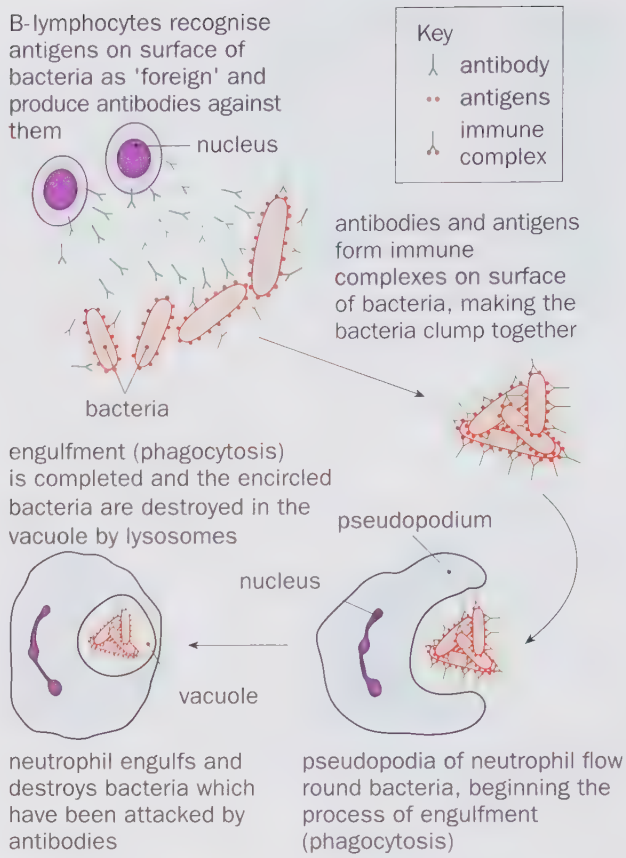
Primary and secondary responses of antibody production

The reaction between antibody and antigen

The antibody becomes attached to the antigen at the antigen binding site like a key in a lock (though neither is an enzyme). This causes the antibody to change from a T shape to a Y shape. This exposes part of the antibody molecule to substances in the plasma that are together known as **complement.** It is the combined effect of antibody and complement that determines the action of the antibody.
For instance the antibody may

- cause the antigens to stick together – **agglutination**,
- stimulate **phagocytosis** by neutrophils, as in the case with **opsonins** which mark the antigen for an immune response,
- act as an **antitoxin** and cause the precipitation of soluble bacterial toxins,
- prevent pathogenic bacteria attaching to cell-surface membranes.

Some of these methods of antigen destruction are shown in the diagram.



Lymphocytes and neutrophils at work

Immunity and immunisation

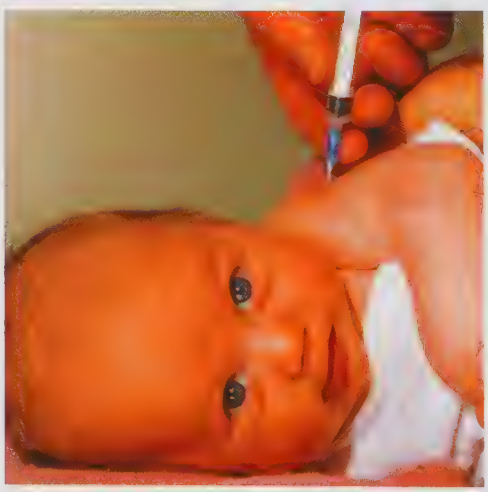
So far we have looked at the type of immunity that occurs during the course of an infection.
The pathogen invades the body, which responds by stimulating the production of T-lymphocytes and B-lymphocytes which are involved in the immune response.
Memory cells are formed which provide long-term immunity to the antigen.

This type of immunity is termed **active** because the lymphocytes are ***activated*** by antigens present on the surface of the pathogen.
Since this activation takes place during the natural course of an infection, we call it **natural active immunity**.

But the immune response can also be triggered ***artificially***.
This involves the injection of antigens into the body.
We call this **artificial active immunity**, although it is more commonly referred to as **vaccination**.

Passive immunity occurs when an individual becomes temporarily immune to an antigen by receiving ready-made antibodies from someone else.

- **Natural passive immunity** occurs when pre-formed antibodies pass naturally from mother to baby across the placenta and in breast milk. Immunity is only temporary since the baby's body does not 'know' how to make more antibodies. But it provides the baby with protection until it develops its ***own*** immune system.
- **Artificial passive immunity** occurs when pre-formed antibodies extracted from one individual are injected into another as a **serum**. This only produces short-term immunity because the injected antibody will possess 'non-self' antigens and so the body will produce antibodies against it.
  However, this sort of immunity can provide a 'quick fix' and is given to people who have been bitten by poisonous snakes or rabid dogs.



Pre-formed antibodies in a serum can act against snake venom

Vaccination

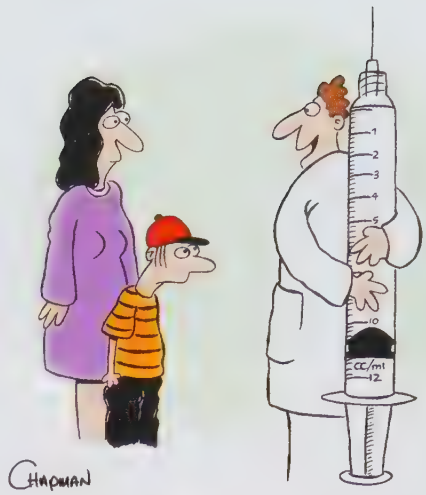
As you have seen, a **vaccine** is an antigen that is injected or swallowed, which causes the development of active immunity in the patient.
The small quantities of antigen introduced into the person's body stimulate the production of antibodies as if infected by the disease.
This type of immunity is long-term since the body is able to produce memory cells in the normal way.
Antigens are treated before being introduced to the body of the patient, in order to make them relatively harmless.

Most vaccines are made in one of the following ways.

- **Killed virulent organisms** such as whooping cough bacteria.
  The microorganism is killed by heat or by use of chemicals, which denature its enzymes.
  So the dead pathogen will not cause the disease, but it will possess antigenic sites on its surface that will be recognised by T- and B-lymphocytes, and result in the production of antibodies in the recipient.
  But there is no chance of the pathogen replicating and causing infection.
- **Live non-virulent strains** such as in the virus causing rubella.
  Vaccines made in this way are often called **attenuated** vaccines.
  The pathogen is deliberately weakened to ensure it does not cause severe infection. Other examples are the BCG vaccine used against tuberculosis and the Sabin vaccine used against poliomyelitis, which is taken orally.
- **Modified toxins** such as the vaccine used against diphtheria and tetanus. In this type of vaccine, the **toxoids** (toxic substances) produced by the bacteria are made harmless.
  They are used to stimulate antibody production, but there is no risk of infection by the pathogen.
- **Isolated antigens** separated from a pathogen, such as influenza.
  The important antigens are separated from the microorganism, in this case by breaking up the pathogen's structure and obtaining glycoproteins.
  The 'flu vaccine' contains a mixture of antigens from various strains of influenza virus, in an attempt to combat the great variation that exists. This **antigenic variation** occurs in microorganisms that have a high mutation rate.
- **Genetically engineered antigens** as in the case of hepatitis B.
  Restriction endonucleases are used to extract from the pathogen the genes that code for a particular antigen. The genes can then be inserted in a harmless plasmid vector using a ligase enzyme. The bacterial cells then replicate to produce large amounts of antigen. (See Chapter 22.)

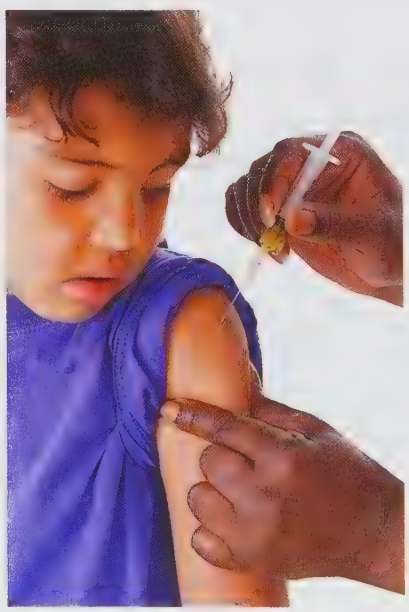
The active immunity that vaccines produce can give protection for a long time. However, several more vaccinations, called **boosters**, may be needed after the initial one. Boosters stimulate quicker production of antibodies and prolong protection. In the case of diphtheria, the first injection lasts 1 year, whereas a second 'booster' injection provides protection for 10 years.

Herd immunity occurs when the vaccination of a significant portion of a population (or herd) provides protection for individuals that have not developed immunity.
As a result, chains of infection are disrupted when large numbers of a population are immune.



"Good news. We have a new shot that combines all shots in one."

Some vaccines can be taken orally



ABO blood groups

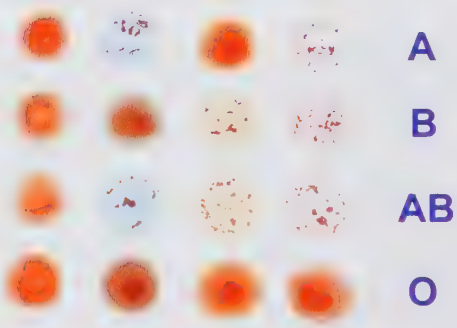
All red blood cells may look the same, but they have different antigens on their cell-surface membranes.
People can be put into one of four blood groups under the **ABO system**.
These blood groups are called **A**, **B**, **AB** and **O**.
They are determined by which antigens are present on the red blood cells.
There are two main antigens: **A** and **B**.

- People in blood group **A** have only **A** antigens on their red blood cells.
- People in blood group **B** have only **B** antigens.
- People in blood group **AB** have both **A** and **B** antigens.
- People in blood group **O** have neither **A** nor **B** antigens.

The plasma will not contain antibodies that will attack its own antigens. So depending upon the blood group, the plasma may contain just **anti-A** antibodies or just **anti-B** antibodies, both of them or neither of them.

Antigen on red blood cells	Antibody in plasma	Blood group	% of UK Caucasian population
A	anti-B	A	46
B	anti-A	B	8
A and B	none	AB	2
neither A nor B	anti-A and anti-B	O	44

- The anti-B antibodies in blood group A plasma do not attack the A antigens of blood group A red blood cells.
  But they will attack the B antigens of blood group B red blood cells.
- The anti-A antibodies in blood group B plasma do not attack the B antigens of blood group B red blood cells.
  But they will attack the A antigens of blood group A red blood cells.
- There are no anti-A and anti-B antibodies in blood group AB plasma to attack the A or B antigens of blood group AB red blood cells.
- Blood group O plasma contains both anti-A and anti-B antibodies but there are neither A nor B antigens on their red blood cells.



Blood of the four different groups tested with anti-sera A (blue), anti-sera B (yellow) and anti-sera A and B (white). Red blood cells clump together if the anti-sera reacts with the A or B antigens on their surfaces

Blood transfusion

When people have a blood transfusion it is important that they receive blood that is compatible with their own.
If a transfusion of incompatible blood is given, then the body will produce antibodies that will unite with the antigens on the surface of the introduced red blood cells resulting in them clumping together.

These clumped red blood cells can block small vessels and cause kidney failure and other potentially lethal reactions.

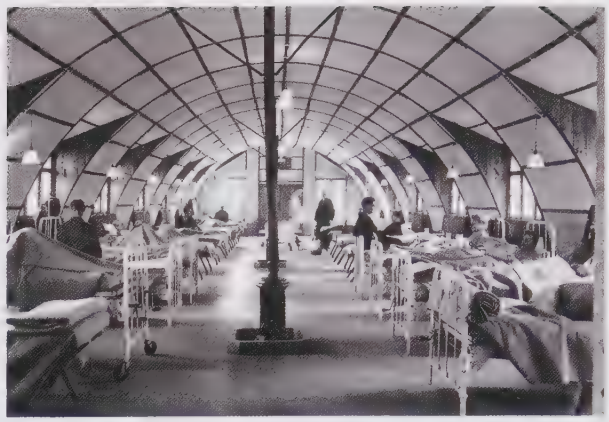
Look at the table which shows blood transfusion compatibility between the donor and the recipient.

- Group O people are called **universal donors**.
  Why are they able to give their blood to anybody?
- Group AB people are said to be **universal recipients**.
  Why are they able to receive blood from any donor?

donor \ recipient	A	B	AB	O
A	✓	✗	✓	✗
B	✗	✓	✓	✗
AB	✗	✗	✓	✗
O	✓	✓	✓	✓

✓ = *safe transfusion*
✗ = *unsafe transfusion*

Antibiotics



Antibiotics are chemicals produced by microorganisms (mainly bacteria and fungi) which ***at low concentrations*** have the ability to inhibit or destroy pathogens.

The first antibiotic was penicillin, which was developed in the 1940s in response to the need to treat soldiers in the Second World War. (You can read about the large scale production of penicillin on page 403.)

Bacteriostatic antibiotics stop bacteria reproducing and slow their growth, for example tetracycline.
Bactericidal antibiotics act by killing bacteria, for example penicillin.

Several species of fungi have been used to produce antimicrobial chemicals and there are now 50–100 commercially available antibiotics.

Antibiotics are characterised by their range of effectiveness and their mode of action.

- **Broad spectrum antibiotics** kill a wide range of bacteria.
- **Narrow spectrum antibiotics** are effective against only a few types of bacteria.

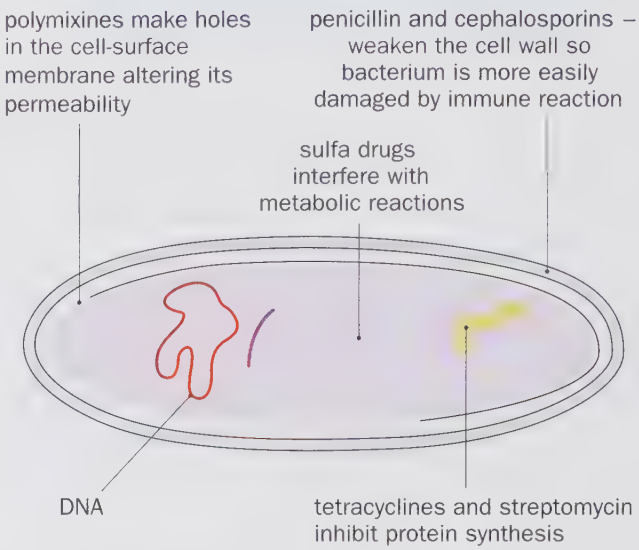
To kill a specific pathogen, you need to use a narrow spectrum antibiotic which is specific for the disease.

All antibiotics must have **selective toxicity**.
This means they should kill or inhibit the growth of bacteria or fungi, but cause little or no damage to the host.

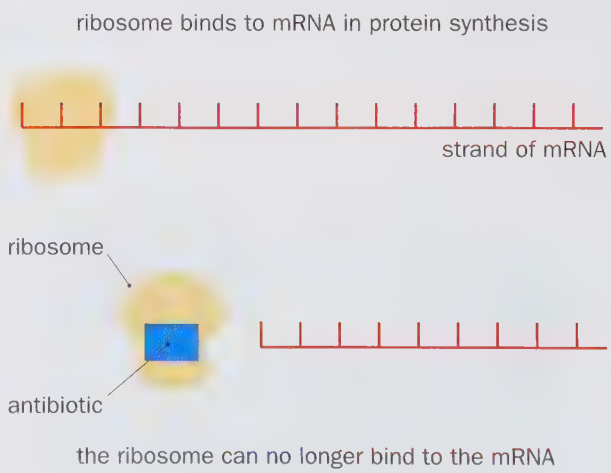
Antibiotics interfere with the growth or metabolism of the pathogen in a variety of ways.

- **Penicillin** inhibits the enzymes that are involved in the formation of the bacterial cell wall.
  Bacteria with weak cell walls die due to leakage of the cell contents.
- **Streptomycin** binds to the bacterial ribosomes, so preventing protein synthesis, including the synthesis of enzymes.
  The lack of protein affects bacterial growth and results in its death.
  Fortunately, bacterial ribosomes are different from human ones. So streptomycin does not interfere with the synthesis of proteins in the cells of the patient taking the drug.
  Tetracyclines also work by interfering with bacterial ribosomes.
- **Polymyxines** damage bacterial cell-surface membranes even in resting cells.

Antibiotics tend to be used against bacterial infections more than fungal infections.
This is because fungal cells work in a similar way to human cells. Consequently, many antifungal agents are highly toxic to humans.



Some antibiotics and the way they work



How streptomycin and tetracycline act

Antibiotic resistance

Many bacteria that were once susceptible to antibiotics have become resistant due to random mutations (see page 214).
This is the case with penicillin-resistant bacteria which are able to synthesise **penicillinase**. This enzyme is able to break down the antibiotic.

Repeated exposure to antibiotics has led to more bacteria surviving and passing on resistant genes.
Antibiotic-resistant genes are often carried by plasmids in bacteria and can be passed on to different strains.
This method of transferring genetic information is called **conjugation**.
For example, gonorrhoea-causing bacteria that can no longer be killed by high doses of penicillin can pass on their resistance to other strains.
Some bacteria have become resistant to two or more antibiotics as a result of conjugation.

Bacterial cells pass on genetic information during conjugation

Perhaps surprisingly, resistant microorganisms often occur in hospitals where antibiotics are used most often, especially to prevent infections occurring from surgery. This has been the case with MRSA, more than 90% of which are now unaffected by penicillin.
Even more worrying is the emergence of more strains of MRSA that are resistant to a growing list of antibiotics.

Resistant strains of *Clostridium difficile* (a bacterium that causes diarrhoea and painful abdominal cramps) have also emerged. It can be prevented by practising good hygiene in healthcare environments.
Washing hands regularly with soap (alcohol hand gel is ineffective against *C. difficile* spores) and cleaning work surfaces with bleach are effective.
In recent years *C. difficile* infections have fallen from 53000 reported cases in 2007 to 14687 in 2013.

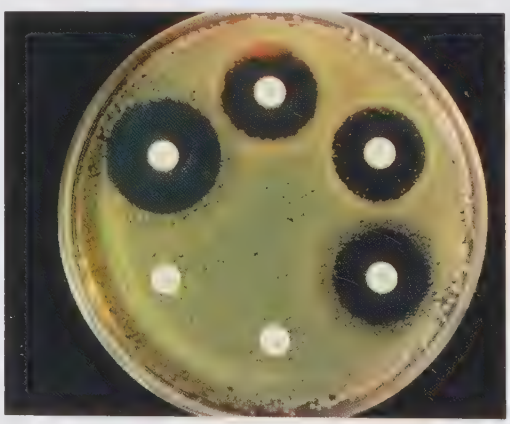
Many doctors have over-prescribed antibiotics in the past

The inappropriate and widespread use of antibiotics should be avoided.
In the past, antibiotics have probably been prescribed too readily by many doctors.
Reducing the number of antibiotics in use means that fewer bacteria are exposed to them and reduces the chances of resistant strains appearing.

Screening antibiotics

It is important that, if an antibiotic is used, it should be the most effective one for a particular strain of bacteria or fungus.
This can be achieved by **screening**.

Samples of the microorganism are taken from the sufferer, or from contaminated food or water, or from faeces. The microorganism is grown on an agar plate. Different antibiotics absorbed onto filter paper discs are then placed on the surface of the agar and the plate is incubated.



Sensitivity of E. coli to antibiotics

The antibiotic diffuses out of each filter paper disc, killing the microbe and producing a clear area: the **inhibition zone**.
The greater the diameter of the inhibition zone, the more effective the antibiotic is against the microorganism.

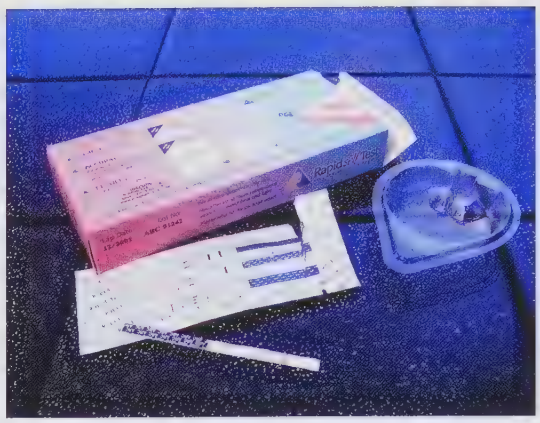
Which antibiotic do you think is most effective against the bacteria tested here?

Biology at work: Antibodies and pregnancy testing

The testing of urine samples for signs of pregnancy goes back to ancient Egyptian times, but the reliable and sensitive tests we have today are a recent innovation.

These tests were developed in the 1960s. They involve the detection of **human chorionic gonadotrophin (hCG)**.

hCG is a hormone produced by the placenta in early pregnancy. It stimulates the ovaries to produce oestrogen and progesterone. These hormones are needed to maintain a healthy pregnancy. hCG is excreted in the urine and high levels act as confirmation of pregnancy. The levels of hCG double about every two days in a pregnant woman.



Commercial pregnancy testing kit

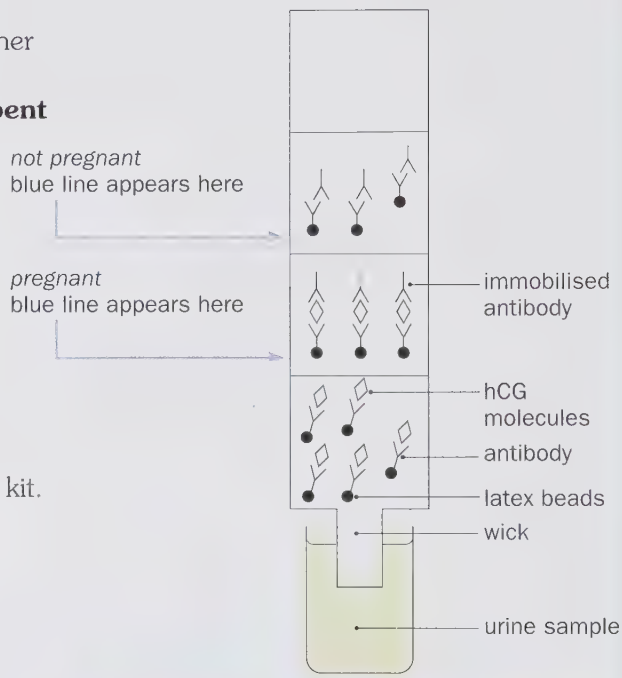
The immune system produces antibodies which target particular cells or chemicals in the body.
Pregnancy tests rely on monoclonal antibodies that bind to hCG only, the presence of other hormones therefore will not give a positive result.
The antibodies bind to hCG molecules making them clump together producing a visible change in the test strip.
This test is a good example of an **enzyme linked immunosorbent assay (ELISA)**, a sensitive biochemical test that uses an enzyme linked to an antibody as a marker for the detection of a specific protein, in this case hCG.

The most common pregnancy test involves a dipstick. This is basically a strip of absorbent material (the wick) on a plastic backing, impregnated with monoclonal antibodies.

The application area is dipped into a sample of early morning urine, which moves up the wick.
As it moves up, hCG in the urine binds to hCG antibodies (which are combined with coloured latex beads) built into the test kit.

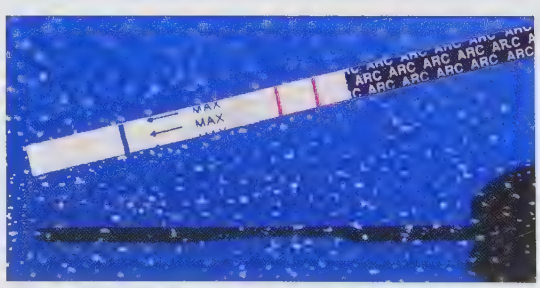
This combination of hCG, antibodies and beads progresses up the wick to an area containing immobilised hCG antibodies. Here the hCG/antibody complex becomes concentrated, and the beads form a coloured band visible on the test strip.

This represents a positive result.



There is a second area on the test strip that confirms the test is working properly. In this area there is a further row of immobilised antibodies which will combine with the antibodies attached to the coloured beads if no hCG is present.

So a negative pregnancy test should still show a result, that is, a coloured band but in a different window.



Test stick showing a positive result

Monoclonal antibodies have a number of other medical related uses, for example

- attaching cytotoxic drugs to antibodies so that they can target cancer cells,
- making use of their specificity in medical diagnosis by enabling the identification of a particular strain of pathogen.

Biology at work: Meningitis

What is meningitis?

Meningitis is an infection of the brain's membranes (**meninges**) caused by both viruses and bacteria.
Viral infection is most common but is rarely life-threatening – most people make a full recovery, but it is estimated that there are about 3200 cases of the more serious bacterial meningitis in the UK every year. This figure represents the true burden of disease, not the number of confirmed cases.

What is bacterial meningitis?

There are two types of bacterial meningitis: meningococcal and pneumonococcal, the former being most common.
One in 10 people who contract bacterial meningitis die and one in seven are left with a permanent disability, including brain damage.

How is it caught?

The disease is cyclic and peaks in incidence occur every 10–15 years.
In 2000, meningococcal meningitis had an incidence of over 2900 cases and this was the highest for over 50 years.
Airborne droplets spread bacteria which can also be transmitted through coughing, sneezing and kissing.
Once breathed in, the bacteria colonise the tissues lining the nose and throat but rarely invade further.

What are the symptoms?

Most people have a natural resistance to meningococcal meningitis. There are, however, small risks of complications arising from the symptoms, which develop within 2–10 days of contact:

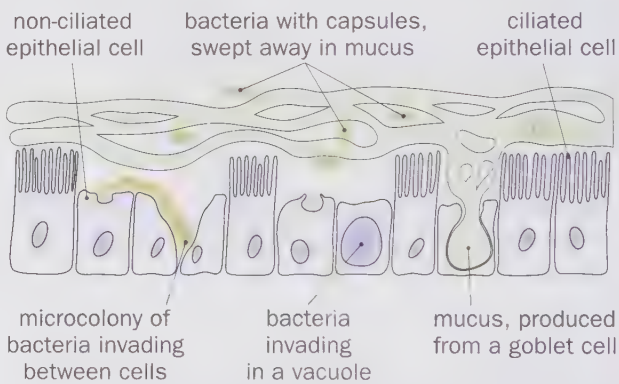
- raised temperature,
- aches and pains,
- headache,
- vomiting.

Many of these are similar to other common infections, which is a problem since a delay in diagnosis can prove fatal once symptoms specific to meningitis emerge.

Bacterial invasion of the mucosal linings in the respiratory tract and eventual passage into the bloodstream leads to capillary leakage and the characteristic red spots that do not fade under pressure when a drinking glass is applied.
Once in the blood of the meninges, bacteria pass into the cerebrospinal fluid, causing inflammation, which then irritates nerve endings.
This leads to the reaction to bright light, and muscle spasms that cause a stiff neck.
Swelling of the brain causes headaches, vomiting, drowsiness and eventual coma, seizure and death.

Year	Laboratory confirmed meningitis B	Laboratory confirmed meningitis C	Laboratory confirmed total
00/01	1951	507	2968
01/02	1754	256	2369
02/03	1384	142	1787
03/04	1490	72	1751
04/05	1459	49	1712
05/06	1331	32	1508
06/07	1165	33	1386
07/08	1248	34	1467
08/09	1211	14	1380
09/10	936	18	1113
10/11	990	26	1219
11/12	720	31	920

Confirmed cases of meningococcal meningitis in England and Wales 2000–2012. Data taken from the Meningitis Research Foundation, Figure 1 (meningitis.org)



Bacteria on the epithelial surface of the nose or throat membranes. Their presence is natural; it is when they invade non-ciliated cells or are phagocytosed that symptoms occur

SEM of meningitis bacteria (×18 000)

Summary

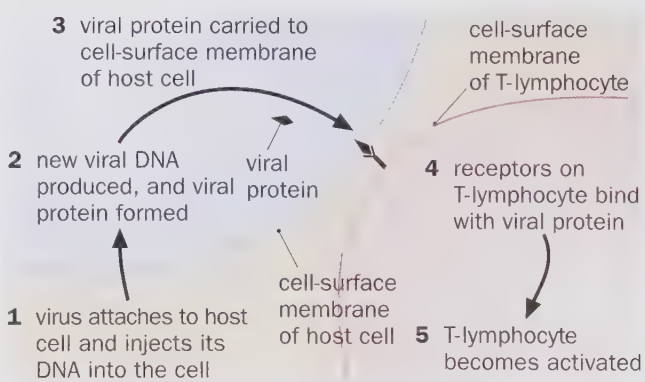
- White blood cells (leucocytes) defend the body against pathogens.
- Bacterial growth can be affected by temperature, pH, oxygen and nutrients.
- Certain safety precautions should be taken and aseptic techniques used when working with microorganisms.
- The growth of bacteria can be monitored by a number of methods, including the use of a haemocytometer, dilution plating or turbidimetry.
- The ability of bacteria to produce disease is affected by pathogenicity, infectivity and invasiveness.
- Viruses are intracellular parasites that cause disease by damaging the host cells that they invade and producing toxins as they invade cells and reproduce.
- AIDS is caused by human immunodeficiency virus (HIV), which attacks the body's immune system.
- Our bodies provide physical, mechanical, chemical and biological defence against microorganisms.
- An immune response is the way in which the body responds to invasion by a specific pathogen or antigen.
- Antibodies bind to specific antigens forming an antibody–antigen complex.
- Cellular response or cell-mediated immunity involves the activities of T-lymphocytes and macrophages.
- Antibody-mediated immunity involves the production of B-lymphocytes, which are activated by antigens that become attached to the macrophage membrane.
- Active immunity may be triggered naturally or artificially. Passive immunity occurs when a person receives ready-made antibodies from someone else.
- Vaccines can be made from killed virulent organisms, live non-virulent strains, modified toxins, isolated antigens or genetically engineered antigens.
- Antibiotics are chemicals produced by microorganisms, which, at low concentrations, can destroy pathogens.
- There is an increasing tendency for bacteria to become resistant to antibiotics as the result of random mutations.

Questions

1 For each of the following infectious diseases, describe the method of transmission, the symptoms of the disease, and the methods by which the incidence of the disease can be reduced:
a) influenza,
b) *Salmonella* food poisoning,
c) AIDS.

2 a) i) Describe how influenza is transmitted from one person to another.
ii) Suggest one reason why epidemics of influenza, such as the one in 1999/2000, tend to be more common in the winter.
b) The incidence of influenza and the death rate from it vary considerably from year to year.
i) In one year the death rate from influenza in the United States was 0.3 per 100 000. The population of the United States is about 250 million.
How many people died from influenza in the United States in that year?
ii) Every few years, major outbreaks of influenza occur as a result of the emergence of a new variety of the influenza virus. Explain how a new variety causes a major outbreak.
c) Describe how viruses such as influenza virus cause the signs and symptoms of disease.

3 The diagram shows some of the events that occur when a particular virus infects a cell in the human body.
a) Describe how viral proteins are produced within the infected cell.
b) Describe how the activated T-lymphocytes respond to the viral infection and prevent further spread of the virus.
c) Explain why a particular type of virus activates only the receptors on the surface of specific T-lymphocytes.



4 a) Describe two ways in which healthy human skin protects the body against invading pathogens.
b) The body has two ways of protecting itself from invading pathogens once they have entered – antibody-mediated immunity and cell-mediated immunity.

i) Name one group of microorganisms against which:
   1) antibody-mediated immunity is most effective,
   2) cell-mediated immunity is most effective.

ii) In each case, name the cell that is activated first in response to a pathogen to which the body is constantly exposed and state the response of each cell type to the pathogen.

5 The graph shows the response of a person to the use of a live vaccine.

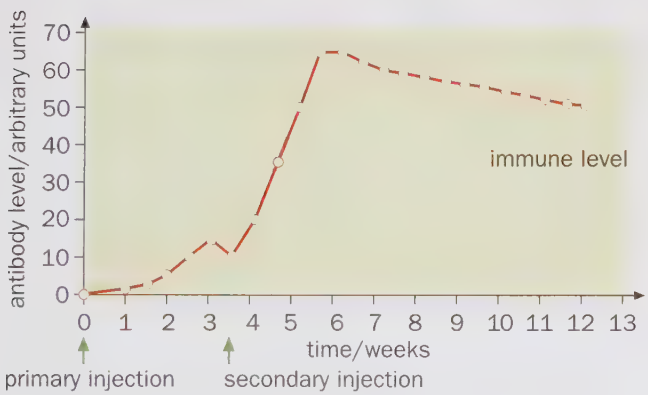
a) i) Explain why the response to the secondary injection was much greater than the response to the primary injection.

ii) Suggest why a 'booster' injection of the vaccine may need to be given every few years.

b) i) Give one example of a live vaccine.

ii) Describe briefly how a live vaccine may be produced.

iii) Suggest one possible risk of using a live vaccine.



6 a) Give three factors that may determine the ability of bacteria to produce disease in the body.

b) Give two ways in which antibiotics affect the functioning of bacteria.

c) Explain one way in which antibiotic-resistant strains of bacteria counteract the effects of antibiotics.

d) Scientists researching ways of dealing with antibiotic-resistant bacteria have found that certain carbohydrates are effective against bacteria that infect epithelial cells lining the throat and lungs. These carbohydrates work by competing with the bacteria for receptor sites on the membranes of the epithelial cells.
Suggest what is meant by 'competing for receptor sites'.

7 a) i) Draw a simple labelled diagram to show the main features of a virus.

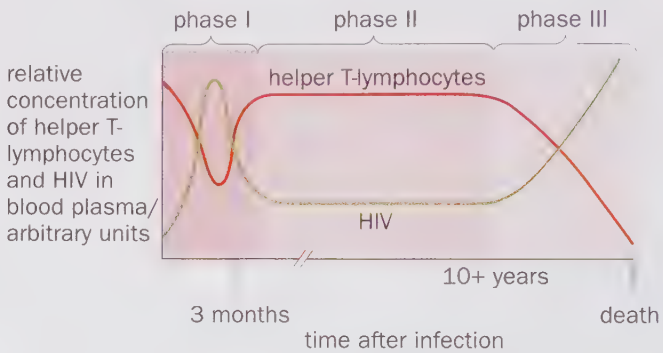
ii) State one way in which viruses differ from all other groups of microorganisms.

b) HIV (human immunodeficiency virus) can reproduce within human helper T-lymphocytes. The graph shows three phases in the relationship between the concentration of HIV in blood plasma helper T-lymphocytes in an affected individual.

i) Use the information in the graph to describe fully the relationship between helper T-lymphocytes and HIV:
   1) during phase I,
   2) during phase II,
   3) during phase III.

ii) Suggest a full explanation for:
   1) the initial rise in HIV during phase I,
   2) the initial fall in helper T-lymphocytes during phase I.

iii) Suggest one function of B-lymphocytes during phase I.

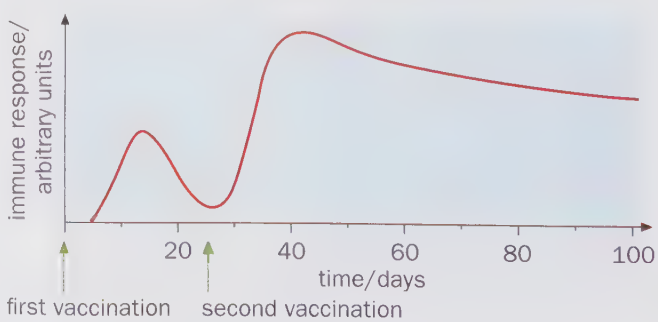


8 The graph below shows the primary and secondary immune response when a child is vaccinated.

a) The vertical axis on the graph is labelled 'immune response'.
Suggest what might have been measured to reflect this.

b) One difference between the result of the two vaccinations is that the peak height of the second immune response is much higher than the first. Give a full biological explanation for this observation.

c) Describe two other differences between the results of the first and second vaccination.



The nature of disease

1 a) What is a pathogen? [1]

b) Give *two* ways pathogens can cause disease when they enter the body of their host. [2]

[3]

2 Basal metabolic rate (BMR) is the amount of energy you require to keep yourself alive when you are at rest, but not asleep. Your BMR varies according to your age, sex and body size.

a) A man has a higher BMR than a woman of the same age, height and body mass. Explain why. [2]

b) A pregnant woman has a higher BMR than a non-pregnant woman of the same age and height. Explain why. [2]

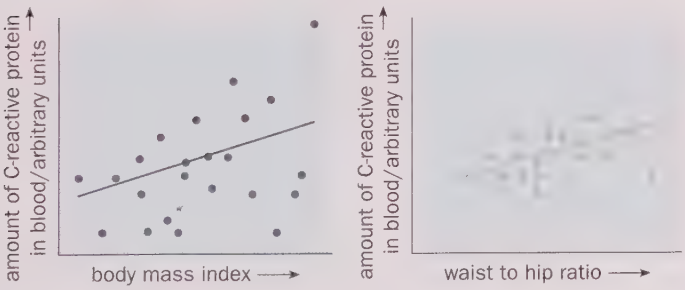
[4]

3 a) Describe how an atheroma forms in the wall of an artery. [2]

Fat cells can release proteins which promote inflammation of arterial walls so increasing the risk of developing atheroma. This protein is known as a C-reactive protein.

Scientists measured the amount of this C-reactive protein in the blood of volunteers. They also calculated the body mass index (BMI) and waist to hip ratio (WHR) for each volunteer. A high BMI or WHR is usually linked to obesity. The graphs show the results and lines of best fit.

The scientists found correlations between the amount of C-reactive protein and BMI, and with WHR.



b) i) What do these data suggest about being obese and the risk of atheroma formation? Give evidence for your answer. [2]

ii) Using these data, would BMI or WHR be more reliable for identifying people who are likely to develop atheromas? Explain your answer. [2]

iii) A person's BMI is calculated using the following equation.

$$\text{BMI} = \frac{\text{mass}}{\text{height}^2}$$

Suggest *one* reason why BMI may not be an accurate indicator of obesity. [1]

AQA **[7]**

Exercise and health

4 a) The table below shows the results of an investigation into the effects of five minutes standard exercise on the pulse rate of four men who were of the same age and mass.

Individual	Pulse rate/beats per minute	
	Resting	Immediately after exercise
John	64	82
David	70	105
Anthony	78	135
Neil	68	98

i) Calculate the mean percentage increase in pulse rate for these men. Show your working. [2]

ii) Explain why it is an advantage for the pulse rate to increase during exercise. [2]

b) John plays football regularly. His resting pulse rate is lower than that of the other men and increases only by 28% after five minutes standard exercise. His blood pressure is lower than that of the other men.

i) Explain how regular exercise has brought about these effects. [3]

ii) Explain why John has a lower risk of developing cardiovascular disease. [1]

AQA (formerly NEAB) **[8]**

5 The table below shows measurements taken from two women, both 45 years of age, on attendance at a health clinic.

Measurement	Woman A	Woman B
Height/cm	175	160
Body mass/kg	65	83
Systolic blood pressure/mmHg	130	162
Diastolic blood pressure/mmHg	87	106
Plasma cholesterol/mg 100 cm^{-3}	207	241

a) The formula for calculating Body Mass Index (BMI) is given in Question 3, where mass is measured in kilograms, and height is measured in metres. Calculate the BMI for both women. Show your working. [2]

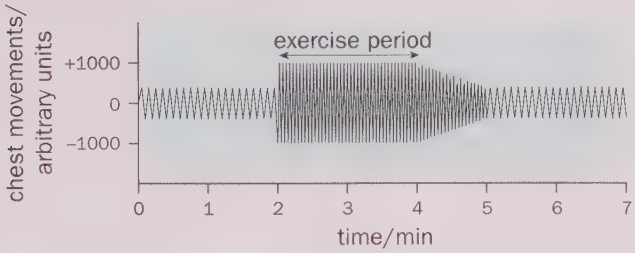
b) Which of the women is suffering from hypertension? Use data from the table to support your answer. [2]

c) There are actions which might help these women reduce the risk of cardiovascular disease (CVD) in later life. Suggest *three* actions they could take to reduce the risk of CVD. [3]

[7]

Further questions on health and disease

6 The breathing movements of an athlete were recorded before, during and after a two minute period of strenuous exercise. The breathing movements were recorded by means of a stethograph, which monitors chest movements. The graph below shows the recording over a seven minute period. The exercise period was during the third and fourth minutes.



a) Describe the changes in chest movements which occurred with the onset of exercise. [2]

b) Explain the chest movements during the minute following the exercise period. [2]

CCEA **[4]**

7 Harry and Bert are both 68-years-old. Bert has never smoked, but Harry has been a heavy smoker since his teens. Harry has now developed emphysema. A doctor carried out a lung volume test on them both, and measured the volume of air in their lungs over a period of 7 seconds whilst they were breathing out.
The results are shown in the table below.

	Volume of air in lungs/dm³	
Time/s	Patient A	Patient B
0	6.6	7.1
1	3.8	6.2
2	3.1	5.7
3	2.3	5.2
4	2.0	4.8
5	1.8	4.5
6	1.6	4.2
7	1.6	3.9

a) Both patients were breathing out during the time shown. Give evidence from the table to support this statement. [1]

b) Calculate the rate at which patient A and patient B breathed out air from their lungs between 0 and 3 seconds. Show your working. [2]

c) Which patient is Harry? Give evidence from the table to support your answer. [2]

d) Emphysema reduces the efficiency of gas exchange in the lungs. Explain why. [4]

AQA **[9]**

Communicable diseases and immunity

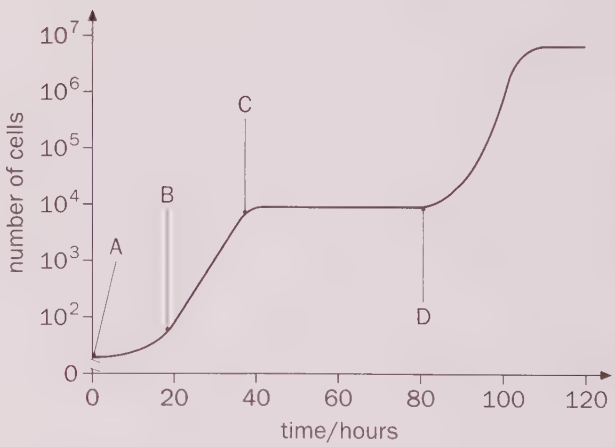
8 Concerns have been expressed that doctors' clothing might transmit bacteria from one patient to another. An investigation in a hospital showed that 50% of the ties worn by doctors had pathogenic bacteria on them. However, only 10% of the ties worn by hospital security staff had pathogenic bacteria on them.

a) Describe how you could use an agar plate to investigate whether a tie has bacteria on it. [4]

b) Hospital security staff were used as the control group in this investigation. Suggest why. [2]

AQA **[6]**

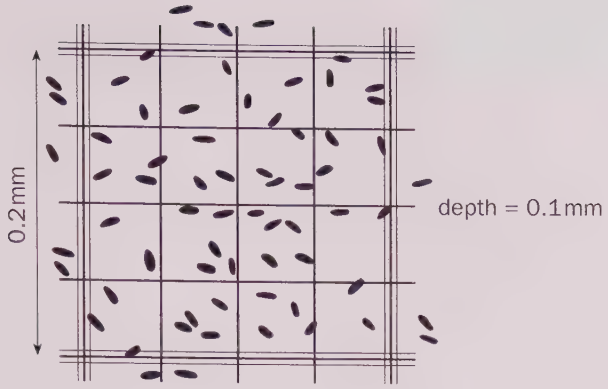
9 a) A student investigated the growth of a culture by counting the number of cells in samples removed at hourly intervals. Each sample was prepared for counting in a standard way. A haemocytometer was used to count the number of cells.
The results are shown in the graphs below.



i) Explain the shape of the curve between the following.
A and B; B and C; C and D. [3]

ii) Suggest why the population increased at point D. [1]

b) One haemocytometer observation taken during the experiment is shown in the diagram at the top of page 299.
Calculate the number of bacteria in 1 cm³ of the sample. Show your working. [3]

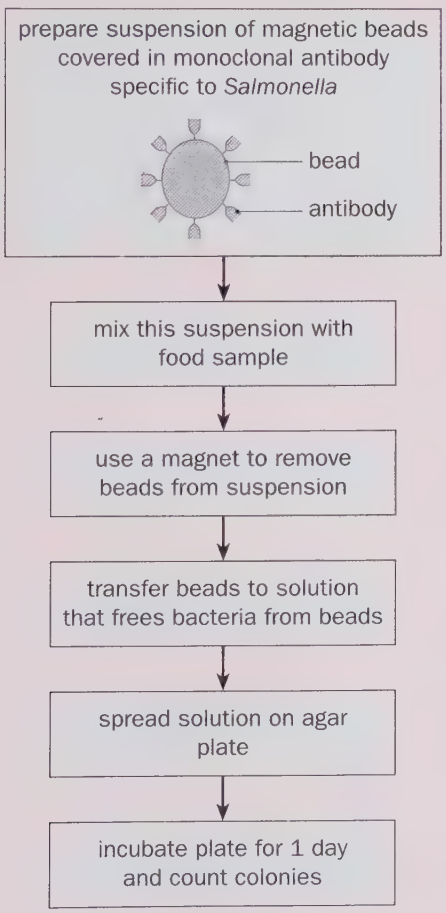


c) Suggest why the use of dilution plating would give a lower estimate of the number of bacteria present, compared with counting with a haemocytometer. [1]

AQA (formerly NEAB) **[8]**

10 a) *Salmonella* bacteria can cause food poisoning. Explain how. [2]

Many species of bacteria can be found on food. It is important to be able to test food to see whether or not it is contaminated with *Salmonella*. The diagram shows a method for isolating and growing any *Salmonella* present on food.



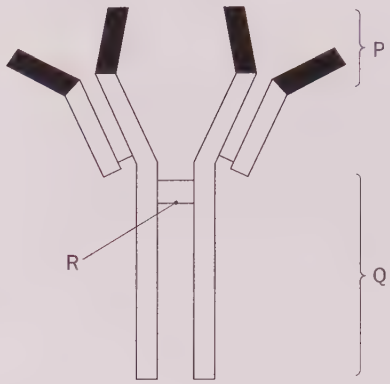
b) i) This method would only allow *Salmonella* bacteria to be isolated from a food sample. Explain how. [1]

ii) This method allows the number of *Salmonella* bacteria in a food sample to be estimated. Explain how. [2]

iii) Describe *two* aseptic techniques a technician would use, to prevent contamination with unwanted bacteria, when spreading the solution on the agar plate. [2]

AQA **[7]**

11 The diagram below shows an antibody molecule.



a) i) Name the region labelled P and state its function. [2]

ii) Name the region labelled Q and state its function. [2]

iii) Name the type of bond labelled R. [1]

b) Shortly after a microorganism, such as a bacterium or a fungus, enters the human body for the first time, the blood contains many different antibody molecules, each produced by a different group of cells. This is known as a polyclonal response.

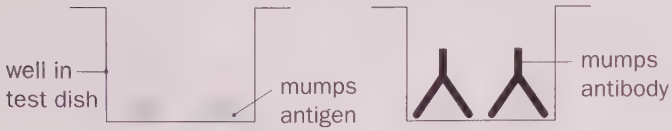
i) Name the type of cells that produce antibodies and releases them into the blood. [1]

ii) Suggest why different groups of cells respond to an infection by a bacterium or a fungus to give a polyclonal response. [3]

OCR (formerly Camb) **[9]**

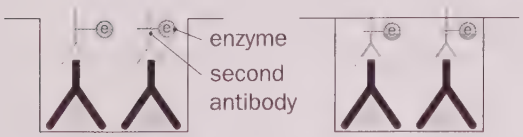
12 a) Define vaccination. [2]

b) A test has been developed to find out whether a person has antibodies against the mumps virus. The test is shown in the diagram.



1 Mumps antigen is attached to a well in a test dish.

2 A sample of blood plasma is added to the well. If mumps antibodies are present, they bind to the mumps antigen.



3 The well is washed. Then a second antibody with an enzyme attached is added. This binds specifically to the mumps antibody.

4 The well is washed again. A solution is added which changes colour if the enzyme is present. A colour change shows that the person has mumps antibodies.

i) This test will detect only mumps antibodies, and no other antibodies in the blood. Explain why. [1]

ii) It is important to wash the well at the start of step 4. Explain why. [2]

iii) There will be no colour change at step 4 if mumps antibodies are not present in the blood. Explain why. [2]

AQA **[7]**

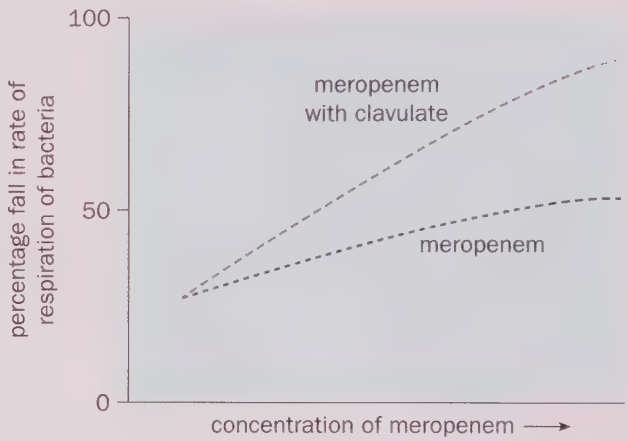
13 Tuberculosis (TB) is a disease caused by a bacterium, which mainly affects the lungs.

a) Name the bacterium which causes TB. [1]

b) Describe how TB is spread from one person to another. [2]

c) Worldwide the highest incidence of TB is associated with low income groups. Suggest *three* reasons why the incidence of TB is higher in low income groups. [3]

d) The antibiotic meropenem is not very effective against the bacterium that causes TB. Scientists investigated whether a substance called clavulate could make meropenem more effective against TB. The scientists grew the TB-causing bacterium on nutrient agar jelly in two sets of Petri dishes. They added different concentrations of meropenem to one set of Petri dishes. To the other set of Petri dishes they added the same concentrations of meropenem together with clavulate. They measured the percentage fall in the rate of respiration of the bacteria in each Petri dish after 2 weeks of treatment. The graph shows their results.



i) The scientists concluded that these results showed that clavulate made meropenem more effective in killing the TB-causing bacterium. Evaluate this conclusion. [2]

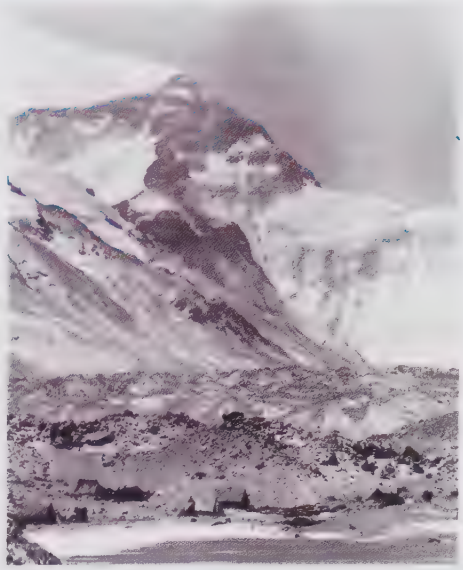
ii) The TB-causing bacterium produces an enzyme called β-lactamase, which breaks down meropenem. Clavulate binds to this enzyme and this stops the enzyme working. Suggest how this stops the enzyme working. [2]

Adapted from OCR and AQA **[10]**

17 Respiration

One of the great mountaineering mysteries of all time neared its resolution with the remarkable discovery of George Mallory's body on Mount Everest. On 8 June 1924, Mallory and his partner, Andrew Irvine, disappeared whilst attempting to be the first climbers to reach the summit. Mallory's perfectly preserved body was located on the upper slopes on 1 May 1999 and shows that he fell and died whilst descending. The fate of Irvine and whether the two actually reached the summit before perishing remains a mystery.

So how did an experienced climber like Mallory fall?
The majority of fatalities, which involve falls above 8000 m, are indirectly attributable to a lack of oxygen and its effects on the brain. Near the summit, climbing is limited by the maximum oxygen consumption rate, which is low. The brain's capacity to think clearly and make simple judgements is then limited. A momentary lapse in concentration can lead to a stumble resulting in a fatal fall.



North face of Everest from base camp, 1924

The need for energy

Living organisms need a constant source of energy to drive the metabolic reactions that take place inside their cells. These metabolic reactions include the following.

- **Movement**

Energy is needed for various types of movement in cells, including
– movement of cilia and flagella,
– muscle contraction,
– movement of chromosomes in cell division.

- **Maintaining a constant body temperature**

Endotherms, which include mammals and birds, need heat energy to keep their body temperature stable and so provide the optimum internal environment for their enzymes to function.

- **Anabolic processes**

These are the processes in which large, complex molecules are built from smaller, simple molecules.
This ***synthesis*** involves an input of energy since new chemical bonds have to be made within the molecule.
Examples of anabolic processes include the synthesis of polysaccharides from sugars, synthesis of proteins from amino acids and DNA replication.

- **Active transport**

Energy is needed to move some molecules and ions across the cell-surface membrane against a concentration gradient.

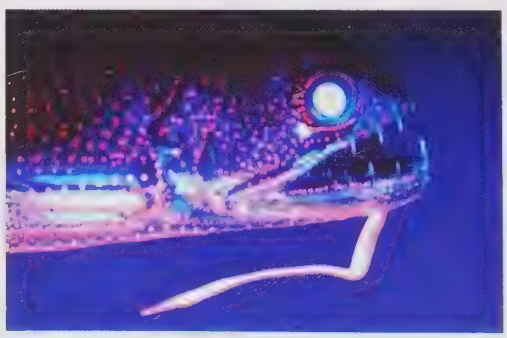
- **Bioluminescence**

Some organisms are able to convert chemical energy into light. Examples include 'glow worms', some phytoplankton and some bacteria.

- **Secretion**

The packaging and transport of secretory products into vesicles in cells such as those in the pancreas requires energy.

Mallory and Irvine prepare to leave Camp V on 6 June 1924



Bioluminescence in Astronesthes, a deep water fish

Cellular respiration

All living cells and organisms get their energy from respiration. This usually involves the oxidation of glucose to release energy and produce carbon dioxide and water.
It is important not to confuse respiration with gas exchange. Let's remind ourselves of the difference.

Gas exchange is the diffusion of gases into and out of cells, which allows respiration to take place.

So gas exchange involves taking oxygen in for respiration and then removing the carbon dioxide produced during the process.

Respiration is a series of oxidation reactions taking place inside living cells, which results in the release of energy from organic compounds such as glucose.

Autotrophs are able to make complex organic molecules, using an energy source such as sunlight, during photosynthesis.
The sunlight energy is transformed into chemical bond energy, which is stored in molecules such as carbohydrates, fats and proteins.
These energy-rich molecules are called **respiratory substrates**, since they can be broken down in respiration.

Most living organisms respire **aerobically**, using oxygen which releases a relatively large amount of energy.

Aerobic respiration is respiration that uses oxygen.

Some organisms respire **anaerobically**.
They do not use oxygen to carry out respiration.

Anaerobic respiration occurs in the absence of oxygen.

Some anaerobic cells switch to aerobic respiration if oxygen becomes available, because aerobic respiration releases far more energy.
However, there are some species of bacteria that carry out only anaerobic respiration and are actually poisoned by the presence of oxygen. These are called **obligate anaerobes**.

Releasing energy

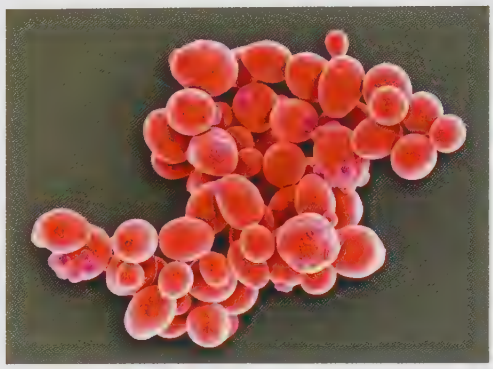
The chemical bond energy in your food could be released by combustion (burning).
The glucose would be converted to carbon dioxide and water but the energy would be released in an uncontrolled way and the increase in temperature would be fatal for living cells.

Respiration involves the gradual release of energy in small steps, rather than the rapid release of energy all at once.
The glucose is broken down in a step-by-step manner, with the controlled release of small amounts of energy at each stage.
It is these reactions that provide the energy to make a molecule called **adenosine triphosphate**, or **ATP**.

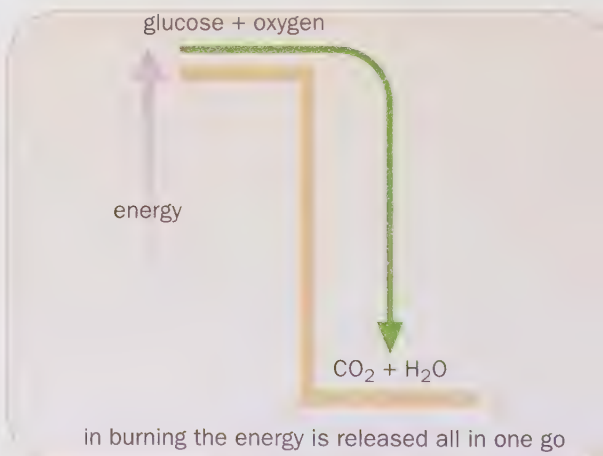
When a molecule of glucose is respired aerobically, it releases enough energy to make 38 molecules of ATP.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 38ATP$$

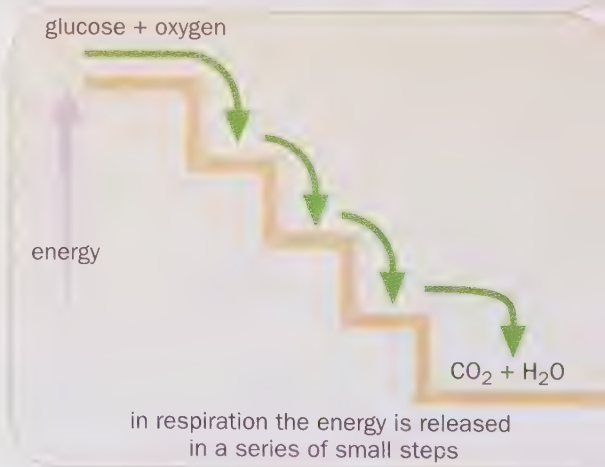
Gas exchange increases with exercise



These yeast cells can respire aerobically or anaerobically



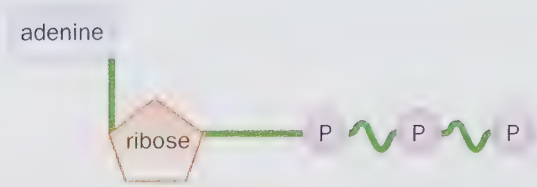
in burning the energy is released all in one go



in respiration the energy is released in a series of small steps

The role of ATP

ATP is the short-term energy store of the cell.
It is often called the 'energy currency' since it picks up energy from food in respiration and passes it on to power cell processes.
ATP is a nucleotide derivative (see page 46).

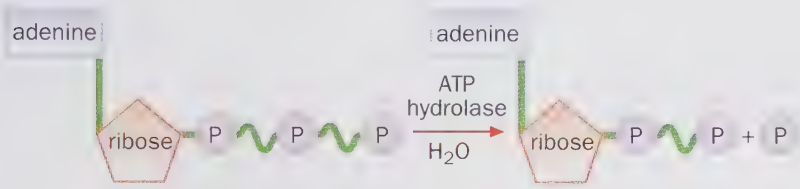


The structure of ATP

If you look at the diagram you can see that ATP is made up of

- a base (adenine),
- a pentose sugar (ribose),
- three phosphate groups.

The three phosphate groups are joined together by two ***high energy bonds***.
ATP can be hydrolysed to release a large amount of energy.
When hydrolysed, ATP produces **adenosine diphosphate** (**ADP**) and phosphate (Pi).
The hydrolysis of ATP to ADP is catalysed by the enzyme **ATP hydrolase**.
The third phosphate group is removed from ATP releasing $30\,kJ\,mol^{-1}$ of free energy.
This energy is used to drive reactions within the cell, such as active transport.

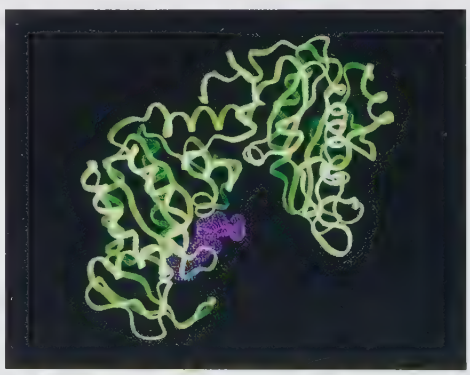


$$ATP \longrightarrow ADP + Pi + 30\,kJ\,mol^{-1}$$

The hydrolysis of ATP

It is also possible to remove the second phosphate group from ADP by breaking another high energy bond.
The hydrolysis of ADP to **adenosine monophosphate** (**AMP**) releases a similar amount of energy.

AMP and ADP can be converted back into ATP by the addition of phosphate molecules. These are condensation reactions catalysed by **ATP synthase**.
Since the hydrolysis of ATP is **exergonic** (energy is released), the production of ATP must be **endergonic** (energy is used up).


Computer simulation of an ATP molecule bound to an enzyme

The production of ATP is called **phosphorylation**.
This can take place in two main forms.

- **Photophosphorylation**, takes place on the membranes of the chloroplasts during photosynthesis.
- **Oxidative phosphorylation**, takes place on the membranes of the mitochondria during aerobic respiration.

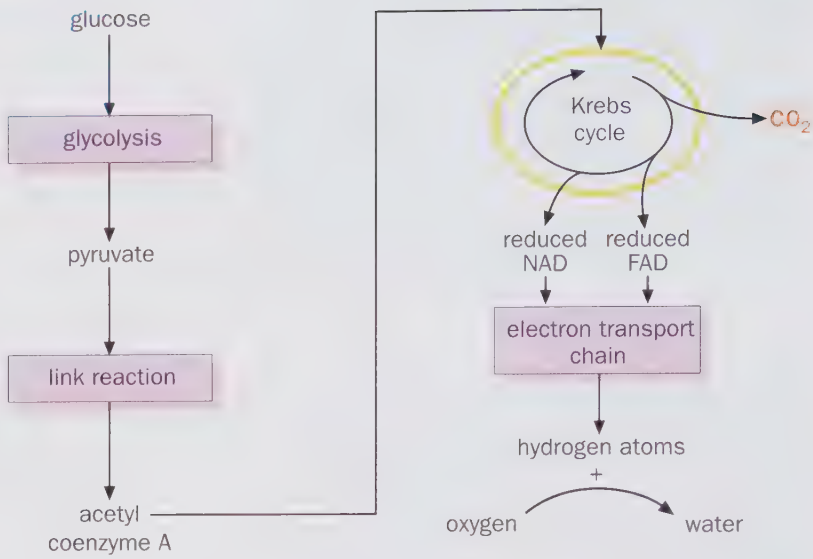
Just as $30\,kJ\,mol^{-1}$ of energy are released when ATP is hydrolysed, so $30\,kJ\,mol^{-1}$ of energy are needed to add each phosphate molecule to make ATP.
This energy is released from various enzyme-controlled reactions at different stages of aerobic respiration.

ATP is the universal energy carrier, but it cannot be transported from cell to cell.
It has to be used inside the cell in which it has been made.

Stages in aerobic respiration

There are four main stages in the breakdown of glucose during aerobic respiration:

- **glycolysis**,
- **the link reaction**,
- **Krebs cycle**,
- **electron transport chain**.



Summary of aerobic respiration

Glycolysis

Glycolysis is the splitting (***lysis***) of glucose. It takes place in a number of enzyme-controlled reactions, starting with the breakdown of the six-carbon (6C) glucose molecule into two molecules of three-carbon (3C) **pyruvate**. The process yields little energy since most is still 'locked up' in the molecules of pyruvate.

Glycolysis takes place in the cytoplasm of cells. Glycolysis does not need oxygen. It is the first stage of aerobic respiration and is, in fact, the ***only*** stage of anaerobic respiration.

- Initially, the glucose is phosphorylated to make **glucose phosphate** (6C).

The phosphate comes from a molecule of ATP. (Glucose does not react easily, so the ATP raises its energy level, making the subsequent reactions easier.)

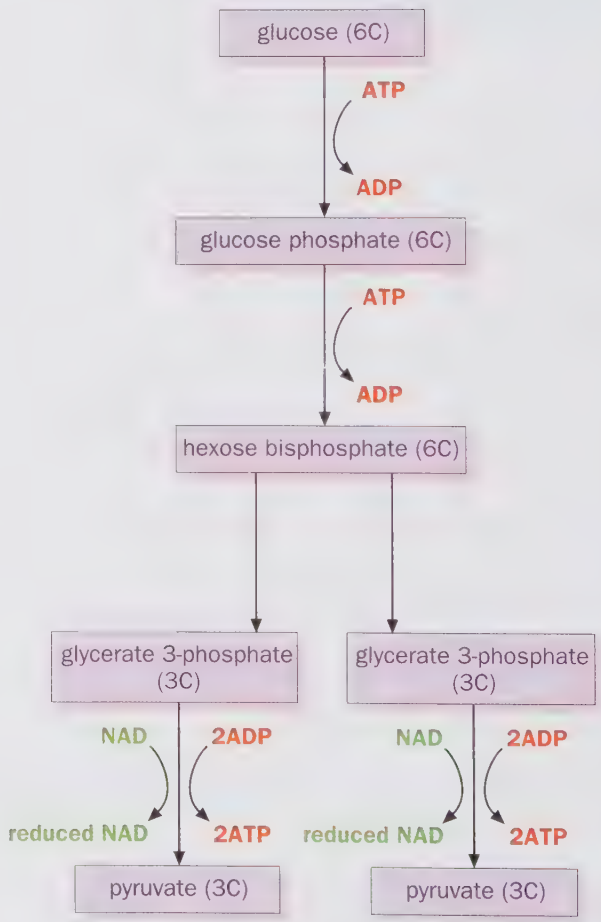
- Glucose phosphate is then phosphorylated to **hexose bisphosphate** (6C) using up another ATP.
- Hexose bisphosphate (6C) splits into two molecules of **glycerate 3-phosphate** (3C) which is a triose phosphate.
- The glycerate 3-phosphate (3C) is converted to pyruvate (3C).

Hydrogen is removed and transferred to the hydrogen acceptor **NAD** (**nicotinamide adenine dinucleotide**). Enough energy is released at this stage to make two molecules of ATP.

Important: Since ***two*** molecules of glycerate 3-phosphate are formed, there will be two molecules of reduced NAD formed (also referred to as NADH) and $2 \times 2 = 4$ molecules of ATP.

So from one molecule of glucose, glycolysis produces the following:

- two molecules of ATP (four ATPs are produced but two ATPs are used up),
- two molecules of reduced NAD (reduced hydrogen acceptor),
- two molecules of pyruvate, which enter the link reaction in aerobic respiration.



two molecules of pyruvate are produced from each molecule of glucose

Glycolysis

The link reaction

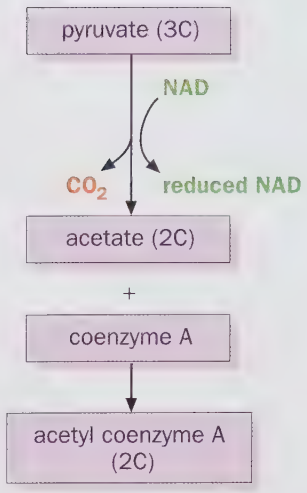
The link reaction links glycolysis to Krebs cycle, the next stage of aerobic respiration. It is sometimes treated as part of Krebs cycle.

- Pyruvate (3C) enters the matrix of the mitochondria from the cytoplasm.
- In the presence of oxygen, three things happen:
  1. The pyruvate is **decarboxylated** (a molecule of carbon dioxide is removed).
  2. The pyruvate is also **dehydrogenated** (hydrogen is removed). The hydrogen is transferred to the hydrogen acceptor NAD to form reduced NAD.
  3. The resulting **acetate** (2C) combines with coenzyme A (CoA) to form the 2C molecule **acetyl coenzyme A**, which then enters Krebs cycle.

  The overall reaction is

  pyruvate + coenzyme A + NAD ⟶ acetyl coenzyme A + CO_2 + reduced NAD

- Since ***two*** molecules of pyruvate are formed from each glucose molecule, there will be also be ***two*** acetyl coenzyme A molecules formed.



The link reaction

Krebs cycle

This series of reactions was discovered by Sir Hans Krebs in 1937. It is also known as the **citric acid cycle** or the **tricarboxylic acid cycle** (**TCA cycle**).
Krebs cycle takes place in the matrix of the mitochondria and includes the following reactions.

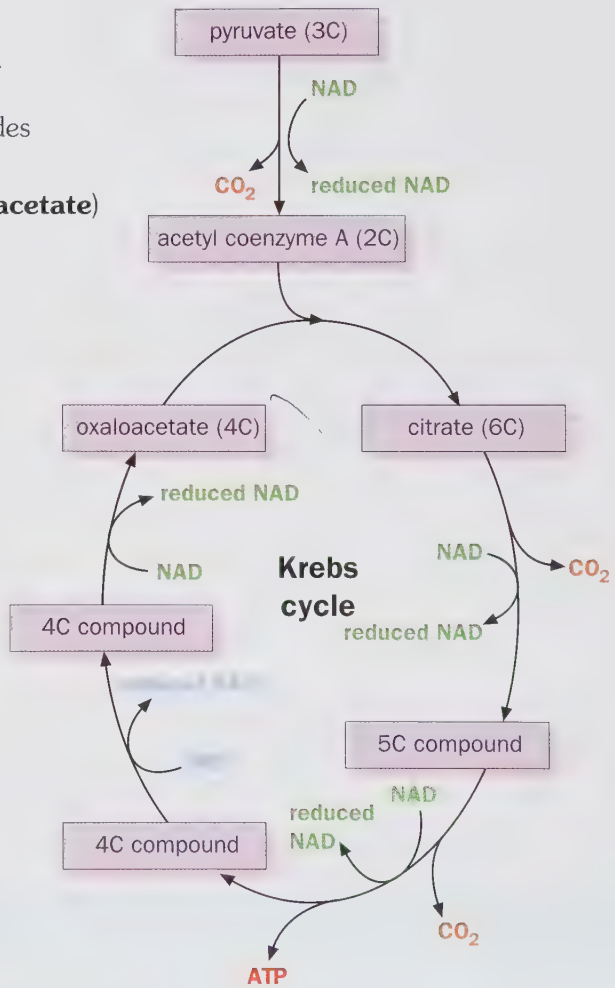
- Acetyl coenzyme A (2C) combines with a 4C compound (**oxaloacetate**) to form a 6C compound (**citrate**).
- A series of reactions take place where the citrate (6C) is both decarboxylated and dehydrogenated.

Carbon dioxide is released as a waste product and the hydrogen atoms are picked up by hydrogen acceptors NAD and **FAD** (**flavine adenine dinucleotide**).

- As a result, oxaloacetate (4C) is regenerated to combine with more acetyl coenzyme A.
- So after ***one*** turn of Krebs cycle, we have:
  – three molecules of reduced NAD,
  – one molecule of reduced FAD,
  – one molecule of ATP,
  – two molecules of CO_2.

But don't forget that two molecules of acetyl coenzyme A enter Krebs cycle for each molecule of glucose.
So the cycle turns ***twice*** for each glucose molecule, so giving 6 × reduced NAD, 2 × reduced FAD, 2 × ATP and 4 × CO_2.

The most important role of Krebs cycle is to provide hydrogen that can be used in the **electron transport chain** releasing energy for the formation of ATP.



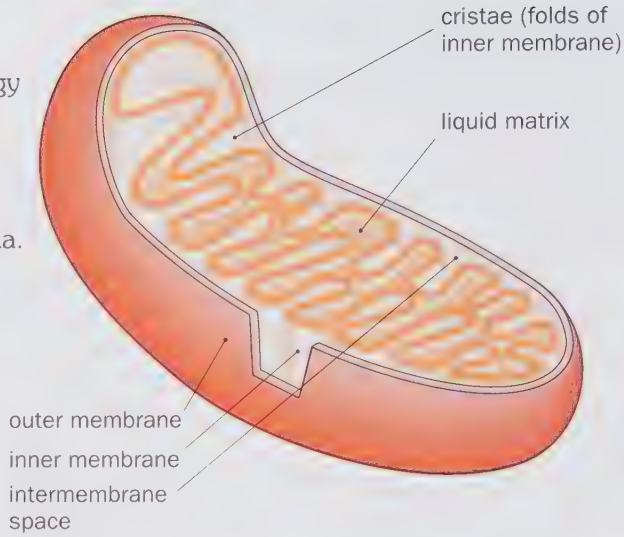
The electron transport chain

The electron transport chain provides the means by which the energy from the hydrogen atoms removed from compounds in Krebs cycle, glycolysis and the link reaction can be used to make ATP.

Oxygen is required for this final stage of aerobic respiration.
The reactions take place on the inner membrane of the mitochondria.

The electron transport chain involves a chain of carrier molecules along which hydrogen atoms and electrons are passed.

- The hydrogen atoms are passed on to other carrier molecules from the hydrogen carriers reduced NAD and reduced FAD.
- Reduced NAD is the first carrier in the chain.
  It passes its hydrogen on to FAD.
- The hydrogen atoms split into hydrogen ions or protons (H^+) and electrons.



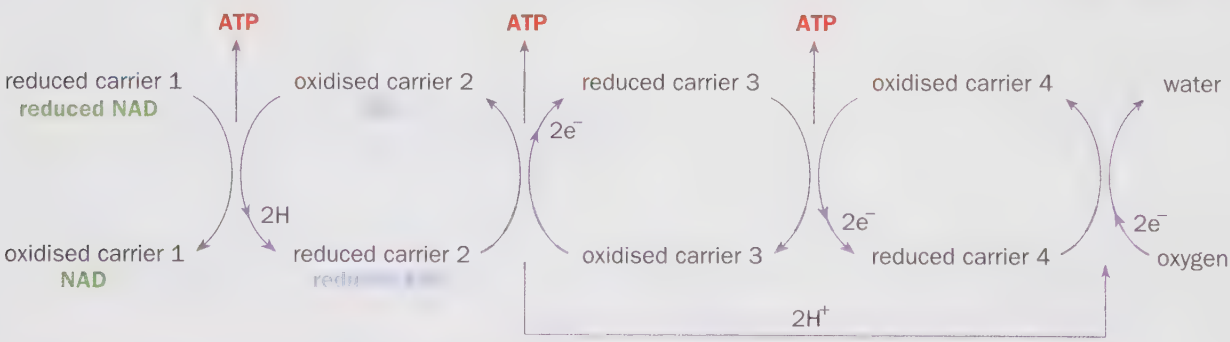
Three-dimensional structure of a mitochondrion

The electrons are transferred along a series of **electron carriers**.
The hydrogen ions stay in solution in the space between the inner and outer membranes of the mitochondria.

- Finally the electrons recombine with protons (H^+) to form hydrogen atoms and are passed on to oxygen to form water.
  Oxygen is therefore the final electron acceptor, the reaction being catalysed by the enzyme **cytochrome oxidase**.
- The transfer of electrons along the chain releases sufficient energy to make ATP from ADP and Pi.

If you look at the diagram below, you can see that this occurs at three points along the chain.
So for each reduced NAD entering the chain, three ATP molecules are made.
You should remember that reduced FAD is also produced by Krebs cycle.
This can enter the chain at stage two but in this case only two ATP molecules will be generated.

- The formation of ATP in this way is called **oxidative phosphorylation**.



Electron transport chain

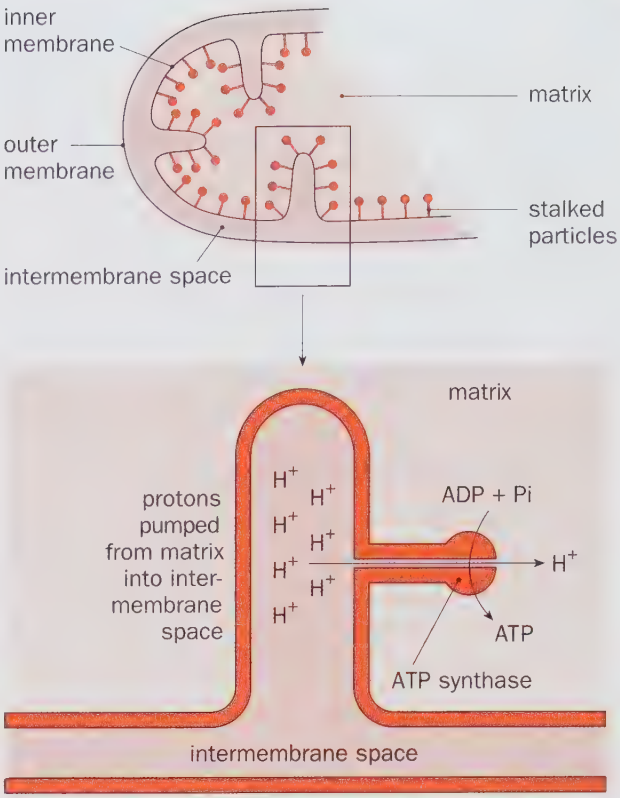
Stage	Site in cell	Net production of ATP
glycolysis	cytoplasm	two (four made, two used) per glucose
link reaction	matrix of mitochondrion	none
Krebs cycle	matrix of mitochondrion	two (one per turn) per glucose
electron transport chain	inner membrane of mitochondrion	34 per glucose

Chemiosmotic theory

In Chapter 4 we looked at the detailed structure of the mitochondrion.
Do you remember that it has a double membrane and that the inner membrane is folded to form cristae?
These cristae give the inner membrane a large surface area, so there is more room for electron carriers and ATP formation.
The cristae are lined with stalked particles.
These stalked particles contain **ATP synthase** enzymes.

The **chemiosmotic theory** provides a model to explain the synthesis of ATP in oxidative phosphorylation.

- The energy released by the electron transport chain is linked to pumping protons from the matrix into the space between the two membranes of the mitochondrion.
- This results in a higher concentration of protons in the intermembrane space than in the matrix of the mitochondrion: an electrochemical gradient is set up.
- The protons pass back into the matrix through the stalked particles, along the electrochemical gradient.
  As they do so, their electrical potential energy is used to make ATP from ADP and Pi.
  ATP synthase catalyses the reaction.



protons diffuse back into matrix through stalked particles and their energy is used to make ATP

Part of a mitochondrion

ATP balance sheet

How many ATP molecules are made from each glucose molecule?

First, let's count the number of ATP molecules made *directly*.
Glycolysis has a net gain of 2 ATPs (four made but two used up).
Krebs cycle makes 2 ATPs (one per turn).
Total = 4 ATPs.

Now let's see how many reduced NAD and reduced FAD molecules are made.
Glycolysis makes 2 reduced NAD.
Link reaction makes 2 reduced NAD.
Krebs cycle makes 6 reduced NAD (3 per turn) and 2 reduced FAD (one per turn).
Total = 10 reduced NAD and 2 reduced FAD.

You know that each reduced NAD can produce 3 ATP molecules, so that gives 10 × 3 = 30 ATPs.
You know that each reduced FAD can make 2 ATP molecules, so that gives 2 × 2 = 4 ATPs.
Now add on the ATPs made directly in glycolysis and Krebs cycle = 4 ATPs.

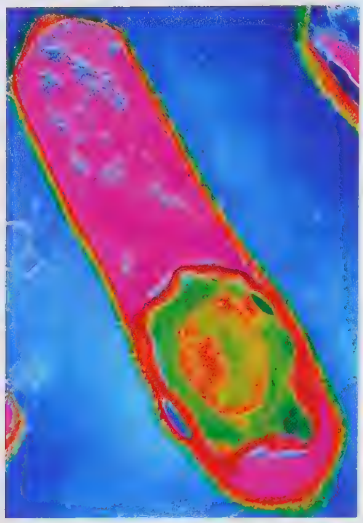
So that gives a grand total of 38 molecules of ATP produced from each glucose molecule.

So you can see that most ATP is made in the electron transport chain by oxidative phosphorylation.

Anaerobic respiration

Anaerobic respiration is respiration in the absence of oxygen.
Some organisms carry out aerobic respiration if oxygen is available but are able to change to anaerobic respiration in its absence.
Most **anaerobes** fall into this category, but there are some bacteria, such as *Clostridium welchii*, which causes gangrene, that thrive in the absence of oxygen. These are called obligate anaerobes.

In the absence of oxygen, only glycolysis can operate.
As a result, the energy yield in anaerobic respiration is low and the pyruvate is converted into waste products.



TEM of Clostridium welchii

Fermentation

This type of anaerobic respiration takes place in **yeast**.

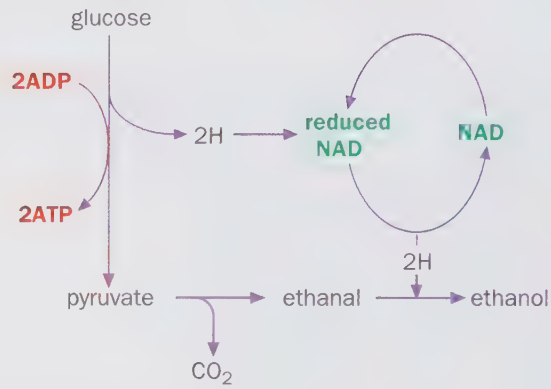
The pyruvate is converted to **ethanol** and carbon dioxide.
The problem is, where does the hydrogen, passed on to NAD in glycolysis, go to?
As you can see in the diagram, it is used to reduce ethanal to ethanol.

glucose ⟶ ethanol + carbon dioxide + 2ATP

Each ATP molecule can release about $60\,kJ\,mol^{-1}$ if it is broken down to AMP, so this is pretty small compared with the $2880\,kJ\,mol^{-1}$ produced in the aerobic respiration of one glucose molecule.

It represents an efficiency of $\frac{120}{2880} \times 100 = 4\%$

The ethanol becomes toxic to the yeast if it accumulates.
Ethanol cannot be broken down to yield any additional energy.



Fermentation

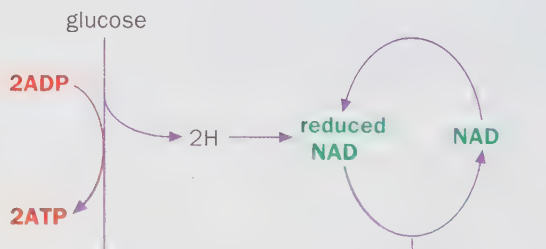
Lactate formation in muscle

As you know, if, during vigorous exercise, we can't get enough oxygen to our muscles, the muscle cells can revert to anaerobic respiration.
In this case, glycolysis again takes place, but the pyruvate is converted into lactate instead of ethanol.
The reduced NAD made in glycolysis again passes its hydrogen on to pyruvate, this time reducing it to lactate.

glucose ⟶ lactate + 2ATP

Again, the efficiency of energy release is low at about 4%.

The build-up of lactate in the muscle causes fatigue and cramp.
When oxygen becomes available again, the lactate is broken down.
First, it is carried in the bloodstream to the liver where it is converted back to pyruvate.
About one-fifth is used to release energy in aerobic respiration and the rest is converted into glycogen.
The oxygen required to break down the lactate is called the **oxygen debt**.
At the end of the activity, the oxygen debt is repaid by continuous deep and rapid breathing.



Lactate formation

Other respiratory substrates

Glucose is not the only substance that can be oxidised to release energy in cells. Other organic molecules can also be used as respiratory substrates to produce ATP, in certain circumstances.

Lipids

Fats provide a store of energy in the body which can be drawn upon when carbohydrate levels are low.

- First, the fats are hydrolysed to fatty acids and glycerol.
- The fatty acids are broken down in the matrix of the mitochondria to form 2C acetyl fragments that combine with coenzyme A to form acetyl coenzyme A. This then enters Krebs cycle.
- The glycerol is phosphorylated and converted into glyceraldehyde 3-phosphate, which is a triose phosphate. This compound is an intermediate in glycolysis and so can enter the pathway and subsequently be broken down in Krebs cycle.

The oxidation of fats produces large amounts of protons, as each triglyceride consists of one molecule of glycerol and three fatty acids molecules. These protons can be picked up by hydrogen carriers and used in the electron transport chain to make ATP.
In fact, 1 g of fat releases more than twice as much energy as 1 g of carbohydrate.

Protein

Protein is another potential respiratory substrate, but is only used in cases of starvation.

- First, the protein is hydrolysed to its constituent amino acids.
- The amino acids are **deaminated** in the liver, with the removal of the amine (NH_2) group from the rest of the molecule.
- This leaves an organic acid that can be fed into Krebs cycle and respired.

A malnourished woman and child collecting spilt grain in Thiekthou during a famine in Sudan

Respiratory quotient

The **respiratory quotient** (**RQ**) is the ratio of carbon dioxide given off, to oxygen used up during respiration.

$$RQ = \frac{CO_2 \text{ given off}}{O_2 \text{ used up}}$$

The RQ gives information about the type of organic molecule being respired.

Maths skills

For carbohydrates, the RQ is 1.0

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

$$RQ = \frac{6CO_2}{6O_2} = 1.0$$

For fats, the RQ is less than 1.0 since more oxygen is needed for complete oxidation.

$$C_{18}H_{36}O_2 + 26O_2 \longrightarrow 18CO_2 + 18H_2O$$

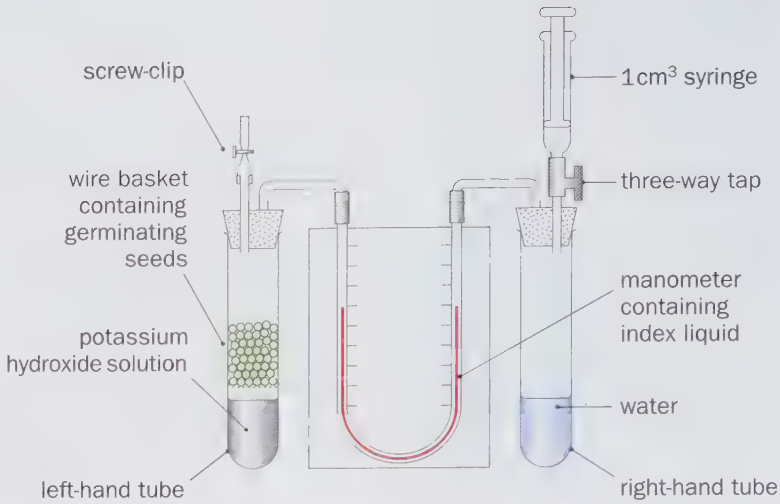
$$RQ = \frac{18CO_2}{26O_2} = 0.7$$

Proteins have an RQ of between 0.8 and 0.9.
In practice, living organisms respire more than one type of substrate, so results for RQ can vary.
Also, substrates are not always fully oxidised.

Respirometers

A simple respirometer can be used to measure the RQ of germinating seeds or even small animals such as woodlice and mealworms.
A respirometer measures the changes in gas pressure inside the apparatus.

- Potassium hydroxide solution is placed in the left-hand tube.
  Potassium hydroxide absorbs carbon dioxide.
- An equal volume of water is placed in the right-hand tube to ensure that the barometric pressure in each tube is equal.
- A wire basket containing the living organisms is positioned inside the left-hand tube.
- Both tubes are then placed in a water bath at 20 °C with the taps open.
- The taps are then closed and the apparatus is left for 15 minutes.
- As the organisms respire, they take up oxygen from the air in the apparatus and give out carbon dioxide.



A simple respirometer

The carbon dioxide is absorbed by the potassium hydroxide solution, causing the gas pressure in the left-hand tube to fall.
The fluid in the left-hand limb of the manometer rises as a result.

- The rise in the fluid is recorded after 15 minutes.
  Provided that the internal diameter of the manometer is known, how could you calculate the volume of oxygen used up by the organisms?
- The potassium hydroxide is then removed from the left-hand tube and replaced with water. The experiment is repeated and any increase or decrease in the gas volume is recorded.
  This gives a measure of the amount of carbon dioxide produced.
- If the volume of carbon dioxide produced is the same as the volume of oxygen absorbed, then there will be no change in the levels of liquid in the manometer.
- However, if more carbon dioxide is produced than oxygen used up, the level of liquid in the left-hand tube will fall.

Maths skills

Uptake of oxygen = 40 mm^3 (as measured in the first experiment, when potassium hydroxide is present in the apparatus).
Carbon dioxide produced − oxygen absorbed = 4 mm^3
(as measured in the second experiment when water is present).
Total carbon dioxide produced = 40 − 4 = 36 mm^3.

$$RQ = \frac{\text{carbon dioxide produced}}{\text{oxygen absorbed}}$$

$$RQ = \frac{36}{40}$$

$$= 0.9$$

Biology at work: Brewing

The basic techniques involved in brewing are centuries old.
Alcoholic fermentation is carried out by yeast and relies upon anaerobic conditions inside the fermenter.
The most common form of yeast to be used is *Saccharomyces cerivisiae*, though *Saccharomyces carlsbergensis* is used in the production of lager.

Over time, the most desirable yeast strains have been selected. These can now be improved by the use of genetic engineering to produce more alcohol.

The ingredients

The initial source of carbohydrate in brewing is barley, although the yeast cannot digest the starch until the barley has been **malted** and **mashed**.
These processes allow the starch to be digested into simple sugars by enzymes found in the sprouting barley.
Hops are also added for flavour and a sugary liquid called **wort** is created.

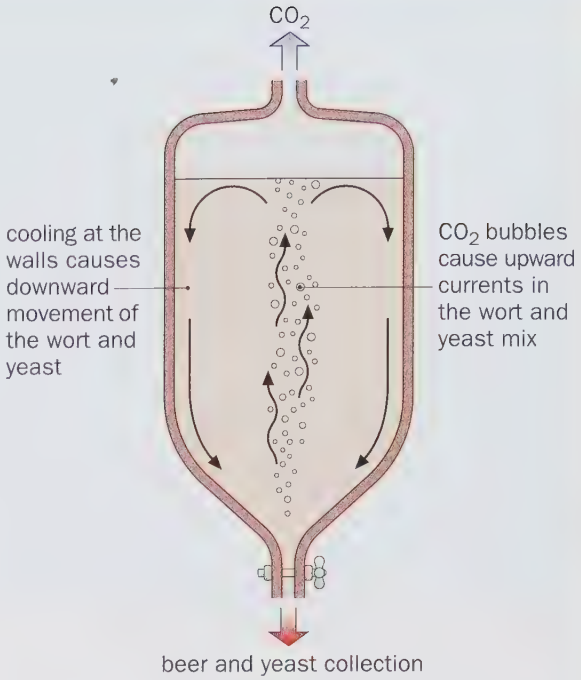
Fermentation

Yeast is added to the wort and uses maltase enzymes to break down maltose into glucose.
Intially the yeast grows aerobically, using oxygen from the air that cools the wort.
However, this oxygen is soon used up and anaerobic fermentation begins.

In the absence of oxygen the yeast uses the glucose as its substrate in glycolysis, resulting in the formation of ethanol.
When the yeast has used up all the available carbohydrate, fermentation ceases and the beer is removed.

This is the so-called **batch culture process**.
To brew more beer in the same fermenter, a new wort and batch of yeast must be added.
Continuous culture has been attempted with brewing but although it saves time, it yields a poorer quality product.

Downstream processing of beer is designed to improve the product and includes a process called conditioning which helps to remove unpleasant tasting compounds.
Depending on the type of beer, conditioning can take from a couple of weeks through to a number of years.
Lagers are conditioned at near-freezing temperatures for up to 6 months.
Some beers are bottle conditioned, which means that they undergo a further fermentation in the bottle resulting in naturally carbonated ale.
Cask ale is conditioned in the casks from which it is dispensed in pubs.
Some beers are pasteurised to remove bacteria and this increases their shelf life. Cask ale is not pasteurised and some people believe that unpasteurised beer has a better flavour.



A modern beer fermenter

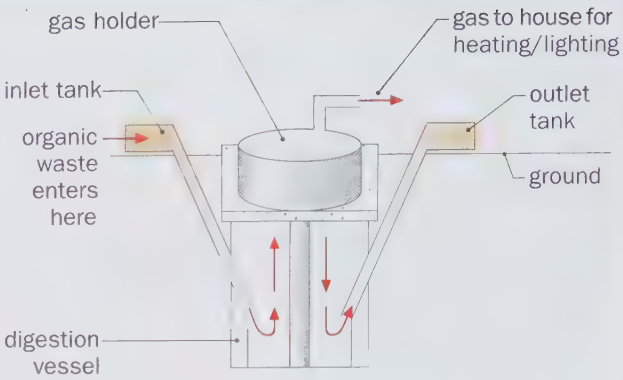

Hops being added in 'coopers' to make wort


Cask conditioning – part of the downstream process

Biology at work: Biofuels

Biofuels are literally fuels that are biological in origin. They exist in a variety of forms, for example alcohol, methane gas, and oil substitutes such as biodiesel. Biofuel production often involves making use of 'waste' biomass. In other words, using the considerable energy locked up in plant and animal material that is neither fully digested nor assimilated in food chains.

Biogas is a gas rich in methane which is used on a small scale to power generators in many parts of the developing world. Cattle dung is digested by anaerobic bacteria in underground vessels, producing a roughly 60 : 40 mix of methane and carbon dioxide.
When the carbon dioxide is removed, the methane is a clean and efficient fuel that can be used for both heat and power. The need to remove carbon dioxide and the relatively slow gas production are two drawbacks to this process.



A simple biodigester

However, in a number of European countries, such as Germany, Austria and France, **biomethane** is being used which has been upgraded to resemble the quality of natural gas and is fed into the mains gas supply.

Bioethanol

In some parts of the world, fermentation of carbohydrate-rich crops, such as sugar cane, is used to produce bioethanol.
This can be used as a petrol substitute in cars or more often as a fuel additive to reduce vehicle emissions.
Blending bioethanol with petrol will help to extend the life of the world's diminishing oil supplies.
By 2011 the USA and Brazil accounted for nearly 90% of world bioethanol production, and most cars on US roads can run on petrol blended with bioethanol. In the UK most standard petrol also contains approximately 5% ethanol. As with brewing, the fermentation of sugar cane involves yeast respiring the sugars under anaerobic conditions to produce ethanol.

In Brazil bioethanol is readily available.

The drawback to ethanol production becoming a worldwide biofuel is the need to pre-treat the tough cellulose found in much 'waste' biomass before it can be fermented.
Biodiesel is an oil substitute manufactured mainly from oil crops such as rapeseed, palm and soyabean. 300 kg of oil can be extracted from 1 tonne of rape and then converted into **rape methyl ester** (**RME**).
Although currently around 90% of rape seed oil is used in the food industry, biodiesel represents an attractive biofuel owing to the absence of oxides of sulfur and the low emissions of particulates, both of which are major drawbacks with diesel of petroleum origin.
The main benefit of biodiesel is that it can be described as 'carbon neutral'. This means that the fuel produces no net output of carbon in the form of carbon dioxide. This happens because when the oil crop grows it absorbs the same amount of carbon dioxide as is released when the fuel is combusted.
Fuels using the energy in biomass may play an important role in replacing finite fossil fuels, as well as being less polluting to the environment.


Oil seed rape is the basic raw material for the production of biodiesel

Summary

- Respiration is a series of oxidation reactions taking place inside living cells, which results in the release of energy from organic compounds such as glucose.
- Aerobic respiration takes place in the presence of oxygen; anaerobic respiration takes place in the absence of oxygen.
- Respiration releases energy to drive the metabolic activities that take place in cells.
- ATP is the short-term energy store in the cell, made from ADP and Pi by phosphorylation.
- Glycolysis involves the splitting of glucose and the conversion of glycerate 3-phosphate to pyruvate, with the production of ATP and reduced NAD.
- The link reaction involves the conversion of pyruvate to acetyl coenzyme A, which enters Krebs cycle.
- Krebs cycle is a series of reactions that results in the formation of reduced NAD, reduced FAD, ATP and carbon dioxide.
- The electron transport chain converts the energy in reduced NAD and reduced FAD into molecules of ATP.
- The chemiosmotic theory involves proton pumps and the formation of ATP by ATP synthase.
- Anaerobic respiration involves the conversion of pyruvate to ethanol or to lactate. In either case, the amount of energy released is far less than in aerobic respiration.
- Lipids and proteins can also be respired; lipids release far more energy than carbohydrates do.
- Respiratory quotient (RQ) is the amount of carbon dioxide released divided by the amount of oxygen used up. RQ can be measured using a respirometer.

Questions

1 Copy and complete the following account of aerobic respiration.
The first stage in the breakdown of glucose is a process called _____, which takes place in the _____ of the cell and eventually results in the production of two molecules of _____ from each molecule of glucose. In most organisms, this product enters the stage of respiration called ___ cycle. This cycle occurs in ____ conditions inside organelles called _____ During these two stages, hydrogen atoms are removed from the substrate and passed on to coenzymes such as __ and __. The final stage of the process is called the ____ _____ _____ and involves the formation of energy-rich molecules of __.

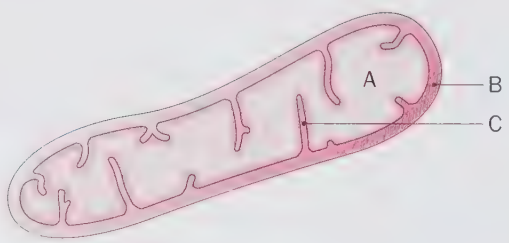
2 ATP can be used as a temporary energy store and supplies energy to cells for a number of processes. During aerobic respiration, ATP is mainly produced in the mitochondria.
a) What is the term given to the production of ATP in respiration?
b) Draw a simple diagram to show the structure of ATP.
c) Describe how ATP is used in processes within cells.

3 Describe the roles of each of the following in Krebs cycle and in the electron transport chain:
a) coenzyme A,
b) NAD,
c) oxygen,
d) ADP.

4 Copy and complete the table putting a tick (✓) if the statement applies to the process or a cross (✗) if it does not apply.

	Glycolysis	Krebs cycle	Oxidative phosphorylation
produces ATP			
involves the production of carbon dioxide			
occurs in mitochondrion			

5 The diagram shows the structure of a mitochondrion.

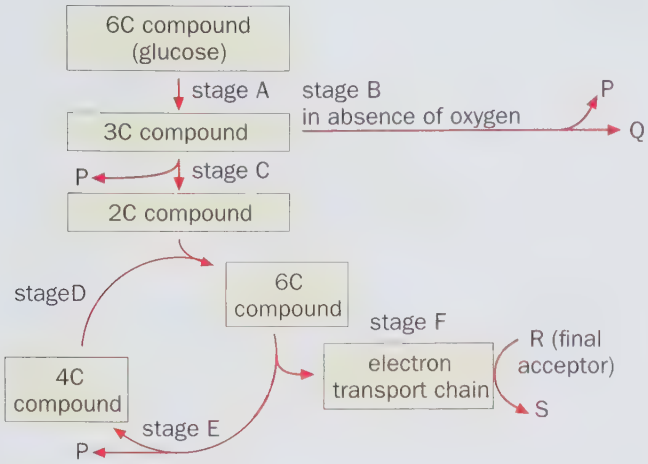


a) Name the parts of the organelle indicated by the letters A, B and C.
b) Describe where in the mitochondrion
   i) the electron transport chain is found,
   ii) the reactions of Krebs cycle occur.
c) List the products formed in Krebs cycle.
d) By what process do protons flow out of space B into space A?

6 The diagram below represents an outline of stages in aerobic respiration.

a) Name the compounds represented by P, Q, R and S.

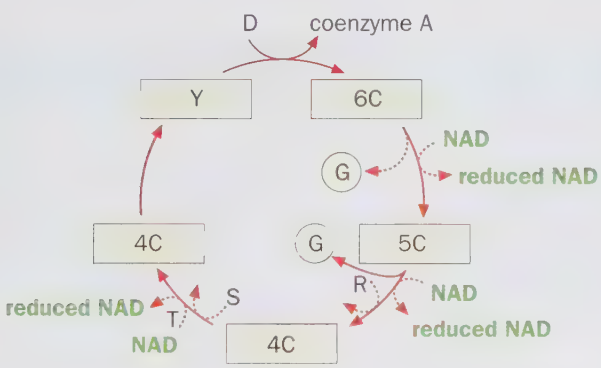
b) Which letter represents the stage at which most ATP is produced?



c) Which two letters represent separate stages where ATP is not produced?

d) Indicate the nature of the reactions in stage F.

7 The diagram shows the series of reactions that take place in Krebs cycle.



a) Where exactly in the cell do these reactions take place?

b) Give the general name for reactions such as T.

c) Name the compounds labelled D and G.

d) Write down the equations for the reactions labelled R and S.

e) How many carbon atoms are found in compound Y?

f) Describe the role of reduced NAD in respiration.

8 a) In the process of glycolysis, the formation of pyruvate involves this reaction:

$NAD + H_2 \longrightarrow$ reduced NAD

What happens to the reduced NAD in

i) an animal cell respiring anaerobically,

ii) a yeast cell respiring anaerobically?

b) Compare aerobic and anaerobic respiration in terms of energy release.

9 a) What is meant by the term respiratory quotient (RQ)?

b) What can you conclude about the respiratory substrates that produce the following RQs:

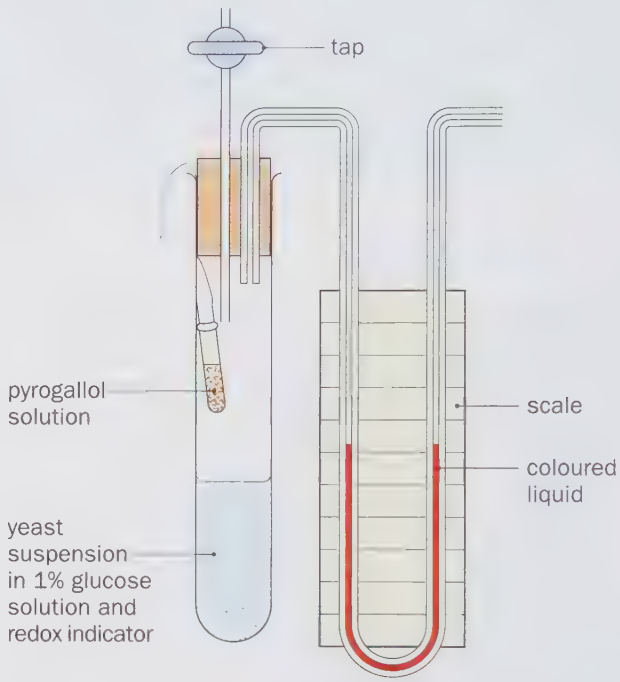
i) 0.7

ii) 1.0

iii) 0.9?

c) Describe in detail, how you would use a respirometer to obtain the RQ of castor oil seeds.

10 The diagram below shows apparatus that can be used to measure the rate of anaerobic respiration in yeast cells. The tube contains a yeast suspension in 1% glucose solution and a redox indicator†. The pyrogallol solution absorbs oxygen.



a) i) Name *one* suitable redox indicator which could be used in this experiment.

ii) Give *one* change you would expect to observe during the experiment as the yeast respires and give a reason for your answer.

b) Describe how you could use the apparatus to investigate the effect of temperature on anaerobic respiration of glucose by yeast.

†*An artificial hydrogen acceptor can be used as a redox indicator to indicate progress of oxidation–reduction (redox) reactions.*

Producing superweeds

Scientists have predicted that the increased levels of carbon dioxide in the atmosphere will result in greater crop yields, because crop plants will be able to convert more carbon dioxide into carbohydrate during photosynthesis. Some plant physiologists have predicted lush plant growth resulting from the beneficial conditions for photosynthesis.

However, other scientists have been examining the possible effect of increased carbon dioxide levels on the growth of weeds.

As global warming increases, weeds could become a growing menace to farmers. The effectiveness of glyphosphate, a widely used weedkiller, is being reduced by increased levels of atmospheric carbon dioxide. Subjecting different types of weed to doses of glyphosphate under current atmospheric carbon dioxide levels resulted in the herbicide stopping weed growth.

However, in a carbon dioxide-rich atmosphere, some weeds were able to resist the herbicide. These weeds were 'C_3' plants whose biochemical pathways are far more efficient if more carbon dioxide becomes available. With carbon dioxide levels expected to double in the next 50 years, it may be that farmers will have to find alternative ways of controlling weeds other than chemical herbicides.

Spraying with chemical herbicides may become a thing of the past

Autotrophic nutrition

Autotrophic nutrition is about making your own food.

Autotrophs are able to use a source of energy to make complex organic molecules from inorganic raw materials. In effect, the energy from the source is transferred into chemical bond energy in organic foodstuffs.

Chemoautotrophs (mainly bacteria) are able to harness the energy released in exergonic chemical reactions to synthesise their organic food.

Photoautotrophs, which include green plants and algae, are able to use light energy to synthesise their own organic materials.

The overall equation for photosynthesis is as follows:

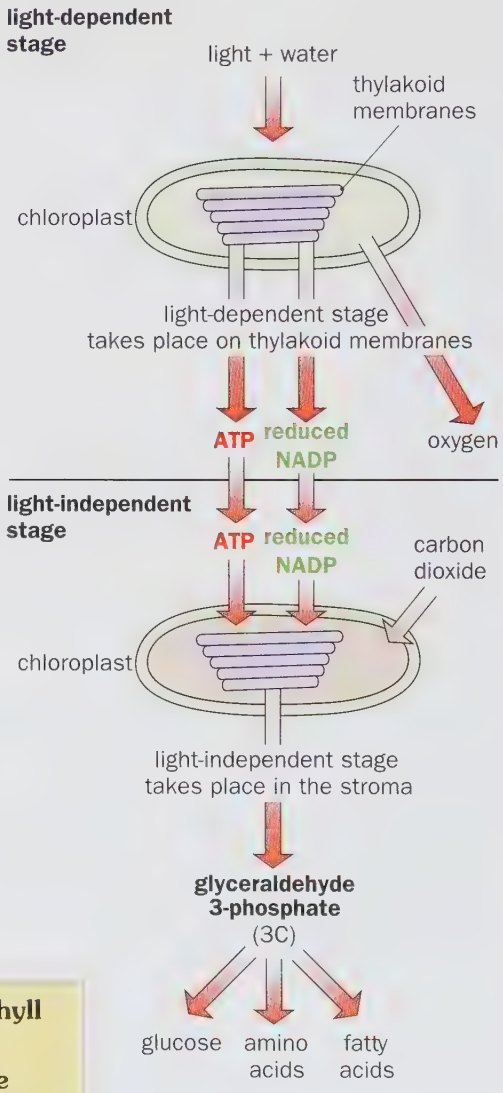
$$\textbf{carbon dioxide + water} \xrightarrow{\text{light energy}} \textbf{glucose + oxygen}$$

$$6CO_2 + 6H_2O \xrightarrow{\text{light energy}} C_6H_{12}O_6 + 6O_2$$

The raw materials for photosynthesis are carbon dioxide, water and light energy. If you look at the reaction, do you think photosynthesis is an exergonic or an endergonic reaction? Does it involve reduction or oxidation?

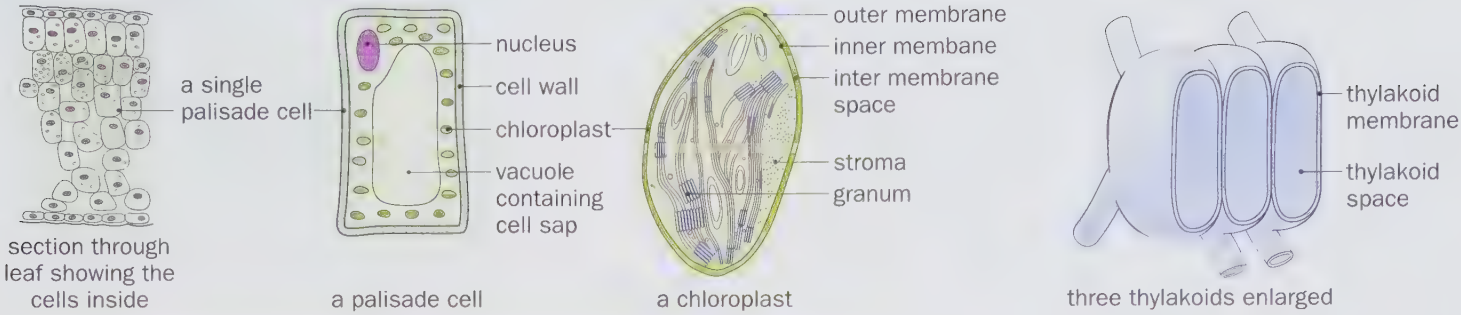
The simple equation conceals the fact that photosynthesis is, in fact, a series of complex biochemical pathways, catalysed by specific enzymes. The two main stages are:

- **The light-dependent stage involving the photoactivation of chlorophyll and the transfer of energy to produce ATP and reduced NADP.**
- **The light-independent stage involving the fixation of carbon dioxide and the use of ATP and reduced NADP to convert it into carbohydrate.**



The site of photosynthesis

We looked at the structure of a leaf in Chapter 8 and the detailed structure of a leaf palisade cell and a chloroplast in Chapter 4.
Look at the diagram to remind yourself of the exact site of photosynthesis.



- **The reactions of the light-dependent stage of photosynthesis take place on and across the thylakoid membranes of the chloroplast.**
- **The reactions of the light-independent stage of photosynthesis take place in the stroma of the chloroplast.**

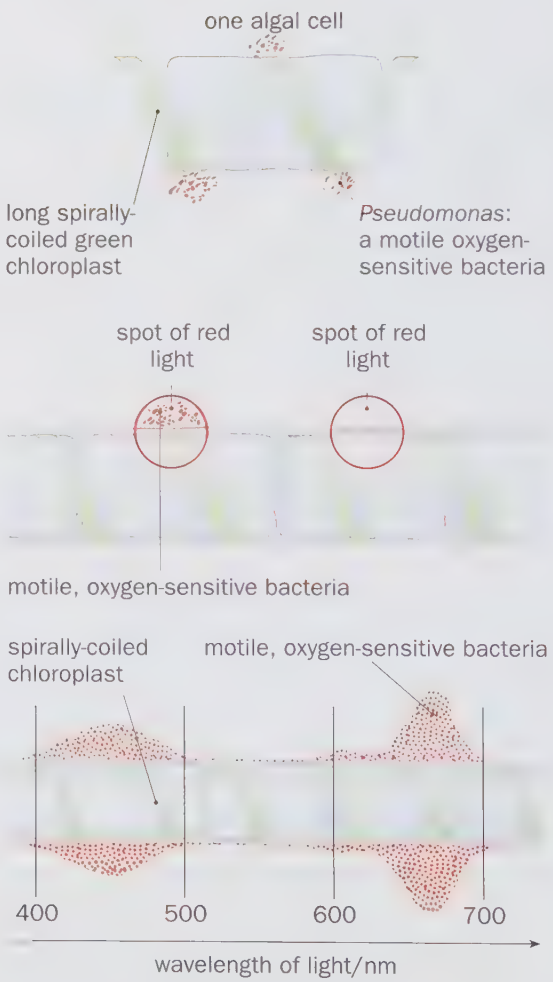
Detecting the site of photosynthesis

As long ago as 1883, the German botanist T. W. Engelmann was able to demonstrate the site of photosynthesis in cells.
He carried out an ingenious series of experiments using a filamentous green alga.
In each of these giant cells there is a ribbon-like chloroplast with a spiral shape.

Engelmann used the fact that oxygen is given off (produced) during photosynthesis to identify the site of the reactions.
He used the motile, oxygen-sensitive bacterium, *Pseudomonas*, which tends to cluster around areas where oxygen concentration is highest.
When the alga was put on a slide and illuminated, the bacteria were seen to cluster around the edge of the cells close to the chloroplast.

Further work was done using this same technique.
This time, the alga was illuminated with light of different wavelengths.
Engelmann noticed that the bacteria clustered in greatest concentration near to the chloroplasts when wavelengths of 450 nm (blue) and 650 nm (red) were used.
These wavelengths resulted in increased photosynthesis, which produced more oxygen and so attracted more of the *Pseudomonas* bacteria.

It has since been shown that green plants photosynthesise most rapidly when illuminated with blue and red light.



Engelmann's experiments on the part played by chlorophyll in photosynthesis

Photosynthetic pigments

Different wavelengths of light are trapped by different photosynthetic pigments.
There are two main groups of photosynthetic pigments in green plants: the **chlorophylls** and the **carotenoids**.

There are a number of different forms of chlorophyll, with **chlorophyll *a*** and **chlorophyll *b*** being the most common. The chlorophylls absorb light in the blue–violet and the red parts of the spectrum.

Why do you think that green plants look green?
It's because they reflect green light instead of absorbing it.

All chlorophylls have a complex ring structure with a long hydrocarbon tail.
Can you see the magnesium atom at the centre of the molecule?
Plants look yellow when they are short of magnesium because they cannot produce enough chlorophyll. This condition is called **chlorosis**.

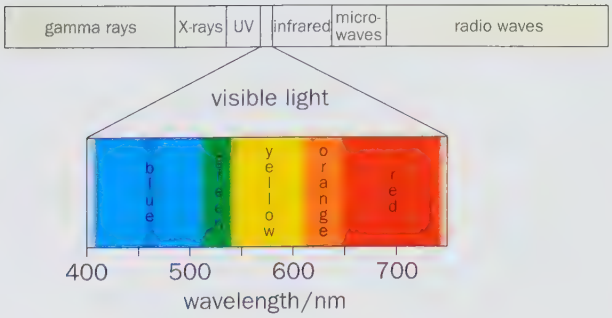
The structure of chlorophyll should remind you of haemoglobin, another molecule with a ring structure.
Can you remember which metal is at the centre of the ring in a molecule of haemoglobin?

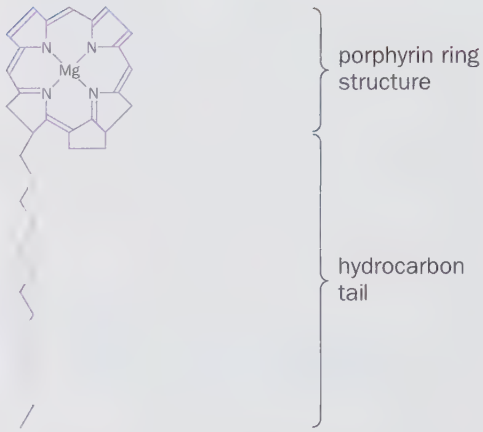
The carotenoids are often referred to as **accessory pigments**. They include **carotene** and **xanthophyll,** and they absorb light from the the blue–violet part of the spectrum.

The **absorption spectrum** is a graph that shows how much light a particular pigment absorbs at each wavelength.
Can you see from the graph that the photosynthetic pigments absorb light mainly from the blue–violet and red parts of the spectrum?

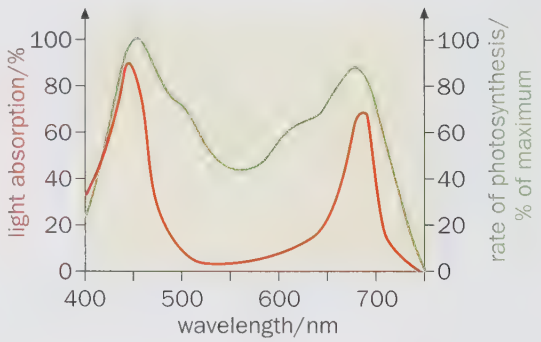
The **action spectrum** is a graph that shows the rate of photosynthesis at different wavelengths of light.

Can you see that the action spectrum of photosynthesis corresponds closely to the absorption spectrum of the chlorophylls and carotenoids?





The shape of a chlorophyll molecule



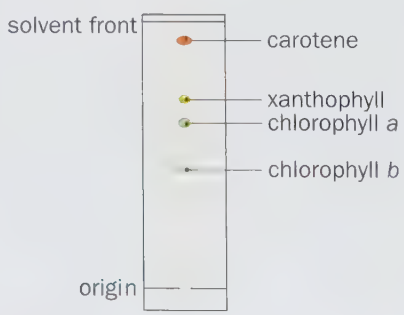
The relationship between the absorption spectrum and the action spectrum (green line)

Separating photosynthetic pigments

Photosynthetic pigments can be separated by **chromatography**.

- First, the pigments are extracted by grinding up a leaf, using a pestle and mortar, with a solvent such as propanone.
- The extract is then 'spotted' onto the origin line of a piece of chromatography paper.
- The **chromatogram** is placed into a glass tank containing a solvent.
- The solvent gradually rises up the chromatography paper and the different pigments separate out depending upon their relative solubility in the solvent and their adhesion to the chromatography paper.
- When the solvent front comes close to the top, the paper is taken out and dried.
- The Rf value for each pigment can then be worked out, and the pigment can be identified. The Rf value of each pigment can be calculated using the following formula:

$$\text{Rf value} = \frac{\text{distance travelled by pigment from origin}}{\text{distance travelled by solvent front}}$$



Separation of plant pigments by chromatography

Harvesting light

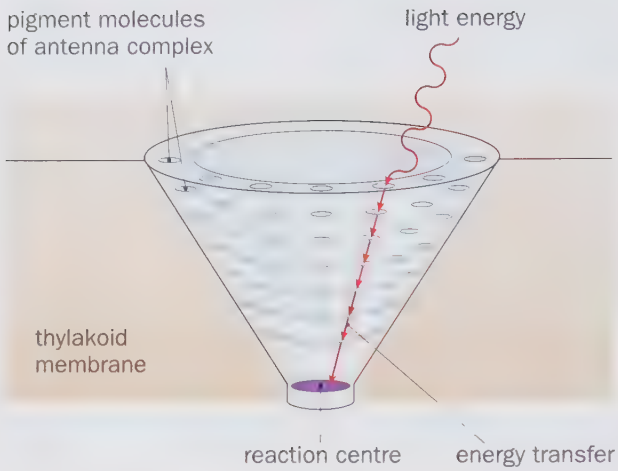
The chlorophylls and the accessory pigments are found in the thylakoid membranes of the chloroplast. They are grouped in clusters of several hundred molecules. Each group is called an **antenna complex**.

Within each antenna complex, there are special proteins which help the pigment molecules to pass absorbed light energy from one molecule to the next.
So photons of light are transferred through the antenna complex until they reach molecules of chlorophyll *a* in the **reaction centre**.

There are ***two*** types of reaction centre:

Photosystem I is arranged around a chlorophyll *a* molecule with an absorption peak of 700 nm.
So the reaction centre of photosystem I is called **P700**.

Photosystem II is arranged around a chlorophyll *a* molecule with an absorption peak of 680 nm.
This reaction centre is called **P680**.



A single antenna complex

Light-dependent stage of photosynthesis

The reactions of the 'light stage' of photosynthesis take place in the thylakoids of the chloroplast.
They involve:

- The synthesis of ATP from ADP and Pi.
  Remember that this is called **phosphorylation**.
  In this case it is called **photophosphorylation**, as light energy is involved.
- The splitting of water molecules by **photolysis** to produce hydrogen ions (protons) and electrons.

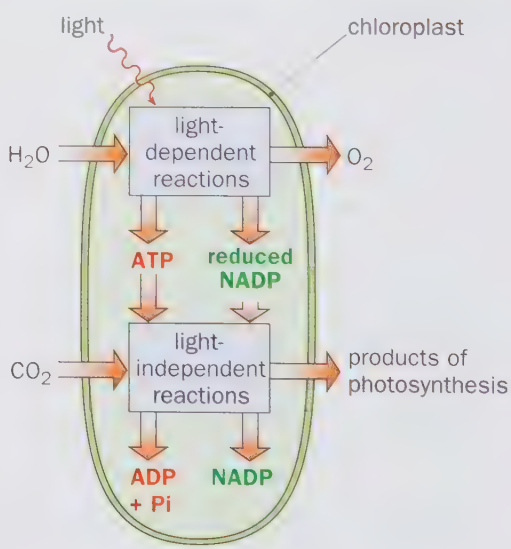
The protons are picked up by the carrier molecule **nicotinamide adenine dinucleotide phosphate** (**NADP**).
The addition of a proton reduces NADP to reduced NADP.

So the products of the light-dependent stage are ATP and reduced NADP and these molecules are needed for the light-independent stage.

There are two different ways in which ATP can be synthesised by photophosphorylation:

- **non-cyclic photophosphorylation**,
- **cyclic photophosphorylation**.

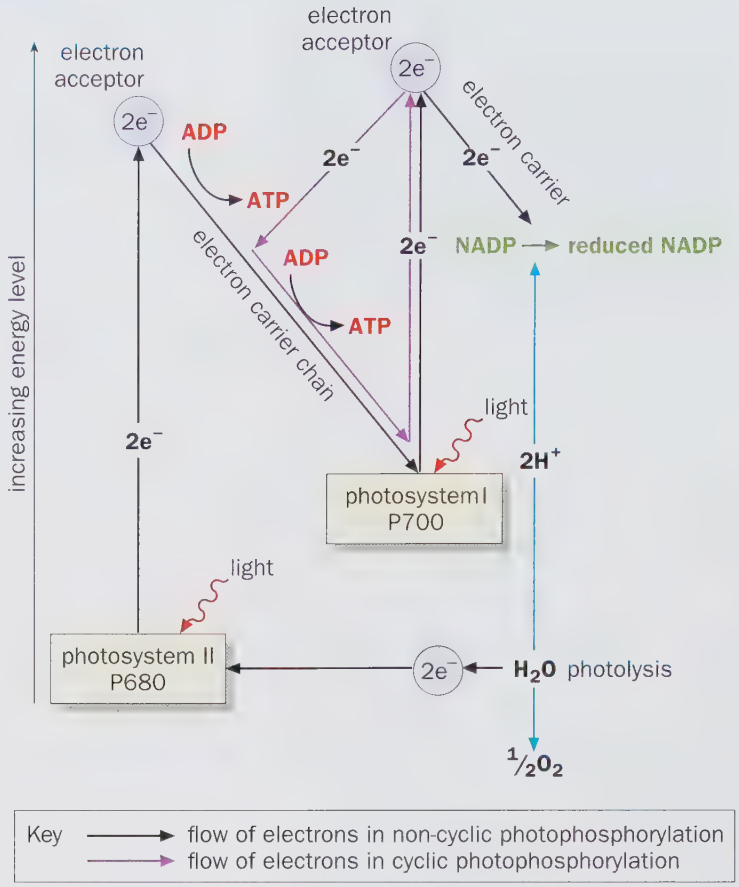
Bioluminescence of chlorophyll



Non-cyclic photophosphorylation

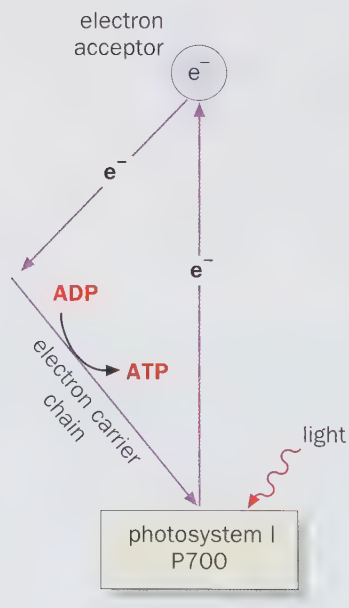
Non-cyclic photophosphorylation involves both photosystem I and photosystem II.
Can you see from the diagram, why the pattern of electron flow in non-cyclic photophosphorylation is often referred to as the **Z-scheme**?

- Light is absorbed by photosystem II and passed on to chlorophyll *a* (P680).
- The irradiated chlorophyll *a* (P680) molecule emits two electrons. This process is called **photoionisation**. These energised electrons are raised to a higher energy level and are picked up by an electron acceptor.
- The electron acceptor passes the electrons along a chain of **electron carriers** (or **electron transfer chain**) to photosystem I.
  The energy released from the electrons is used to make ATP from ADP and Pi.
- Light is absorbed by photosystem I and passed on to chlorophyll *a* (P700). It emits two electrons.
- The energised electrons rise to a higher energy level and are picked up by a second electron acceptor.
- Since both chlorophylls (P680 and P700) have now lost electrons, they will both be positive and unstable.
- The two electrons released from the chlorophyll *a* (P680) of photosystem II go to replace the two that have been lost by chlorophyll *a* (P700) of photosystem I.
- P680 of photosystem II receives its replacement electrons from the splitting of water (**photolysis**).
- During photolysis, the water molecule dissociates into electrons, protons (H^+) and oxygen. As we have said, the electrons go to photosystem II. The oxygen is released as a waste gas.
- The protons combine with electrons held by the second electron acceptor to give reduced NADP.
  This passes to the reactions of the light-independent stage.
- So the products of the light-dependent stage are reduced NADP, ATP and waste oxygen gas.



Cyclic photophosphorylation

Cyclic photophosphorylation involves photosystem I only.
Light is absorbed by photosystem I and passed on to chlorophyll *a* (P700).
This causes the chlorophyll *a* molecule to emit an electron.
This 'energised' electron is raised to a higher energy level and is picked up by an **electron acceptor**.
The electron is then passed along a chain of **electron carriers** before it is returned to the chlorophyll *a* molecule (P700).
As the electron passes along the electron carrier chain, enough energy is released to make ATP from ADP and Pi.
This ATP is needed for the light-independent stage.
No reduced NADP is made during cyclic photophosphorylation.

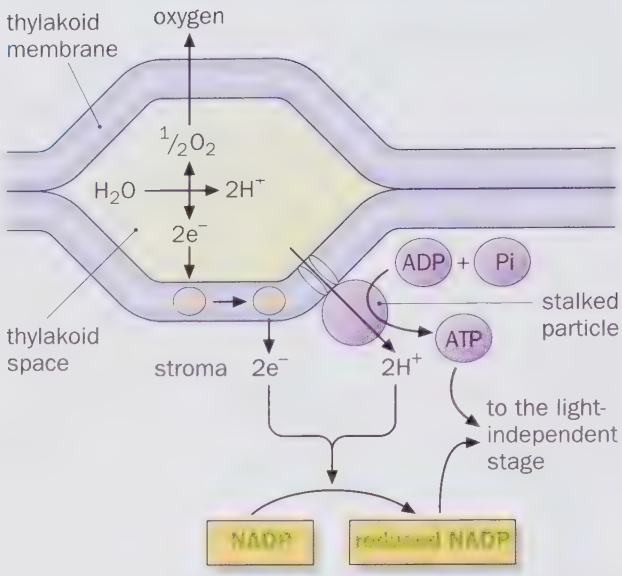


Cyclic photophosphorylation

Chemiosmosis in the chloroplast

As you have seen, the reactions of the light-dependent stage of photosynthesis take place in the thylakoid membranes of the chloroplast.

In the 'Z-scheme', electrons flow along the chains of electron carriers from photosystem II and photosystem I.
As they do so, they provide energy to pump protons (H^+) from the stroma, across the thylakoid membrane, into the thylakoid space.
This sets up an electrochemical and a concentration gradient, since there are more protons inside the thylakoid space than there are outside in the stroma.
Protons diffuse along this gradient out across the thylakoid membrane through protein channels.
This drives the formation of ATP by **ATP synthase**.
This process is very similar to the formation of ATP that we came across in respiration in Chapter 17.



ATP production in the chloroplast by chemiosmosis

The light-independent stage

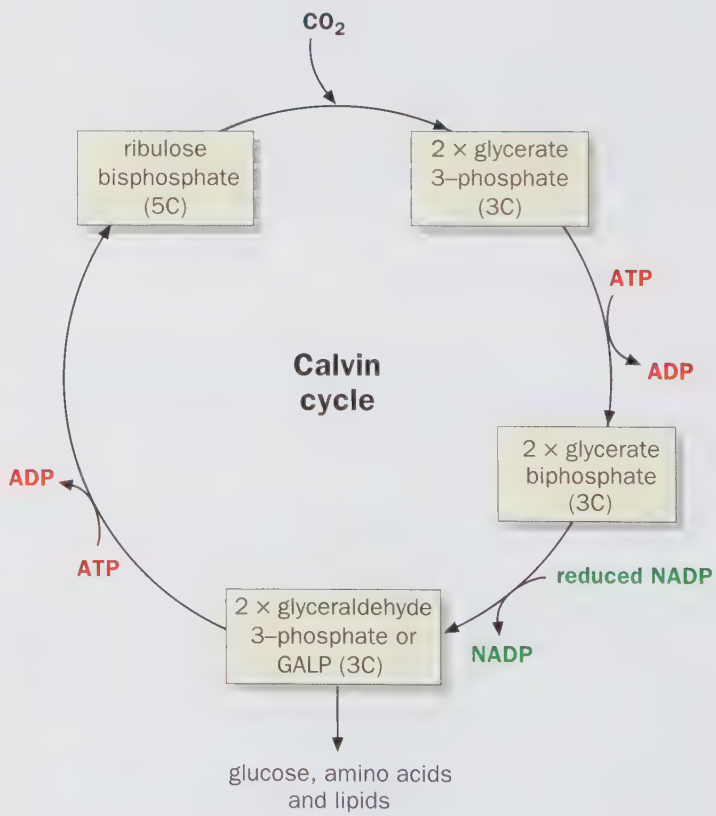
This cyclic pathway is sometimes called the **Calvin cycle**.
The diagram shows a simplified version of the cycle.
In reality there are many enzyme-controlled reactions involved.
The important stages are:

- Carbon dioxide combines with a five-carbon (5C) compound, **ribulose bisphosphate** (**RuBP**).

The reaction is catalysed by the enzyme **RuBP carboxylase** (**Rubisco**), the most common enzyme in the world.

- The product is an unstable 6C compound that breaks down to form two molecules of 3C **glycerate 3-phosphate** (**GP**).
- ATP is used to phosphorylate the two molecules of GP to form two molecules of 3C **glycerate biphosphate**.
- The next stage involves the use of reduced NADP to reduce each molecule of glycerate biphosphate to **glyceraldehyde 3-phosphate** (**GALP**), a triose phosphate (TP).
- For every six molecules of GALP formed, five are used in a series of reactions to regenerate ribulose bisphosphate, which can then combine with more carbon dioxide.
- One of the six GALP (or TP) molecules is converted to glucose and other carbohydrates, amino acids and lipids.

Can you see where the products of the light-dependent stage (ATP and reduced NADP) are used in the light-independent stage?



The use of isotopes

The Hill reaction

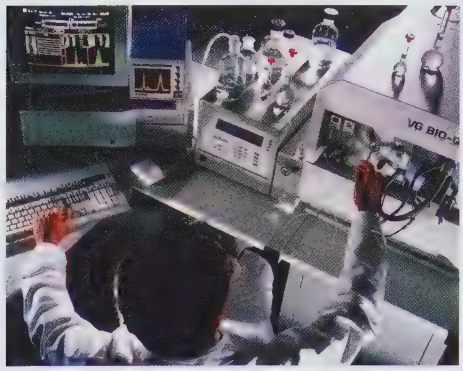
$$6CO_2 + 6H_2O \xrightarrow{\text{light energy}} C_6H_{12}O_6 + 6O_2$$

How do we know that the oxygen released in photosynthesis actually comes from the water molecule and not from the carbon dioxide?
In 1939, the British biochemist Robert Hill showed that isolated chloroplasts give off oxygen in the presence of a hydrogen acceptor.

Then, in 1941, he placed cells of the green alga *Chlorella* into water containing the heavy isotope ^{18}O.
(^{18}O is an isotope of oxygen with a mass number of 18 rather than 16.)
By using a mass spectrometer, he was able to show that the oxygen produced in photosynthesis was the isotope ^{18}O, which must have come from the water.

$$CO_2 + H_2{}^{18}O \longrightarrow (CH_2O) + {}^{18}O_2$$

When Hill repeated the experiment with carbon dioxide containing the heavy ^{18}O instead, the oxygen produced was 'normal' ^{16}O, so confirming his earlier results.
The splitting of water or photolysis is sometimes called the **Hill reaction**.



A scientist working with a mass spectrometer

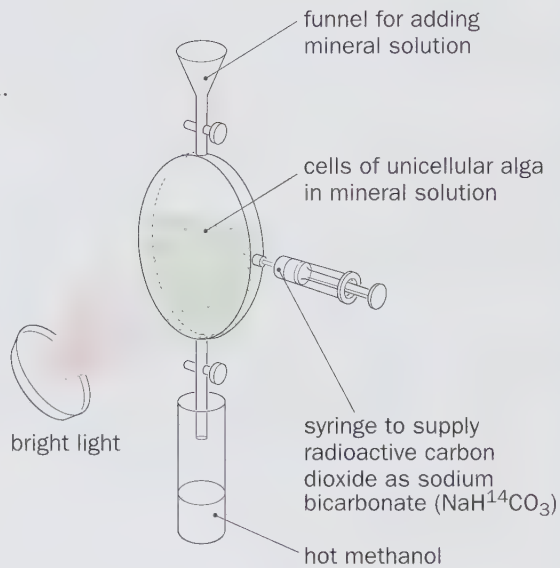
The Calvin cycle

The events of the light-independent stage of photosynthesis are also known as the **Calvin cycle**, after the American biochemist Melvin Calvin.
In the 1940s he was able to make use of the newly-available radioactive isotope of carbon, ^{14}C (normal form is ^{12}C).

Calvin grew cultures of the unicellular alga *Chlorella* in flat glass containers called 'lollipops'.
He exposed *Chlorella* to carbon dioxide labelled with the radioactive isotope $^{14}CO_2$, for varying periods of time.
They were then killed by dropping them into hot methanol.
The products of photosynthesis were extracted and separated by chromatography.
X-ray film, which is sensitive to $^{14}CO_2$, was then placed over the chromatograms, and left to develop for a few days.
When the X-rays were developed, the radioactive compounds showed up as a patterns of dark spots on the sheets.
This technique is known as **autoradiography**.
The radioactive spots were cut out and analysed to identify the compounds present.
The first product formed was glycerate 3-phosphate.

The table shows the subsequent compounds that were formed after further periods of time.

The technique enabled Calvin to piece together a pathway showing the reactions that take place in the light-independent stage.

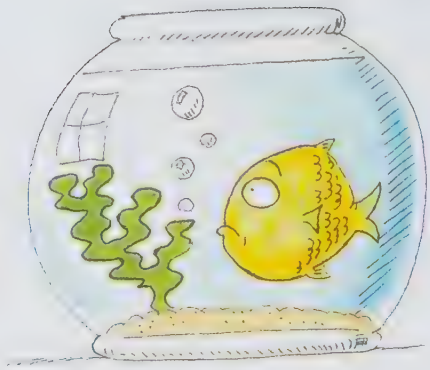


Calvin's 'lollipop' apparatus

Time of exposure to light after isotope added/s	Substances containing ^{14}C
5	glycerate 3-phosphate (GP)
15	GP, hexose phosphates
60	GP, hexose phosphates, sucrose, amino acids
300	GP, hexose phosphates, sucrose, starch, amino acids, proteins, lipids

Substances in Chlorella extract labelled with radioactive carbon after different periods of photosynthesis in the presence of $^{14}CO_2$

Measuring the rate of photosynthesis



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There are a number of ways of measuring the ***rate*** of photosynthesis. If you look at the equation for photosynthesis, you can get some ideas.

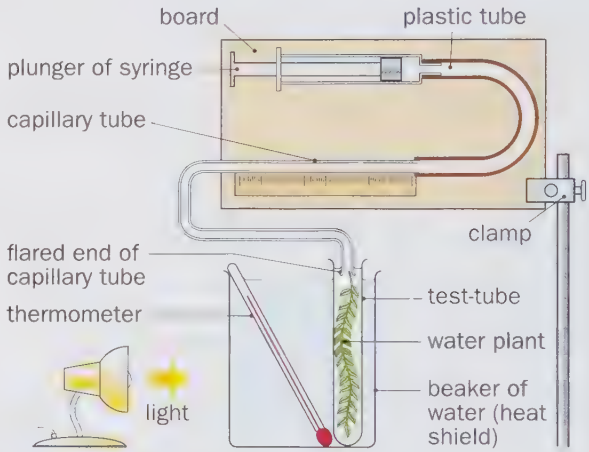
$$\text{carbon dioxide} + \text{water} \xrightarrow{\text{light energy}} \text{glucose} + \text{oxygen}$$

You could find out how fast photosynthesis is taking place by measuring

- the rate at which carbon dioxide is used up,
- the rate at which glucose is produced,
- the rate at which oxygen is produced.

The most convenient method is collecting the bubbles of gas given off by pondweed over a given time and then measuring the total volume of gas produced. This can be done accurately using an apparatus known as a **photosynthometer**.

- A piece of well illuminated Canadian pondweed (*Elodea*), about 10 cm long, is cut underwater and fixed into the flared end of the capillary tube.
- Depending upon which environmental variable you decide to investigate, you need to make sure the other factors are kept constant.
  For instance, if you are investigating the effect of light intensity on the rate of photosynthesis, it is important to keep
  - the concentration of carbon dioxide in the water in the boiling tube constant,
  - the temperature of the water in the water bath constant.
- The apparatus is flooded with water and a bench lamp placed at various distances away from the plant in order to change the light intensity.
- Bubbles of gas collect in the flared end of the capillary tube and are collected for a set time (usually 5 minutes).
- After this time, the gas collected can be drawn into the capillary tube by gently pulling the plunger on the syringe, and the volume measured.
- The light intensity is inversely proportional to the distance of the lamp from the plant, $I \propto \frac{1}{d^2}$ (where I is the light intensity and d is the distance between the light source and the plant).
- After taking readings of the length of the bubble collected from the plant when the lamp is at different distances away, you can draw a graph showing the amount of gas produced per unit time against light intensity.

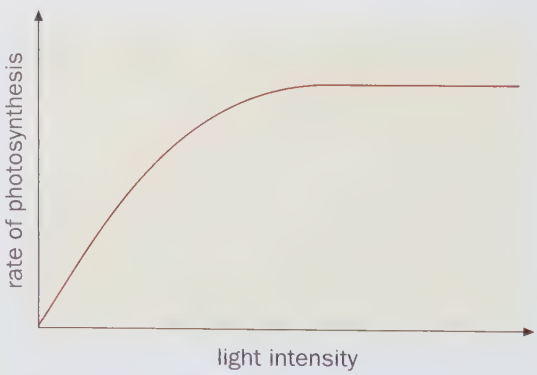


A photosynthometer set up to measure the effects of light intensity on photosynthetic rate

The variables that you can investigate using this technique include light intensity, temperature, carbon dioxide concentration and light quality (wavelength).

How would you vary the temperature, carbon dioxide concentration, and light quality?

The graph shows the effect of increasing light intensity on the rate of photosynthesis. As you can see, the rate increases with increasing light intensity but only up to a certain point.
At this point, the **law of limiting factors** means that one of the ***other*** factors, such as carbon dioxide or temperature, must be limiting the rate of photosynthesis. This factor must be increased if the rate of photosynthesis is to increase further.



The relationship between light intensity and photosynthetic rate

When a process (for example photosynthesis) is influenced by several factors, the rate at which the process proceeds is determined by the factor in shortest supply.

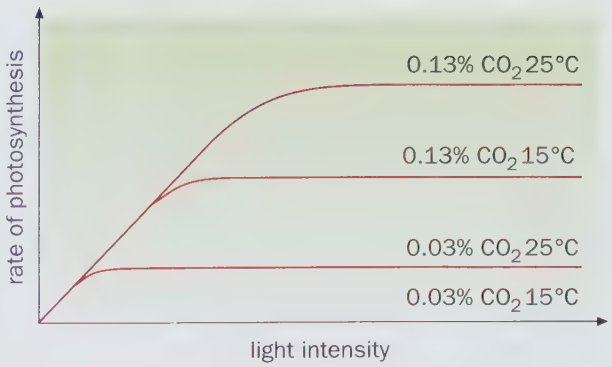
Biology at work: Manipulating photosynthesis

To obtain the best possible yield from a crop, a grower needs photosynthesis to proceed at its maximum rate. The rate of photosynthesis is limited by three key factors: **light** in terms of both **intensity** and **wavelength**, **temperature** and the **carbon dioxide concentration.**

Manipulating these so-called **limiting factors** is only really feasible in a glasshouse situation.

Light intensity

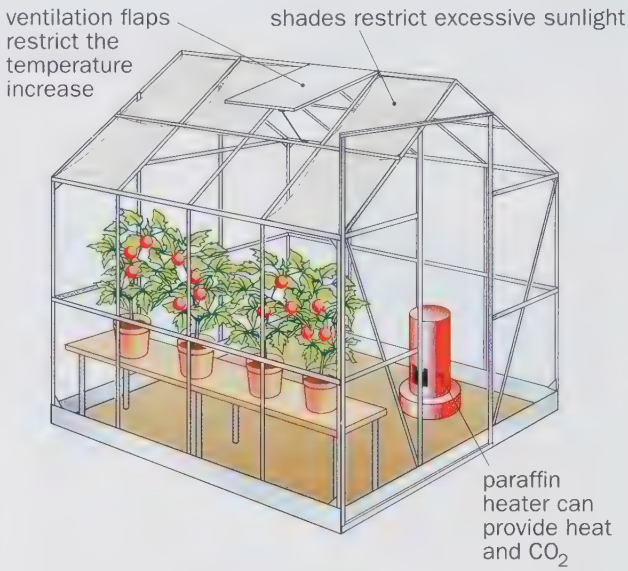
Artificial lighting can be used when natural light intensity is low, although in the summer months, even in the UK, the natural light intensity is often above 10 000 lux, which is the maximum useable intensity for photosynthesis.



The effect of light intensity on photosynthetic rate at different carbon dioxide concentrations and at different temperatures

Adjusting the atmosphere

Carbon dioxide is often a limiting factor for crop growth because the atmospheric level of 0.03% is lower than the optimum for photosynthesis.
Over long periods, the optimum carbon dioxide level is around 0.1%.
An increase in carbon dioxide levels in a glasshouse can be achieved by burning high-quality fuel like paraffin.
Paraffin burns without producing unwanted fumes and it also increases the temperature at the same time.



Regulating the temperature

The importance of temperature is related to the fact that photosynthesis is an enzyme-controlled process.
If other factors are not limiting then a 10 °C rise in temperature (within the range 10–35 °C) will lead to a doubling of the rate of photosynthesis.
A temperature of around 25 °C is often quoted as an optimum for photosynthesis.
Paraffin heaters, for example, can provide this environment in winter but in summer additional heating is rarely required.

Glasshouse cultivation can provide increased yields and 'out of season' crops.
However, it is not simply a matter of increasing light, heat and carbon dioxide levels indefinitely.
For example, very intense light can damage chloroplasts, and carbon dioxide levels of 0.5% over long periods can cause stomatal closure.
Therefore glasshouses require adequate shading and ventilation systems to ensure that the potential limiting factors are maintained at an **optimal** level.

In large-scale commercial operations, these parameters can be closely monitored and controlled by computer.
Close control is important because the cost of manipulating the environment must not outweigh potential profit increase.

Large scale conventional glasshouses

Biology at work: Feed the world

Advances in agricultural science and technology have contributed to remarkable increases in food production in the last 50 years. This has allowed food production to keep pace with population growth over this period. However, progress towards reducing hunger in the new millenium is variable due to unequal access to food.
The simple answer is to produce more food, but new land for agricultural development is limited. Yields are also restricted by the deteriorating quality of the agricultural environment.

- Soils are being eroded and are losing fertility as micronutrients are exhausted.
- Water supplies are being used up and wasted.
- Grassland for stock animals is being overgrazed.
- Excessive use has been made of fertilisers and pesticides, which can cause health problems.

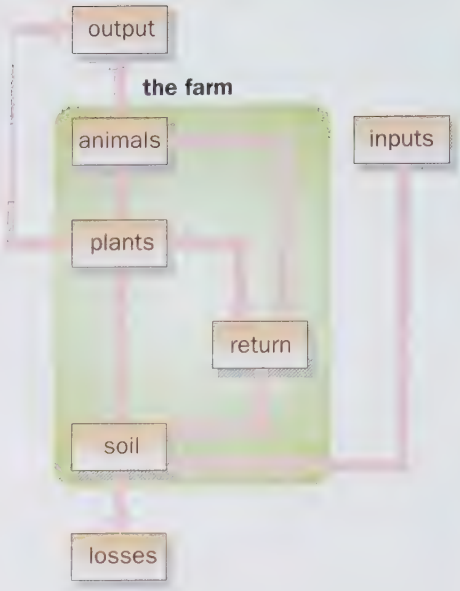
Greater food production will come from targeting local agricultural systems by:

- making the most of local resources, knowledge, and analysis,
- seeking the participation of local farmers.

In Kenya, the production of maize and beans requires the replacement of depleted nutrients such as nitrogen and phosphate,
to avoid soil degradation and erosion. The beans are inter-cropped with the maize since they are legumes, which fix atmospheric nitrogen for use by the maize. This technique has spread to semi-arid areas as the population has increased. But research has shown that under these semi-arid conditions, beans do not fix atmospheric nitrogen. The whole agricultural system is now under threat.

A solution involves using the cowpea legume which is more suited to the semi-arid environment. It can fix substantial amounts of nitrogen even in very dry soils.
Cowpeas can be inter-cropped with maize and supplemented by small amounts of nitrogen fertiliser. The use of energy intensive inputs such as fertiliser can be justified to save the soil before it becomes too exhausted. Greater plant biomass leads to greater levels of humus being deposited. This integration of traditional and new technologies is called **fertiliser-augmented soil enrichment** (**FASE**).

The progress of sustainable agricultural development in developing countries cannot rely on advances in genetic engineering and applied ecology. The active participation of local farmers and the integration of traditional and alternative practices are equally important to being able to feed the world.



The use of nutrients on a farm can be represented as a balance sheet of inputs and outputs

Region	People (in millions)
Southern Asia	295
Sub-Saharan Africa	223
Eastern Asia	167
South-Eastern Asia	65
Latin America and the Caribbean	47
Western Asia and Northern Africa	24
Developed regions	16
Caucasus and Central Asia	6
Oceania	1

Note: All figures are rounded

This shows that a total of 842 million people around the world in 2011–13 were still suffering from undernourishment (Source: FAO)

Building small stone walls around land contours can reduce soil erosion and conserve water

Summary

- Photosynthesis involves the use of light energy to synthesise organic molecules.
- The light-dependent stage involves the photoactivation of chlorophyll and energy transfer to produce ATP and reduced NADP.
- The light-independent stage involves the fixation of carbon dioxide and the use of ATP and reduced NADP to convert it into carbohydrate.
- The light-dependent stage takes place on and across the thylakoid membranes of the chloroplast.
- The light-independent stage takes place in the stroma of the chloroplast.
- The photosynthetic pigments include chlorophylls *a* and *b*, carotene and xanthophyll.
- The absorption spectrum is a graph showing how much light a pigment absorbs at each wavelength.
- The action spectrum is a graph showing the rate of photosynthesis at different wavelengths.
- Chlorophylls and accessory pigments are grouped together to form antenna complexes. They funnel photons of light to the reaction centre.
- There are two types of reaction centre, photosystem I (P700) and photosystem II (P680).
- Both cyclic and non-cyclic photophosphorylation involve the synthesis of ATP from ADP and Pi.
- The products of the light-dependent stage, reduced NADP and ATP, are used in the light-independent stage, when carbon dioxide is fixed and then reduced to carbohydrate.
- The rate of photosynthesis can be measured using a photosynthometer.
- The rate at which photosynthesis proceeds is determined by the factor that is in shortest supply. These factors include light intensity, light quality, temperature and carbon dioxide concentration.

Questions

1 Copy and complete the following account.
Photosynthesis is a type of ___ nutrition involving the synthesis of organic molecules from inorganic materials. The process involves two types of reactions, light-dependent and ___ ___. In the light-dependent reactions, light energy is absorbed by molecules of ___ located on the ___ of the chloroplasts; ___ and ___ are produced and oxygen is given off as a waste product. In the light-independent reactions, ___ accepts molecules of carbon dioxide, which together with the products of the light-dependent reactions results in the formation of ___. This compound can be converted to ___ or used to regenerate the carbon dioxide acceptor molecule.

2 a) Draw a fully labelled diagram of a chloroplast.
b) Where in the chloroplast do the following take place:
i) photoactivation of chlorophyll,
ii) regeneration of ribulose bisphosphate?
c) i) Explain what is meant by the photoactivation of chlorophyll.
ii) Explain how, as a result of photoactivation, ATP and reduced NADP are formed in the chloroplast.
iii) What is the other product of the light-dependent stage?

3 The following account contains **eight errors**. The first has been circled.
Copy and complete the account, substituting the **correct** terms in place of the errors.

The second stage of (respiration) occurring in the light is the oxidation of carbon dioxide to the phosphorylated three-carbon sugar. This conversion is brought about in part of a cyclic series of enzyme reactions known as the Hill cycle. ATP and reduced NAD produced during the light harvesting and energy transduction are essential components. The primary carboxylation reaction is between a two-carbon compound and carbon dioxide. The reaction is catalysed by a hydrolase enzyme. The first stable product is a six-carbon compound, which is phosphorylated and reduced using ADP and reduced NADP to form a different three-carbon compound. For every three molecules of carbon dioxide and three molecules of the five-carbon compound, six molecules of this three-carbon compound are synthesised. One of these six is the net production of photosynthesis, the other five molecules being used in the regeneration of three molecules of the five-carbon acceptor, which re-enters the cycle.

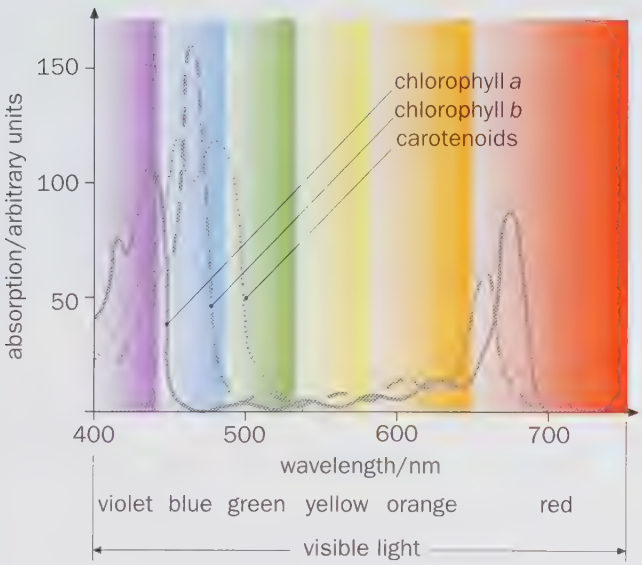
4 a) Chlorophyll *a* is found in all plants that can photosynthesise. What is its function?

b) The graph below shows the absorption spectra of three types of pigment found in the leaf of a plant.

i) State the wavelength that is most effectively absorbed by chlorophyll *a*.

ii) Name the technique you would use to isolate chlorophyll *a*.

iii) Use the information in the graph to explain why it is an advantage for a leaf to contain more than one pigment.



5 Copy and complete the diagram below of the light-dependent stage of photosynthesis by

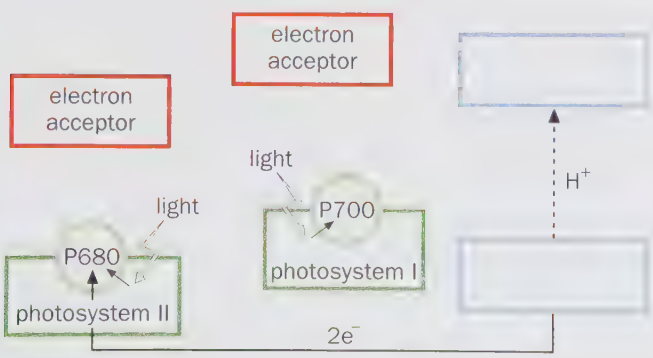
a) drawing four arrows to show the flow of electrons,

b) indicating with a letter P the site of non-cyclic photophosphorylation,

c) writing, in the appropriate boxes, equations to show

i) the photolysis of water,

ii) the formation of reduced NADP.

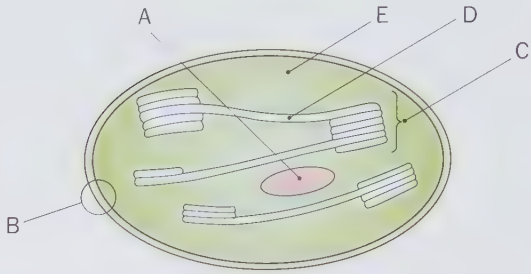


6 The diagram shows the structure of a chloroplast as seen with an electron microscope.

a) Name the features labelled A, B, C, D and E.

b) i) State the exact region of the chloroplast where chlorophylls *a* and *b* would be found.

ii) Name two other pigments that may be found inside a chloroplast.



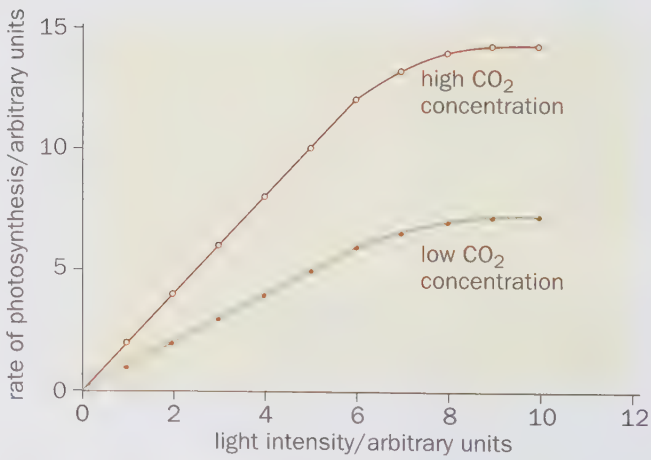
7 The graph shows the effect of light intensity on the rate of photosynthesis of an aquatic plant measured at two different carbon dioxide concentrations.

a) i) Describe the effects of increasing the carbon dioxide concentration on the rate of photosynthesis.

ii) Explain why the curve for low carbon dioxide concentration flattens out above a light intensity of six arbitrary units.

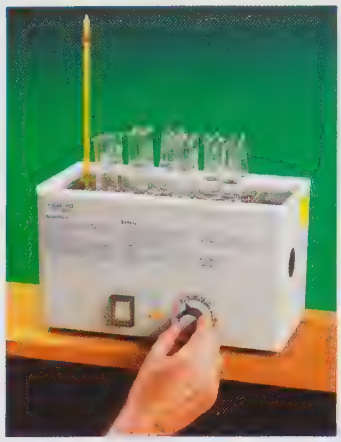
iii) Suggest how the rate of photosynthesis might have been measured.

b) Describe briefly how amino acids may be produced from the products of photosynthesis.



19 Homeostasis

Have you ever used a thermostatically controlled water bath? Perhaps in an investigation into enzyme activity, you needed to keep your reacting mixtures at a constant temperature. You set the dial on the water bath to the temperature that you need. When the water in the water bath reaches that temperature, it will stay constant throughout the experiment. You don't have to keep re-adjusting the temperature.

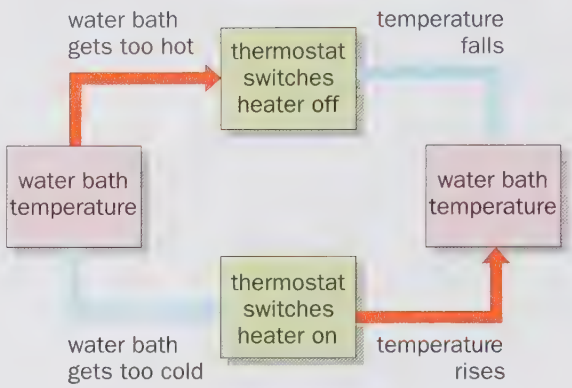


How do you think this happens?

Inside the water bath is a thermostat that detects changes in temperature. Once the required temperature is reached, the thermostat signals the heating element to switch off.

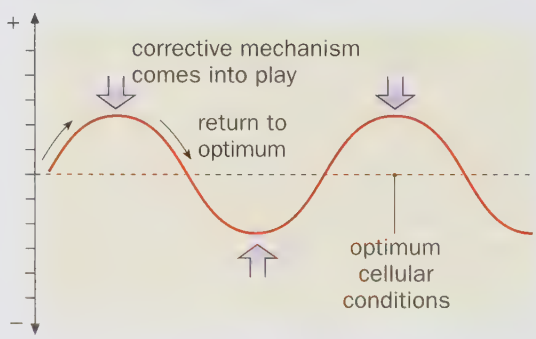
The temperature of the water bath starts to fall. If it falls too low, then this is detected by the thermostat, which switches the heater back on, so the temperature of the water rises again.

The heating element is switched on or off depending on the temperature of the water detected by the thermostat. In other words, the system relies on **feedback**. And since the change in water temperature results in the heater producing an ***opposite effect***, this is known as **negative feedback**.



The enzymes that control the chemical reactions in our cells operate best within fairly narrow limits of temperature, pH, substrate and product concentration. In a mammal, the immediate environment of the cells is the tissue fluid. So if cellular enzymes are to work to their optimum, then it is important that the composition of tissue fluid is kept as stable as possible.

Homeostasis is the maintenance of a constant internal environment within a living organism within restricted limits.

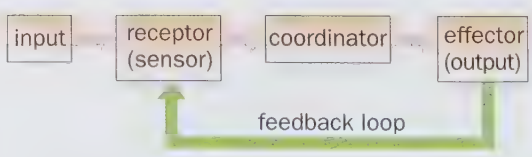


A change in optimum conditions in the cells is detected by receptors. Corrective mechanisms are activated which restore conditions to the optimum

You have already seen examples of homeostatic control systems operating for blood pressure and gas composition of the blood. In earlier studies, you probably looked at how blood sugar and body temperature were controlled and maintained within narrow limits.

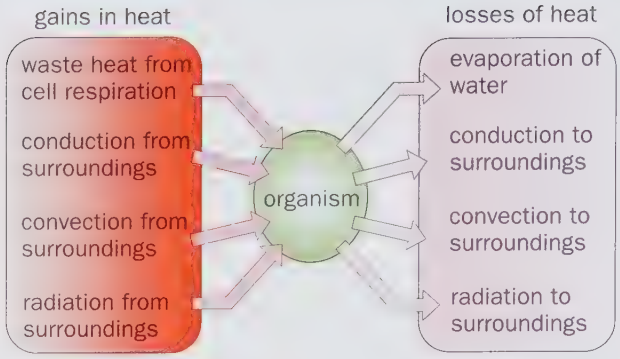
Each control system must have

- a **receptor** (sensor), which detects a **stimulus**. A stimulus is a change in the level of the factor being regulated. This detectable change is called the **input**.
- a **coordinator**, which receives and controls information from the receptor and triggers the action that will correct the change.
- an **effector**, which carries out the action that brings about the change (often called **the corrective mechanism**).



Heat gain and loss

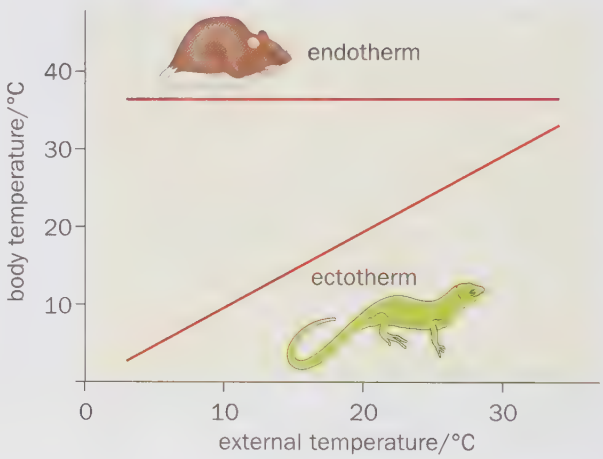
Living organisms exist in habitats where the temperatures range from 115 °C in volcanic vents to −40 °C at the poles.
An organism's temperature varies according to the amount of heat it gains and the amount of heat it loses.
If heat gain is greater than heat loss, then the temperature of the organism will rise, and vice versa.
To maintain a stable body temperature, an organism must to balance its heat gain with its heat loss.
This regulation of body temperature is called **thermoregulation**.



How an organism gains and loses heat

Organisms gain heat as a waste product of respiration and from their environment, if it is warmer.
There are three ways in which heat can be transferred to and from an organism.

- **Radiation** accounts for most of the energy that is lost and gained by organisms. Heat can be radiated to and from the air, water and ground.
- **Conduction** is the transfer of energy by contact from molecule to molecule.
  Air does not conduct heat as well as liquids and solids, because the molecules are spread out.
  So conduction is greatest when an organism is in contact with water or with solids such as the ground.
- **Convection** transfers heat energy by currents of air or water.
  Heated water and air rise and cooled water and air sink.
  This sets up convection currents around the organism.
  Heat can also be lost from an organism when water evaporates from a surface, for example during sweating.



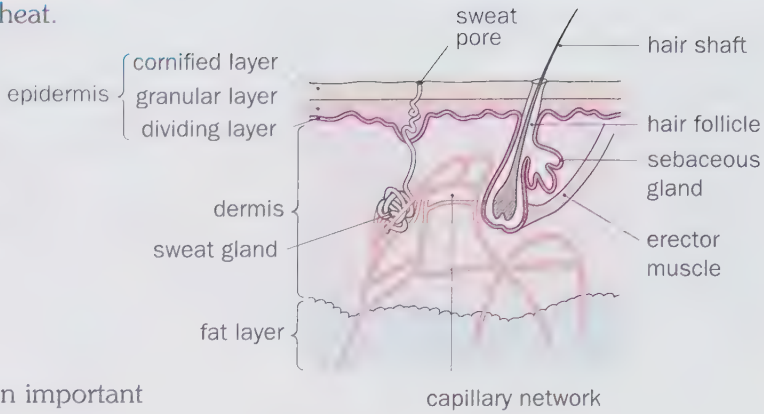
Thermoregulation in ectotherms

Ectotherms are animals that do not generate much body heat.
All animals are ectothermic except for birds and mammals.
In many ectotherms, such as fish and amphibians, their body temperature fluctuates more or less with that of their environment.
However, other ectotherms, such as lizards, control their body temperature by their behaviour or by increased activity.

Many lizards live in habitats that are hot during the day but cold at night.
Lizards spend the night under cover of rocks or in a burrow.
When they emerge in the morning, their body temperature will be well below that needed for normal activity.
During this time, the lizard's movements will be slow and this means it is less efficient than normal at avoiding predators or catching prey.
So it basks in the sun, positioning itself at right angles to the sun's rays, to raise its body temperature.
Soon the lizard is fully active and able to hunt for food.
During the hottest part of the day, the lizard finds shade under a rock to prevent itself from overheating.
Towards the end of the day, the lizard again basks in the sun.
It then returns to its burrow, and its body temperature again drops to nearer that of its surroundings.

Thermoregulation in endotherms

Endotherms are animals that generate their own body heat.
They are able to regulate their body temperature and keep it relatively constant.
Mammals and birds are endothermic, deriving heat from metabolic processes taking place inside the body, mainly waste heat from respiration.
You are able to maintain your **core body temperature** at about 37 °C even though the environmental temperature varies.



Section of human skin

The role of the skin in thermoregulation

Most heat exchange occurs through the skin and it has an important role in controlling body temperature.
If you look at the section of the human skin, you can see that it has a complex structure.
The structures involved in thermoregulation are found in the dermis.

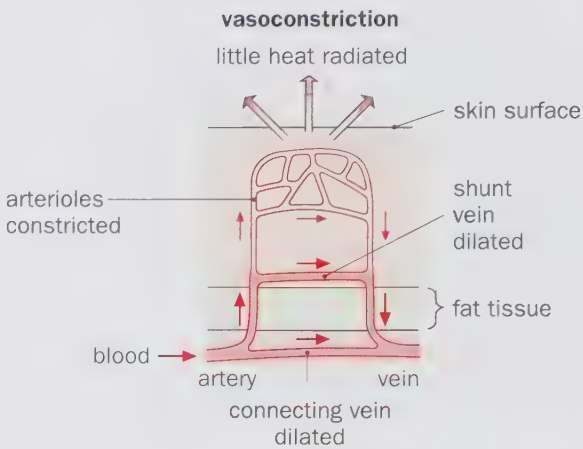
Capillaries provide the cells of the epidermis and dermis with food and oxygen but they are also involved in regulating heat loss.
The more blood that flows through the capillaries, the greater will be the amount of heat lost from the skin.

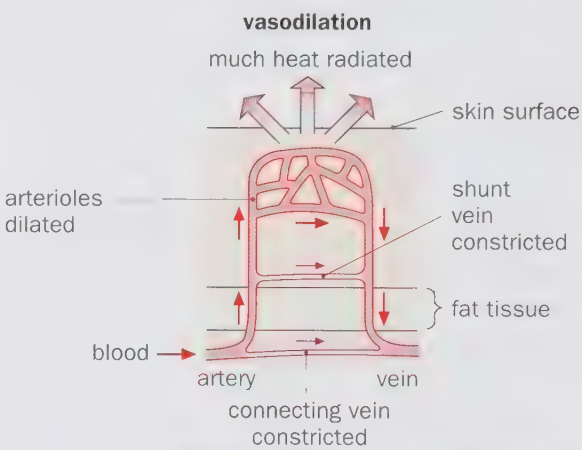
There are many capillary networks in the dermis, often forming loops.
As you know, capillaries have no muscle in their walls, but the arterioles that bring blood to them do.
When this muscle contracts, it causes the arteriole to **constrict**, that is, it becomes narrow, reducing the blood supply to the capillary.
Blood is diverted along a **shunt vessel**, so less blood gets into the capillary network and less heat is lost.
This is called **vasoconstriction**. The skin becomes paler.
When the muscle in the arteriole relaxes, it **dilates**, becoming wider and allowing more blood into the surface capillaries.
This **vasodilation** allows more heat to be lost from the capillaries.

When do you think vasoconstriction and vasodilation happen?

Sweat glands each have their own capillary blood supply.
A salty solution of sweat is secreted along the sweat duct and passes out of the sweat pore to lie on the skin surface.
The skin becomes flushed.
As the sweat evaporates, it takes heat out of the skin, so cooling it.

Connected to each hair follicle is an **erector muscle**.
When this contracts, it causes the hair to stand upright, trapping a stationary layer of air close to the skin surface.
Since air is a good insulator of heat, this reduces heat loss.
This is of little use to humans, but is effective in mammals that have thick fur.
Birds effectively do the same thing on a cold day when they fluff up their feathers and trap air close to the skin.
On hot days, the erector muscles relax and the hairs lie flatter against the skin surface, reducing the insulating layer of air so that it has far less effect upon reducing heat loss from the skin.



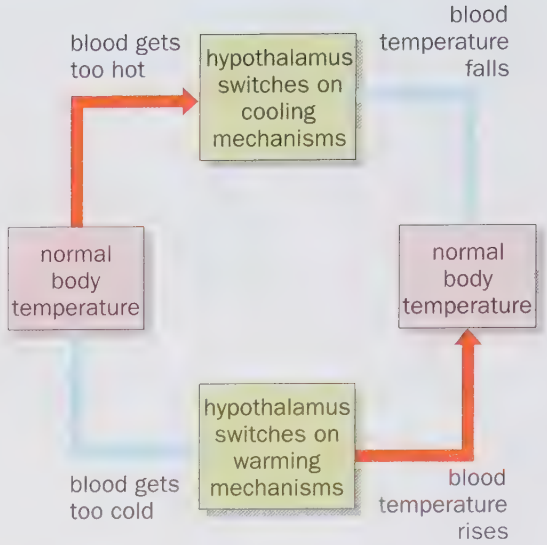



Robin on a cold day

Controlling body temperature

No matter what the environmental temperature is, your core body temperature stays at around 37 °C (unless you have a fever or hypothermia). How is this homeostatic control achieved?

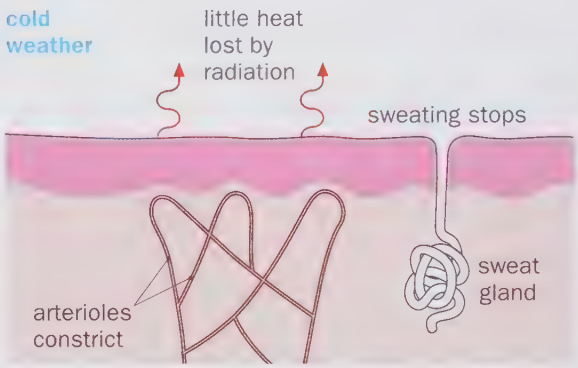
Your body temperature is controlled by the **hypothalamus**, a small structure at the base of the midbrain.
The hypothalamus acts as the body's thermostat, along with its other functions.
The hypothalamus monitors the temperature of the blood passing through it.
If the blood temperature is high, the hypothalamus sends out nerve impulses that switch on 'cooling mechanisms', such as increased sweating and vasodilation.
If the temperature of the blood is low, then the hypothalamus sends out nerve impulses that switch on 'warming mechanisms'.
In addition, the hypothalamus receives nerve impulses from hot and cold temperature receptors in the dermis of the skin.
These respond to changes in environmental temperature.



Overcooling

When the surrounding environment is cold, the hypothalamus detects a decrease in the temperature of its blood supply and receives impulses from the skin's cold receptors. It send out nerve impulses that bring about the following responses.

- **Vasoconstriction** of arterioles diverts blood away from the skin surface, so less heat is lost by radiation.
  That's why you look paler when you are cold.
- **Sweating** is reduced, or stops altogether.
- **Erector muscles** contract, raising your hairs and trapping a stationary layer of insulating air close to the skin surface.
- **Shivering** is the rapid, involuntary contraction and relaxation of muscles, which results in increased heat production.

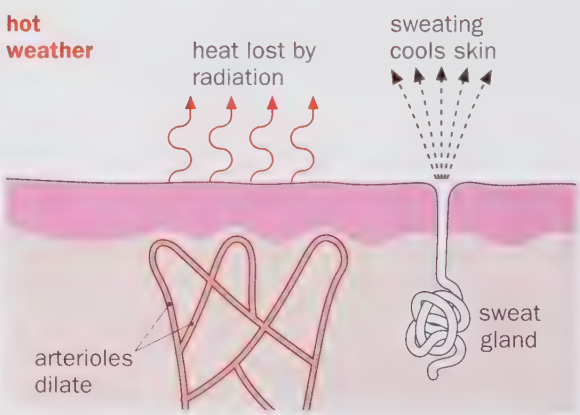


These responses have the effect of conserving body heat and producing more heat.

Overheating

When the environment is hot, the hypothalamus detects an increase in the temperature of its blood supply and receives nerve impulses from the skin's heat receptors.
It sends out nerve impulses that bring about the following responses.

- **Vasodilation** of arterioles allows more blood to reach the capillaries near the skin surface, so more heat is lost by radiation.
  That's why you look flushed when you are hot.
- **Sweating** increases, so more sweat lies on the skin surface.
  Evaporation of this sweat cools the skin.
- **Erector muscles** relax and the hairs lie flatter against the skin.
  This reduces the stationary layer of insulating air.



In addition, ***behavioural adaptations*** play a part in thermoregulation.
Desert mammals avoid high daytime temperatures by being nocturnal.
Some animals hibernate during the hottest months, a process called **aestivation**.
In cold areas, animals are active during the day, often huddling together in groups to reduce heat loss, as in the case of penguins.

Time for a bit of aestivation

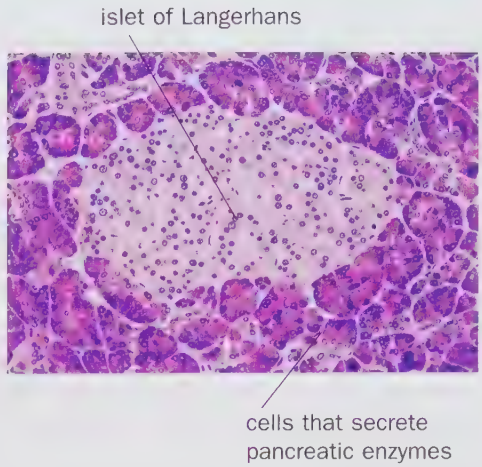
The pancreas

The **pancreas** is both an exocrine and an endocrine gland.
Exocrine glands release secretions along ducts or tubes.
The pancreas secretes pancreatic juice down the pancreatic duct into the duodenum.
Endocrine glands secrete hormones directly into the bloodstream.
A **hormone** is a chemical that coordinates certain activities in the body.

The pancreas secretes the hormones **insulin** and **glucagon** into the blood to regulate the blood sugar level.
The pancreas plays a central role in the control of blood glucose.
If you look at the photomicrograph of a section through the pancreas, you can see that scattered amongst the cells that produce digestive enzymes are groups of different cells called **islets of Langerhans**.
The islets of Langerhans are made up of two different types of cells.

- The **alpha cells** (α-cells) are sensitive to low levels of glucose in the blood and secrete the hormone **glucagon**.
- The smaller **beta cells** (β-cells) detect increases in blood glucose levels above the normal and secrete a hormone called **insulin**.

Glucagon and insulin act in opposite ways to maintain a constant blood glucose level.



Section of the pancreas

Control of blood glucose

The blood of a normal person contains between 80 and 90 mg of glucose per 100 cm^3. Blood glucose levels will rise due to the following.

- The absorption of carbohydrates from the alimentary canal.
- The conversion of stored **glycogen** to glucose by a process called **glycogenolysis**. Glycogen is stored in the liver and muscles and can be quickly converted into glucose to meet the body's needs.
- The conversion of amino acids and glycerol to glucose by a process called **gluconeogenesis**.

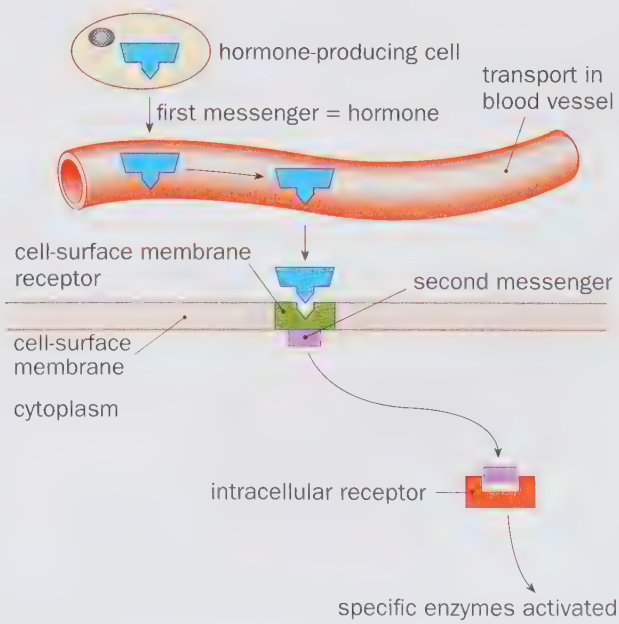
Excess amino acids are broken down in the liver by **deamination**.
The amino part of the molecule is excreted, but the remainder can be converted into glucose.
During fasting, the blood glucose level is maintained by the conversion of lipid stores.
Animals do not store proteins.
So starvation may lead to proteins being used, resulting in muscular wastage.

Glucagon

If the blood glucose level gets too low, the α-cells of the islets of Langerhans detect the change and secrete glucagon.
This hormone fits into **receptor sites** on the cell-surface membranes of liver cells and leads to the activation of the enzymes inside to

- convert glycogen to glucose,
- increase the rate of gluconeogenesis (the conversion of amino acids and glycerol into glucose).

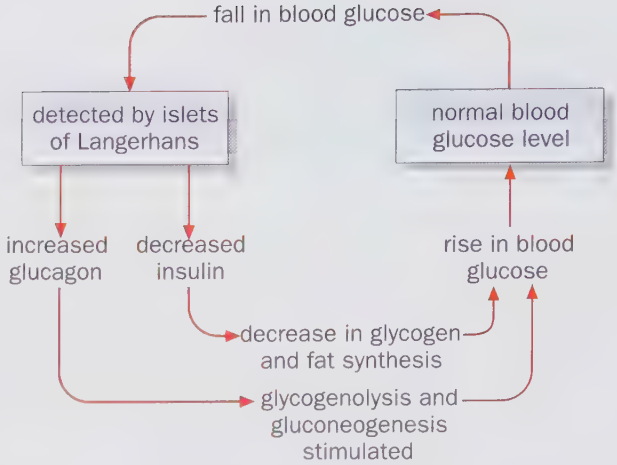
The diagram shows the action of glycogen by the 'second messenger model'. See page 359 for details of how this works in adrenaline.



The role of a hormone in activating specific enzymes

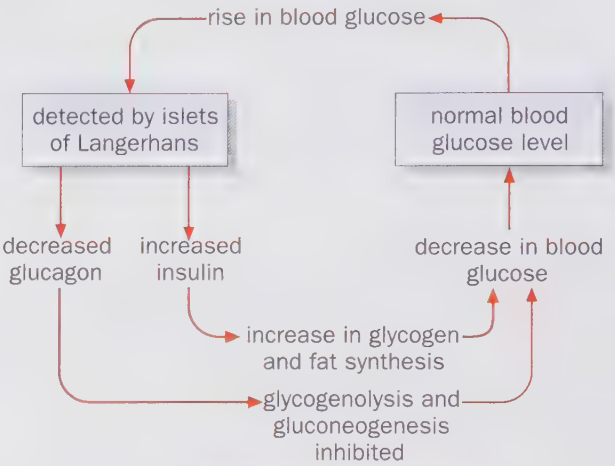
Insulin

If the level of glucose in the blood gets too high, it enters the β-cells of the islets of Langerhans by facilitated diffusion. Glycolysis results in the breakdown of glucose and increase in ATP. ATP-sensitive potassium ion (K^+) channels close. The increase in potassium ions causes depolarisation of the membrane, calcium ion (Ca^{2+}) channels open and the influx of calcium ions causes the release of stored insulin. This hormone circulates around the body in the bloodstream and attaches to receptor sites on the cell-surface membranes of liver, muscle and adipose (fat-storing) cells. This changes the permeability of these target cell-surface membranes to glucose, by increasing the activity of channel proteins which allows glucose to pass across the cell-surface membrane.



The secretion of insulin results in the following changes.

- The rate of respiration in the cells increases, since there is now more of the respiratory substrate, glucose, available.
- The rate of conversion of glucose to glycogen increases. The glycogen is stored in the liver and muscles (**glycogenesis**).
- The rate at which glucose is converted to fat and stored in adipose tissue increases.



Diabetes

Some people cannot control their blood glucose level properly because they are unable to produce sufficient insulin. This clinical condition is called **diabetes mellitis**. There are two types of diabetes: **type 1** and **type 2**. Type 1 diabetes, also known as 'early onset diabetes' usually occurs rapidly before the age of 20. Amongst its possible causes are

- the insulin receptor sites on the cell-surface membranes of the liver, muscle and adipose cells fail to recognise insulin. So despite the fact that insulin is produced, its effects are blocked.
- the β-cells of the islets of Langerhans are destroyed by the body's autoimmune system and so are no longer able to secrete insulin.

After a carbohydrate meal, the blood glucose level rises. In diabetic patients there is insufficient insulin to increase the permeability of the cell-surface membranes to glucose, so the cells are starved of their fuel, even though the blood glucose level is high. The cells have to respire using proteins and fats instead, which results in the patient losing weight. Another symptom is thirst, caused by the increased water potential of the blood. The patient needs more water to dilute the blood and lower its water potential. One indicator of diabetes is the presence of glucose in the urine. This is because the kidneys are unable to reabsorb the high levels of glucose filtered into the tubules.

Type 1 diabetics may need daily injections of specific amounts of insulin. As you will see in Chapter 22, human insulin can now be produced on a large scale by genetic engineering.

Type 2 diabetes, also called 'late onset diabetes', is more common than type 1. It tends to develop after the age of 40 and is linked to obesity. Fortunately it is not as serious as type 1 and can usually be controlled by a combination of a low-glucose diet and physical exercise. The cause is usually due to a drastic decrease in the ability of the β-cells in the pancreas to make insulin.

Child injecting insulin

An insulin dosage for injection

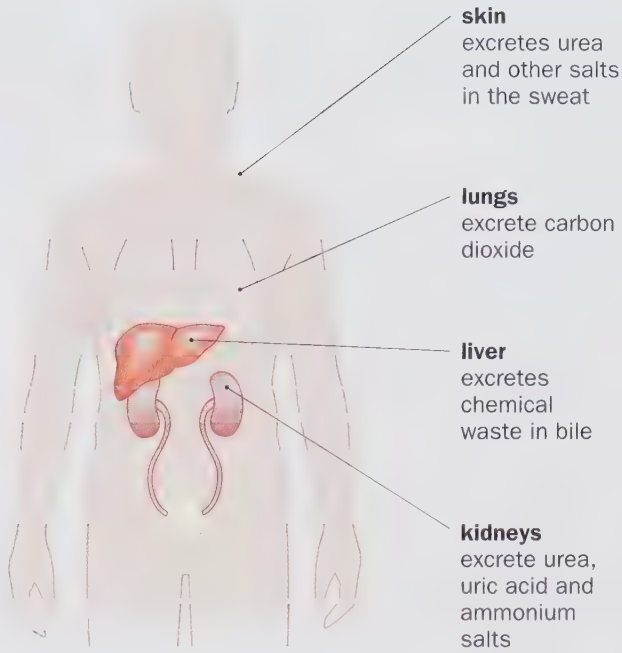
Excretion

Many of the metabolic reactions that take place inside the bodies of living organisms produce waste products which can be toxic if they are allowed to build up.

Excretion is the removal from the body of waste products made in the cells during metabolism.

In humans these **excretory products** include

- carbon dioxide from respiration, which is excreted by the lungs,
- **nitrogenous waste products**, such as urea, uric acid and ammonium salts, are formed from the breakdown of excess amino acids and nucleic acids.
- bile pigments, which are made in the liver and excreted in the bile via the small intestine. They are formed from the breakdown products of haemoglobin from old red blood cells. Any useful iron is removed and used to make new haemoglobin before the bile pigments are excreted.



Human excretory organs

Excreting nitrogenous waste

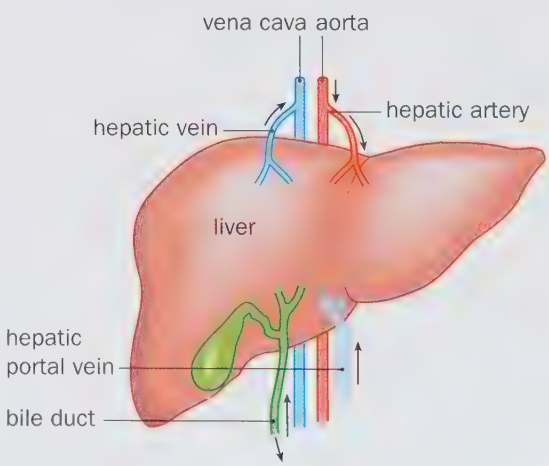
Different animals deal with the excretion of nitrogenous waste in different ways, depending upon the environment which they inhabit.

- **Fish** release their nitrogen as **ammonia**, a small, highly toxic molecule. Ammonia is extremely soluble and quickly diffuses out across the gills, at no energy cost to the fish. The ammonia is diluted down to non-toxic levels.
- **Birds and insects** excrete their nitrogen as **uric acid**, which is made from ammonia at a large energy cost to the animal. Uric acid is almost insoluble in water and is non-toxic. Very little water is needed to excrete uric acid and it is removed from the bodies of birds and insects as a white paste. This is important in conserving body water and allows them to inhabit arid environments.
- **Mammals** excrete their waste nitrogen as **urea**. If more protein is eaten than the body needs, excess protein or amino acids cannot be stored.


Fish release ammonia across their gills

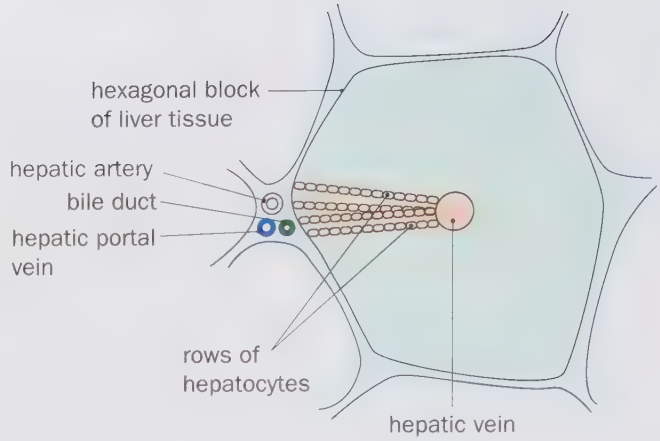
Structure of the liver

The liver is a large organ that is situated just below the diaphragm. Inside it is divided up into hexagonal blocks of tissue called **lobules**. Blood is carried to the liver into the following blood vessels: the **hepatic artery**, which carries oxygenated blood, and the **hepatic portal vein**, which brings soluble food molecules from the ileum. The **hepatic vein** transports blood away from the liver. In addition, the **bile duct** carries bile secreted by the liver to the duodenum. Inside the liver, the branches of the hepatic artery and hepatic portal vein combine to enter a series of channels called **sinusoids**. These sinusoids are lined by numerous liver cells or **hepatocytes**. As the blood passes along the sinusoids, exchange of materials occurs with the hepatocytes. The hepatocytes have microvilli to increase the surface area for exchange of materials, and possess many mitochondria, an indication of their high metabolic rate.



Structure of liver and its blood supply

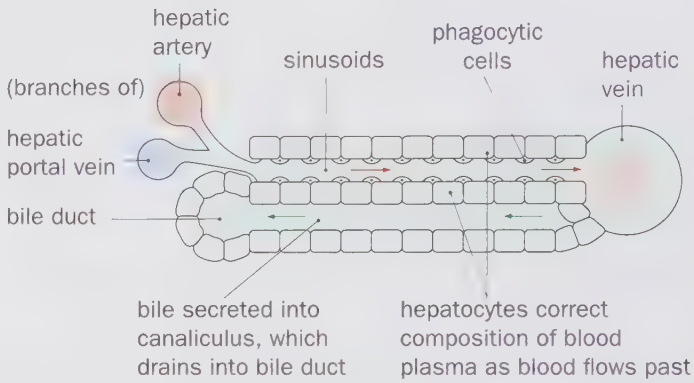
Blood with adjusted metabolites drains out of the sinusoids into a branch of the hepatic vein.
Between the sinusoids are fine tubes called **canaliculi** into which the hepatocytes secrete bile. The canaliculi carry bile to the bile duct which leads to the gall bladder where bile is stored.



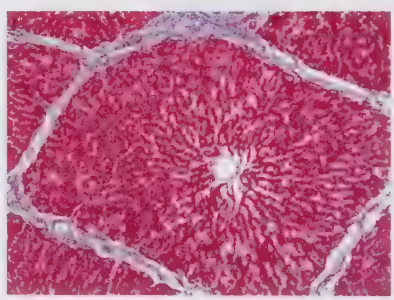
Functions of the liver

- **Deamination** – involves the breakdown of excess amino acids in the liver. The amino group is removed from the amino acid and the rest of the molecule is used in respiration (see page 309). The nitrogenous waste product of deamination is ammonia. Urea is made in the liver by combining two molecules of ammonia with one molecule of carbon dioxide. But this is not a single reaction. It occurs as a series of reactions which involve intermediate molecules. The urea molecule builds up as a result of the **ornithine cycle**. The synthesis of urea requires a lot of energy, but its advantage is that it is far less toxic than ammonia and so the tissues are able to tolerate it in high concentrations.
- **Blood glucose regulation** – as we have seen, the liver converts excess glucose absorbed from the ileum into glycogen and stores it (glycogenesis). If the blood sugar level falls, the stored glycogen can be reconverted into glucose (glycogenolysis). These two processes are controlled by the two hormones insulin and glucagon which are secreted by the islets of Langerhans in the pancreas.
- **Regulation of lipids** – lipids that arrive in the liver may be broken down or modified and transported to other parts of the body for storage. If the store of glycogen in the liver reaches its upper limit, the excess is converted into fat. Excess cholesterol in the blood reaching the liver is excreted in the bile. The liver is also capable of synthesising cholesterol if the level in the body becomes inadequate.
- **Production of bile** – the liver produces bile salts and bile pigments. These go on to form bile along with sodium chloride, sodium hydrogencarbonate, cholesterol and water. Bile is a yellow-green fluid which is stored in the gall bladder before it is released into the duodenum and involved in digestion (see page 155).
- **Maintenance of red blood cells** – some 2 million red blood cells are destroyed and replaced everyday. Along the sinusoids of the liver are phagocytic cells which breakdown redundant red blood cells after their 120-day lifespan. The result is bile pigments, which are excreted, and iron which is either stored or passes to the bone marrow where new red blood cells are formed.
- **Detoxification** – the phagocytic cells lining the sinusoids ingest foreign organisms, whilst the hepatocytes breakdown toxic chemicals such as alcohol.

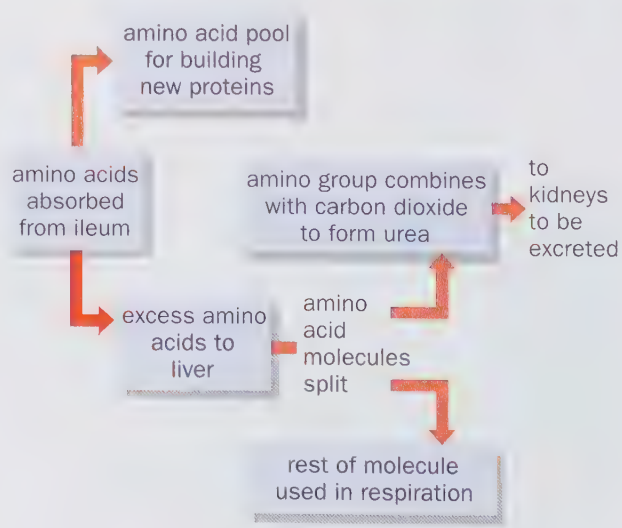
Other functions of the liver include the storage of vitamins and minerals, the synthesis of plasma proteins, such as albumins, globulins, and those involved in blood clotting. The liver also breaks down hormones after they have fulfilled their functions.



Detailed structure of liver tissue



Photomicrograph of liver tissue



The process of deamination

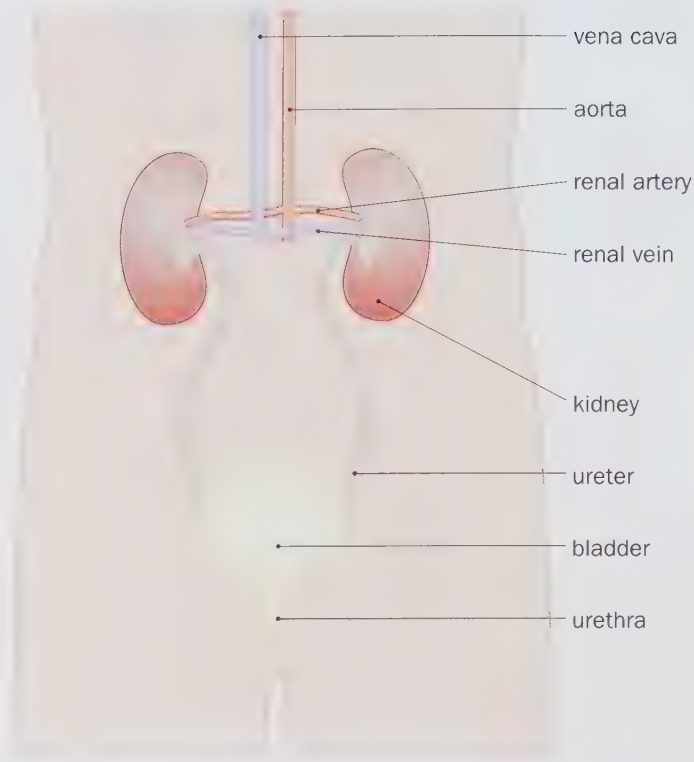
The kidneys

You have two **kidneys** positioned at the back of your abdomen. If you put your hands on your hips, your kidneys should be where your thumbs are.

The kidneys are the main organs in the **urinary system**. They filter waste products out of the blood.
Each kidney receives its blood supply from a branch of the aorta called the **renal artery**.
The kidney consists of millions of filtering units called **nephrons**.
Blood comes to the kidney under high pressure making filtration efficient.
The filtered blood leaves the kidneys along the **renal veins**.

The filtered waste products are excreted by the kidney as **urine**.
The urine passes down a muscular tube called the **ureter**.
There is a ureter connecting each kidney to the **bladder**.
The bladder is a muscular sac that stores the urine.
When **urination** occurs, a ring of muscle called the **sphincter muscle** relaxes and urine passes out of the body along the **urethra**.
In females, the urethra is relatively short, whilst in males it is longer and acts as the passage for both urine and semen at different times.

Each day the kidneys filter about 180 dm^3 of fluid, but, as you will see, most of this is reabsorbed.
On average, about 1 dm^3 of urine is produced each day.



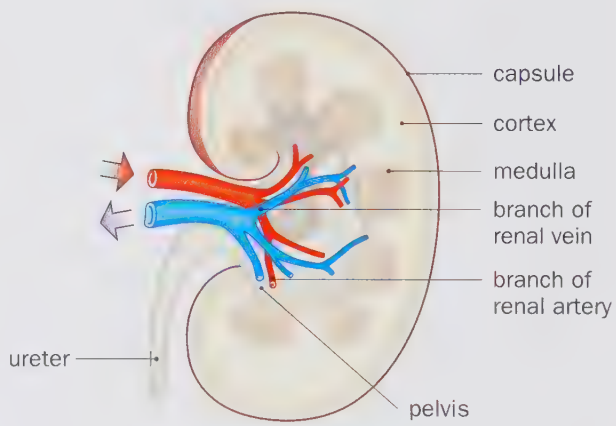
Human urinary system

Kidney structure

Each kidney is surrounded by a layer of adipose (fat) tissue and a layer of fibrous connective tissue.
These keep the kidneys in position and protect them from mechanical damage.
If you cut a section through the kidney, three main areas can be seen.

- The dark, outer region is called the **cortex**.
  It is here that filtration is carried out by the nephrons.
  It has a dense capillary network which receives blood from the renal artery.
- The lighter, inner region is called the **medulla**.
  Each nephron extends across the medulla to form structures called **renal pyramids**.
- The renal pyramids project into a central space called the **pelvis**.
  Urine passes out into the pelvis before it passes down the ureter.

The kidneys have a major homeostatic role in the body. They remove nitrogenous waste products, and they also help to control the water content and pH of the blood.

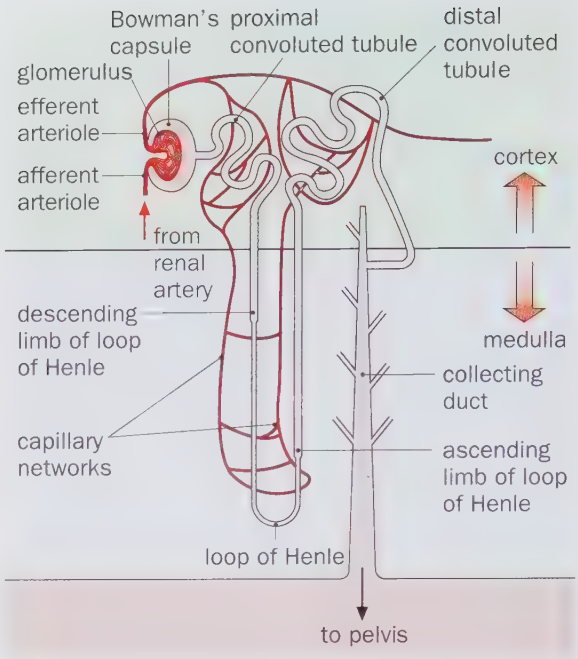


Vertical section of the kidney

Structure of the nephron

The **nephron** is the functional unit of the kidney.
Under the microscope, kidney tissue can be seen to be made up of thousands of these tiny tubes.
At one end of the nephron, in the cortex, is the cup-shaped **Bowman's capsule**.
Immediately below the capsule is a twisted region called the **proximal convoluted tubule**.
This leads into the long, hairpin-like **loop of Henle,** which runs deep into the medulla and then back out to the cortex, where it forms another twisted region called the **distal convoluted tubule**.
This is joined to a **collecting duct**, which carries urine through the medulla to the pelvis of the kidney.

Each nephron has a rich blood supply.
Blood is brought to the kidney by the renal artery, which branches many times to form arterioles. Each Bowman's capsule is supplied with blood by an **afferent arteriole**.
This branches inside the Bowman's capsule to form a knot of capillaries called the **glomerulus**.
These join up again to form the **efferent arteriole**, which takes blood away from the Bowman's capsule.
Can you see from the diagram that the afferent arteriole is much wider than the efferent arteriole? This means that more blood is carried to the glomerulus than is carried away from it.



The structure of a nephron and its blood supply

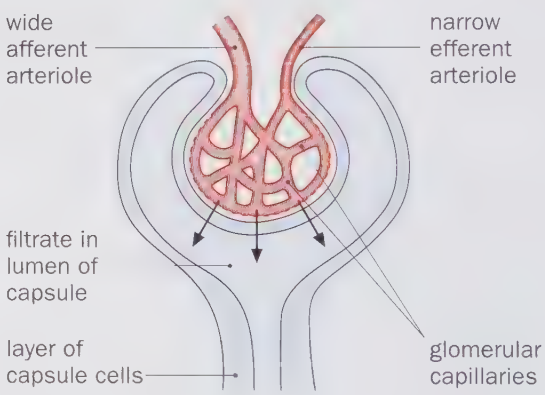
Ultrafiltration

Ultrafiltration involves the filtering (under pressure) of small molecules out of the blood and into the Bowman's capsule.
To understand how ultrafiltration works, it is necessary to know about the microstructure of the Bowman's capsule.
The blood entering the glomerulus is separated from the space inside the Bowman's capsule, by two cell layers and a basement membrane.

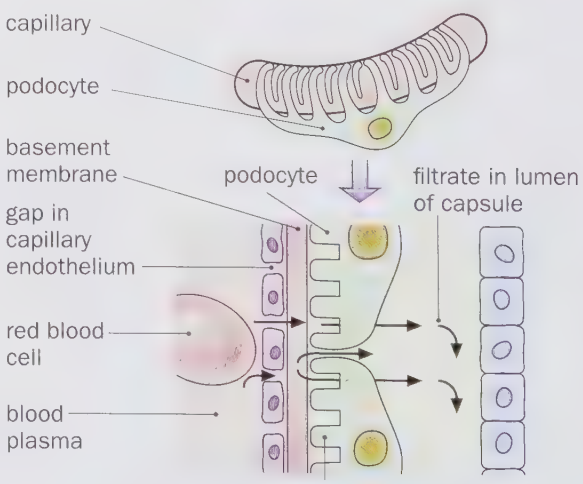
- The first cell layer is the wall or **endothelium** of the capillary.
  In the glomerulus, this single layer of cells has thousands of gaps.
- The **basement membrane** between the two cell layers is composed of glycoprotein and collagen fibres.
  Its mesh-like structure acts as the filter during ultrafiltration.
- The second cell layer makes up the wall of the Bowman's capsule.
  The epithelial cells in this wall are called **podocytes**.
  They have foot-like processes and, like the cells of the capillary, they do not fit tightly together, so there are gaps between them.

The gaps in the capillary endothelium and in the Bowman's capsule wall will allow most molecules to pass through.
But the basement membrane prevents large molecules, such as proteins and also blood cells, from passing through and so acts as the filter.
Only small, soluble molecules can pass through the basement membrane.
The blood pressure in the kidney is higher than in other organs.
This high pressure is maintained in the glomerulus because the afferent arteriole has a wider diameter than the efferent arteriole.

More blood goes to the glomerulus than comes away. A hydrostatic pressure builds up, forcing substances through the endothelial pores, across the basement membrane and into the Bowman's capsule.



Ultrafiltration in the Bowman's capsule



Microstructure of glomerulus and Bowman's capsule

Reabsorption

Ultrafiltration is so efficient that up to 20% of the water and solutes are removed from the plasma as it passes through the glomerulus.
If you look at the table you can see that the filtrate in the renal capsule is identical to the blood plasma, apart from containing no plasma proteins. These are too large to pass through the basement membrane.

You can also see that useful substances such as amino acids and glucose are filtered out of the blood.
These are needed by the body and so they are reabsorbed back into the blood as the filtrate flows along the nephron.
This process is called **selective reabsorption** since only certain molecules are reabsorbed.
All the glucose, amino acids, vitamins and many sodium and chloride ions are actively transported out of the proximal convoluted tubule and back into the blood.

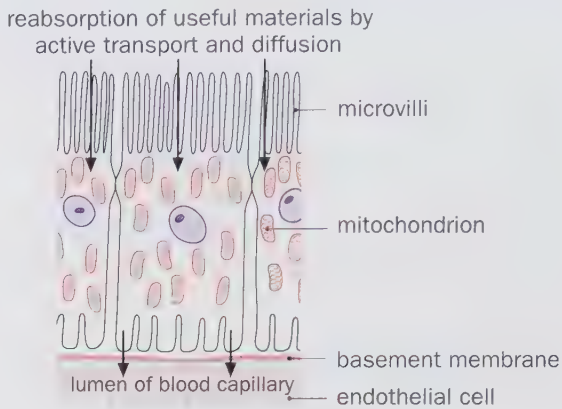
If you look at the structure of the cells making up the wall of the proximal convoluted tubule, you will see many of the adaptations associated with active transport:

- microvilli provide a large surface area for absorption,
- numerous mitochondria provide ATP for active transport.

The uptake of these substances means that the blood in the capillaries surrounding the nephron now has a relatively high solute concentration.
So a large amount of water passes out of the filtrate in the proximal convoluted tubule and back into the blood by ***osmosis***.

Molecule or ion	Approximate concentration/g dm^{-3}	
	Plasma	Filtrate
water	900.0	900.0
protein	80.0	0.0
glucose	1.0	1.0
amino acids	0.5	0.5
urea	0.3	0.3
inorganic ions	7.2	7.2

Mean composition of human plasma and filtrate



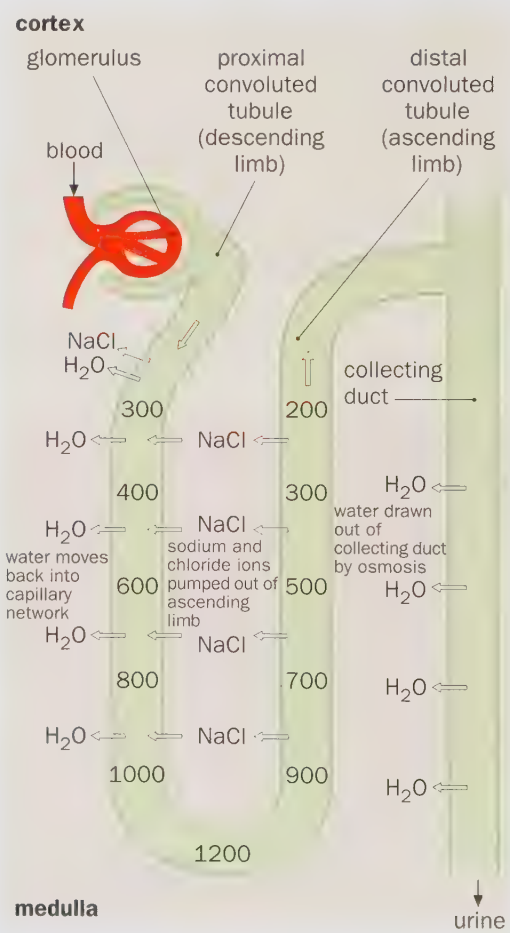
Cells of the proximal convoluted tubule

The loop of Henle

The **loop of Henle** is a hairpin loop that runs deep into the medulla and then turns and goes back to the cortex again.
The first part of the loop is called the **descending limb** and the second part of the loop is called the **ascending limb**.

The function of the loop of Henle is to create an area of high solute concentration deep in the medulla.
The collecting ducts of each nephron pass through this area and so a lot of water can be reabsorbed from the collecting ducts by osmosis. A concentrated urine can be produced as a result.

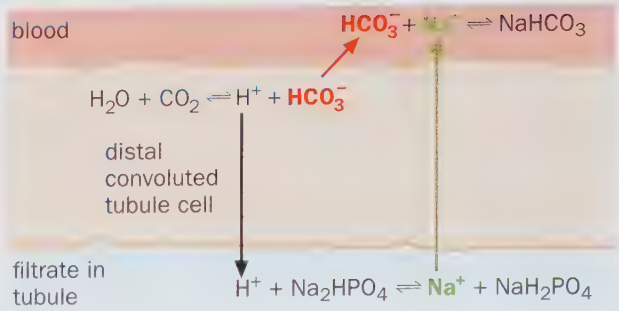
The diagram shows you how the loop of Henle works.
The ascending limb is more permeable to salts and less permeable to water.
As the filtrate moves up, sodium and chloride ions move out passively at first and are then actively pumped out into the surrounding tissue.
This causes water to pass out of the descending limb by osmosis.
As a result the filtrate becomes more concentrated as it passes down the descending limb of the loop.
The net result is that the solute concentration at any part of the loop is lower in the ascending limb than it is in the descending limb.
This mechanism is called the **countercurrent multiplier mechanism**.
As the collecting ducts pass through the medulla to the pelvis, they pass through this region of high solute concentration.
So water is drawn out of the collecting ducts by osmosis, resulting in a far more concentrated urine.



The countercurrent multiplier mechanism

The distal convoluted tubule and collecting duct

The distal convoluted tubule is made up of cells with a similar structure to those of the proximal convoluted tubule.
They have microvilli on their surfaces and have many mitochondria.
These cells can actively pump sodium ions out of the nephron and into the blood.
Hydrogencarbonate ions dissociate from carbonic acid and then also pass into the blood.
This raises the pH of the blood when necessary.
So the distal convoluted tubule is able to control the acid/base balance of the blood.



Acid/base balance controlled in the distal convoluted tubule

The second part of the distal convoluted tubule acts as the collecting duct.
The permeability of the walls of both the distal convoluted tubule and the collecting duct is affected by hormones.
This regulates how much water passes out into the medulla, and consequently how concentrated the urine will be.

Mammal	Relative thickness of medulla	Maximum urine concentration/ arbitrary units
beaver	1.0	52
pig	1.3	110
human	2.6	140
rat	5.2	300
kangaroo rat	7.8	550

Water balance in desert animals

As you have seen, the loop of Henle is concerned with water reabsorption.
The longer the loop of Henle, the greater the solute concentration set up in the medulla. So the more water is reabsorbed and the more concentrated the urine becomes.
Mammals that inhabit arid areas such as deserts, have particularly long loops of Henle, since they need to conserve as much water as possible.

The thicker the medulla, the longer the loop of Henle that passes through it.
So the thicker the medulla, the more concentrated the urine is.
The kangaroo rat (*Dipodomys deserti*) lives in desert regions and can produce urine that is ten times more concentrated than the beaver.
The kangaroo rat is well adapted to desert conditions and is able to survive with little or no water intake. How does it do this?

- If you look at the table you can see that most of its water gain comes from its food.
  The food may be completely dry, but 'metabolic water' is produced from it during respiration in the cells.
  So all the water needs of the kangaroo rat are satisfied directly or indirectly from its food.
- Kangaroo rats remain underground during the day.
  The air in their burrows is cooler and more humid, so less water is lost from the body by evaporation.
- The kangaroo rat is able to conserve body water by producing a highly concentrated urine.
  It is also able to produce very dry faeces.
- Its nasal passages are adapted to cool the air before it is breathed out. So respiratory moisture condenses in the nasal passages before it can be exhaled.

The kangaroo rat

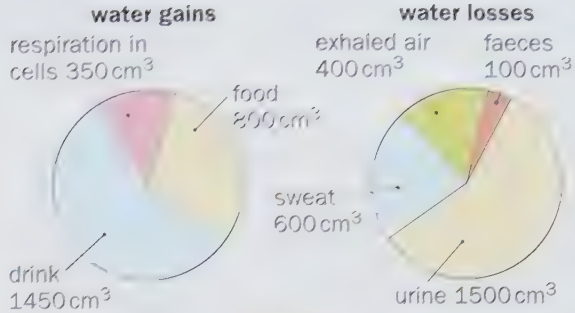
Water gain/cm³	
metabolic water	54.0
water in dried food	6.0
Total water gain	60.0

Water loss/cm³	
urine	13.5
faeces	2.6
evaporation (mainly breath)	43.9
Total water loss	60.0

Osmoregulation by the kidney

Osmoregulation is the homeostatic control of body water.
We need to balance our water intake with our water loss.
As you can see from the pie-charts, we get most of our water from drinking and from the food we eat.
We get a small proportion from metabolic reactions such as respiration.

Most of the water we lose is lost as urine. The amount lost as sweat depends upon environmental temperature and our activities.
Since gas exchange surfaces need to be moist, water will inevitably be lost when we breathe out.
Some water is also lost in the faeces.



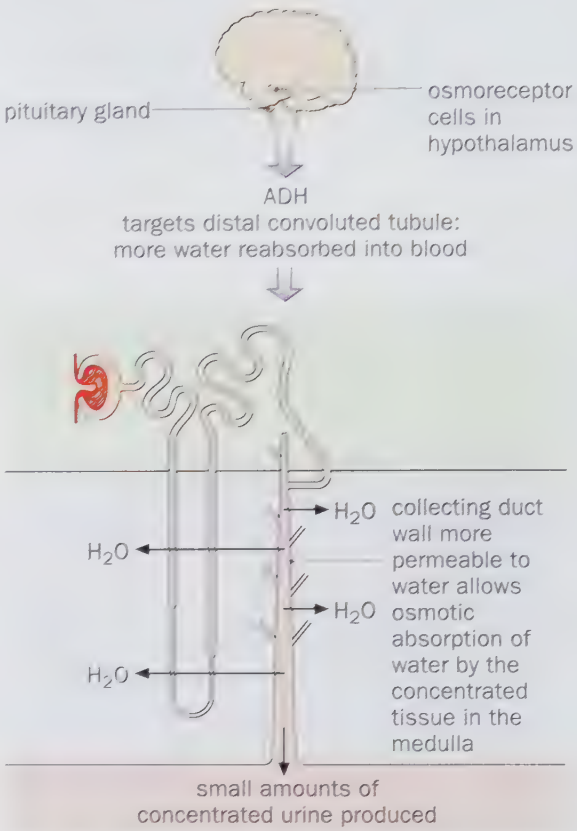
Controlling body water

Control of body water is another example of homeostasis operating on a principle of negative feedback.
In this case, the receptors responsible for detecting changes are located in the **hypothalamus** of the brain.
These **osmoreceptors** react to changes in the solute concentration of the blood as it flows through the hypothalamus.

If you haven't had a drink for a while, your blood will have a low water potential (it becomes more concentrated).
This is detected by the osmoreceptors in your hypothalamus, which stimulates your pituitary to release **antidiuretic hormone** (**ADH**).
The release of ADH into the bloodstream brings about the following.

- ADH makes the distal convoluted tubule and the collecting duct ***more*** permeable to water.
- This allows more water to be reabsorbed from the distal convoluted tubule and the collecting duct into the region of high solute concentration in the medulla.
- This produces a smaller volume of more concentrated urine.

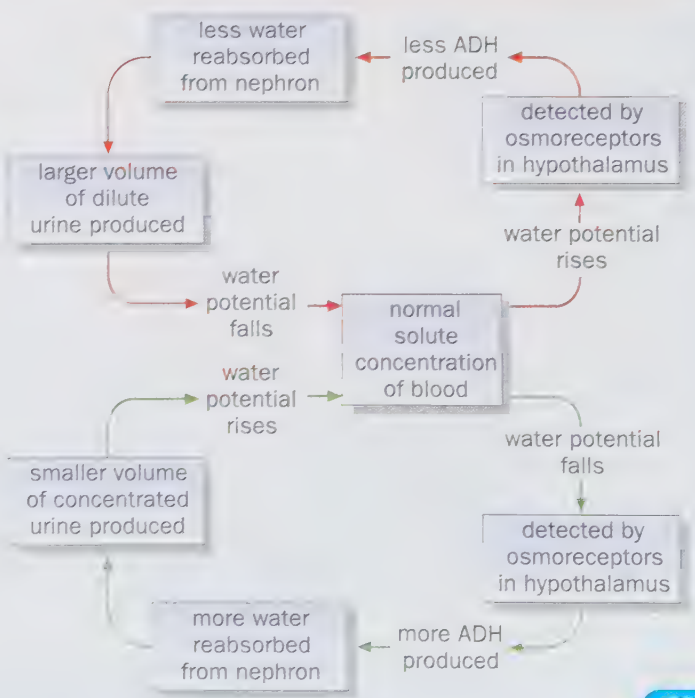
So the action of ADH is to ***conserve*** body water.



What happens if you drink lots of fluids?
Your blood will have a high water potential (it becomes more dilute).
This is detected by the osmoreceptors in your hypothalamus and results in a decrease in the amount of ADH secreted by the pituitary.
This decrease in the amount of ADH in the bloodstream results in the following.

- Lack of ADH makes the distal convoluted tubule and the collecting duct ***less*** permeable to water.
- Less water is reabsorbed into the medulla.
- Larger quantities of dilute urine are produced.

In addition to stimulating the release of ADH when the blood is concentrated, the hypothalamus also activates your **thirst centre** in the brain. As a result, you feel thirsty and take in additional water to dilute the blood. Drinking stops the stimulation of your thirst centre almost immediately. This is thought to be due to the thirst centre responding to the stomach filling with water.



Biology at work: Kidney dialysis

The kidneys have an important role in homeostasis. They maintain the salt and water balance of the body fluids, as well as excreting waste products.

Kidney failure is accompanied by an imbalance of the electrolytes in the blood, for example high levels of sodium and potassium often result.
Glomerular filtration rate (**GFR**) is a measure of the rate of flow of filtered fluid through the kidney.
It can be used to indicate the degree of kidney disease.
Low rates indicate severe kidney failure.
In these severe cases kidney function needs to be taken over by the process of dialysis.

There are two methods of kidney dialysis, **haemodialysis** and **peritoneal dialysis**.

Haemodialysis is where the blood is passed through an artificial kidney machine, either in hospital or at home. Each haemodialysis session lasts between 2 and 6 hours and a patient may need two or three sessions a week.

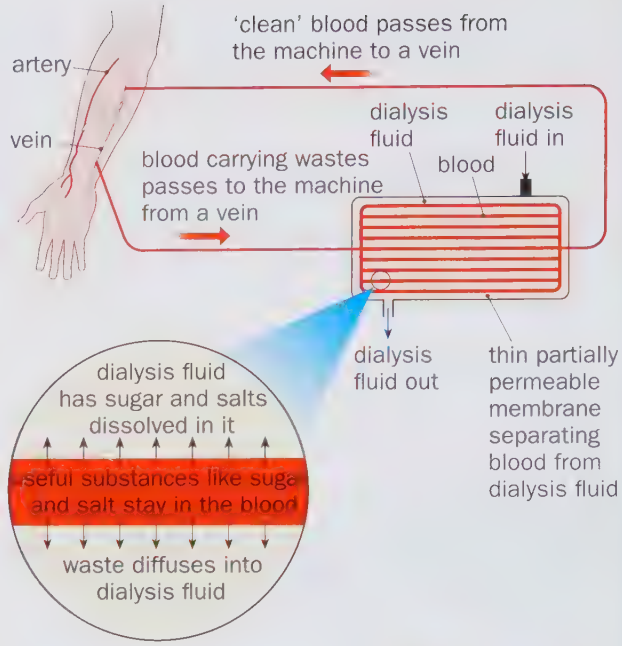
Access to the bloodstream is obtained using a **shunt**, which is a special tube that connects an artery to a vein. Blood flows from this tube into the machine and back to the patient.

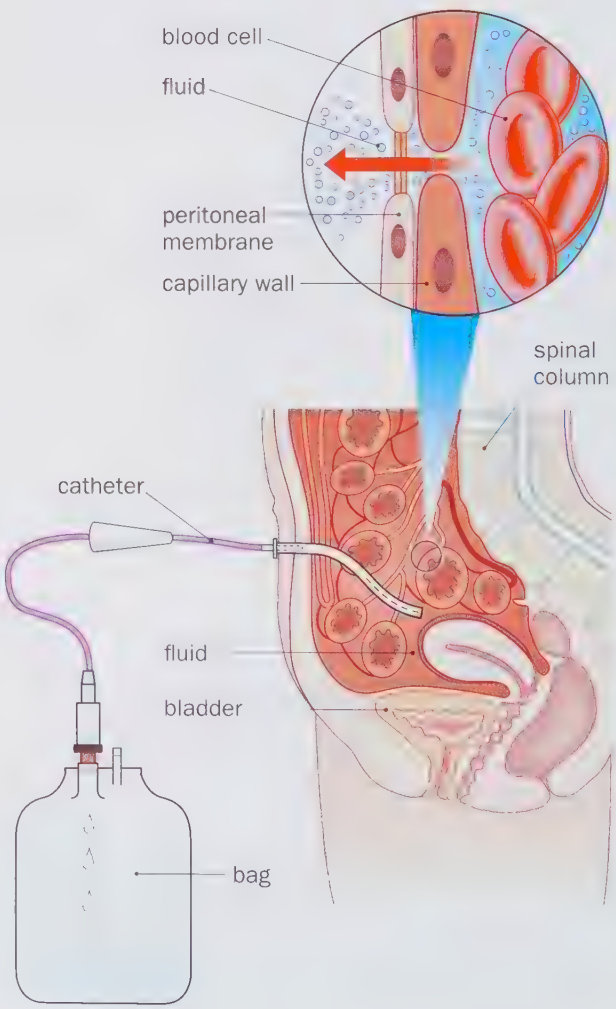
Inside the machine, the blood flows over the surface of the partially permeable dialysis membrane. This membrane separates the blood from the dialysis fluid.

The dialysis fluid has the same composition as that desired in the blood plasma. As a result, wastes, toxic molecules and excess fluid pass (by diffusion) from the blood into the dialysis fluid. To aid this process, the blood and dialysis fluid flow in opposite directions. This so-called **countercurrent principle** ensures that a fairly constant diffusion gradient is maintained between the blood and the fluid.

The 'purified' blood is returned to the patient and the dialysis fluid is discarded.
Being connected to the machine is clearly restrictive but at least for this period of time the patient can eat and drink more or less what they want.

Peritoneal dialysis makes use of natural filtering within the abdomen (the peritoneum) and is usually carried out at home. This technique involves inserting dialysis fluid into the peritoneal cavity using a catheter.
Waste products and excess water pass through the peritoneal membrane into this cavity.
After several hours, the fluid is drained out and discarded.
During this period of time the patient can carry on with normal activities.

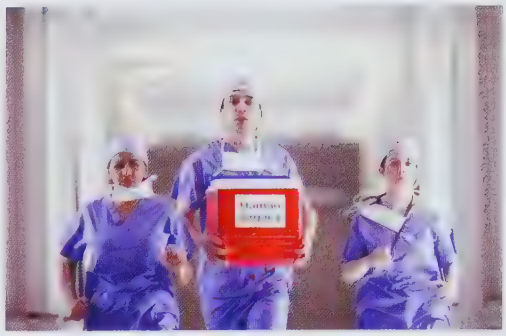




Biology at work: Kidney transplantation

Inevitably, the health of patients with long-term kidney failure suffers, and many doctors look upon a kidney transplant as preferable to long-term dialysis.

Only one functional donor kidney is needed to restore the health of a patient, and a kidney transplant is the most common and straightforward type of transplant operation.



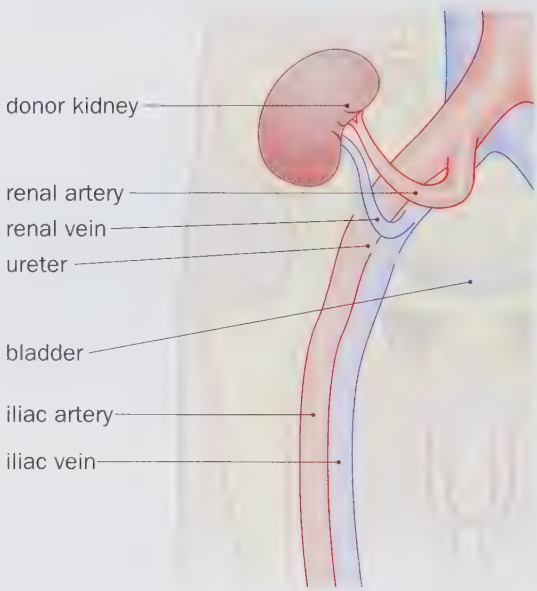
Rushing a transplant organ to a recipient

Obtaining a kidney

To prevent the recipient's immune system rejecting the kidney, the tissue type and blood group of recipient and donor must be a close match.
This will often be the case where a close relative donates a kidney.
Often though, kidneys come from people who have consented to the medical use of their organs after death.
An operation has a reasonable chance of success as long as the transplant is completed within 48 hours of the removal of the donor kidney.
During this time, a machine passes a cool saline solution through the kidney to keep it viable.

The transplant operation

The operation involves the donor kidney being placed into the pelvis of the recipient.
Often the renal artery and vein are joined to the **iliac** blood vessels in this region.
The ureter is connected directly to the bladder of the recipient.



The danger of rejection

There is an 85–95% likelihood of a donated kidney surviving for a year, and even after 15 years it is likely that 50–60% of donated kidneys will still be working.
The general rate of success with kidney transplantation is particularly high when the donor is a close relative.
However, if the kidney is rejected, then the patient must return to dialysis before further transplants are undertaken.
Usually, all kidney transplant patients take special drugs to reduce the risk of rejection. These are called **immunosuppressants** and they usually have to be taken for the rest of a patient's life.

From April 2012 to April 2013 approximately 3000 kidney transplant operations were carried out in the UK. But by the end of this period there were still approximately twice as many people on the waiting list for a transplant operation.

Why do you think that kidney transplantation is a better option for a patient than kidney dialysis?

Kidney transplantation is more expensive than kidney dialysis in the short term, but less expensive in the long term. Try to explain this statement.

Summary

- Homeostasis is the maintenance of a constant internal environment within a living organism.
- Many homeostatic systems rely upon negative feedback to produce an opposing change.
- Some ectotherms are able to control their body temperature by their behaviour.
- The skin has a major role in maintaining a constant core body temperature.
- In mammals, the hypothalamus monitors body temperature and switches on a number of corrective mechanisms if the body temperature rises or falls too much.
- The pancreas secretes glucagon to raise the blood glucose level, and insulin if the blood glucose level needs to be reduced.
- Diabetes can be treated by moderating carbohydrate intake or injecting genetically engineered insulin.
- The liver has many important functions including deamination, blood glucose regulation and detoxification.
- Excretion is the removal from the body of waste products made in the cells during metabolism.
- Fish, birds, insects and mammals produce different excretory products.
- The kidneys are the main organs of the urinary system and are composed of numerous nephrons.
- The Bowman's capsule of each nephron is well adapted for ultrafiltration of the blood.
- Useful substances are reabsorbed into the blood as the filtrate passes along the nephron.
- The loop of Henle creates a region of high solute concentration deep in the medulla by the countercurrent multiplier mechanism. This enables water to be reabsorbed back into the blood.
- Osmoreceptors in the hypothalamus monitor the solute concentration of the blood.
- The secretion of antidiuretic hormone by the pituitary increases the reabsorption of water from the nephron.
- Desert-living mammals, such as the kangaroo rat, have adaptations for conserving their body water.

Questions

1 a) i) What is meant by the term 'homeostasis'?
ii) Why is homeostasis important to the functioning of the human body?

b) Hill walkers often encounter extreme changes in environmental conditions. Describe the changes involved in thermoregulation when a walker responds to a rapid fall in environmental temperature.

2 a) What is meant by
i) an ectotherm,
ii) an endotherm?

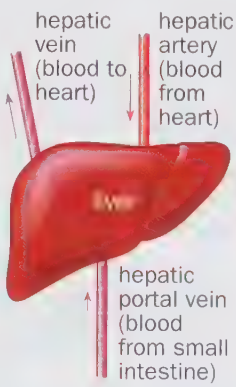
b) Explain how ectothermic animals try to prevent their bodies from overheating.

c) How does an endotherm prevent its body from overheating?

3 The diagram shows the main blood vessels going to and coming from the liver.

a) In a healthy person, the blood glucose level in the hepatic vein fluctuates much less than that in the hepatic portal vein. Explain why this is so.

b) Blood glucose level is more or less constant, even if a person has not eaten for several days.
How does gluconeogenesis help to maintain this constant blood glucose level?

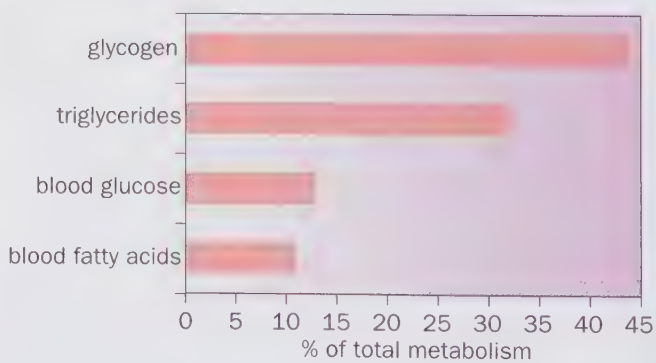


c) Suggest why people suffering from diabetes are advised to eat their carbohydrate in the form of starch rather than in the form of sugars.

4 The chart shows the sources of energy for 1 hour of cycling.

a) What percentage of the energy comes from carbohydrates?

b) Glycogenolysis and gluconeogenesis both contribute to the formation of respiratory substrates during exercise.
What is meant by
i) glycogenolysis,
ii) gluconeogenesis?

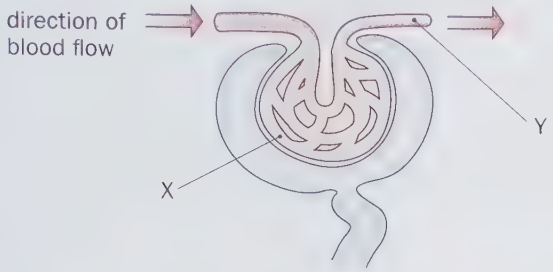


5 a) i) What is the end-product of nitrogen metabolism excreted by terrestrial insects?
ii) What is the advantage to terrestrial insects of excreting this substance?

iii) Explain why fish are able to excrete ammonia, which is highly toxic.

b) Describe how urea is formed from surplus amino acids in the mammalian liver.

6 The diagram shows a Bowman's capsule.



a) i) What is the name given to the network of capillaries labelled X?

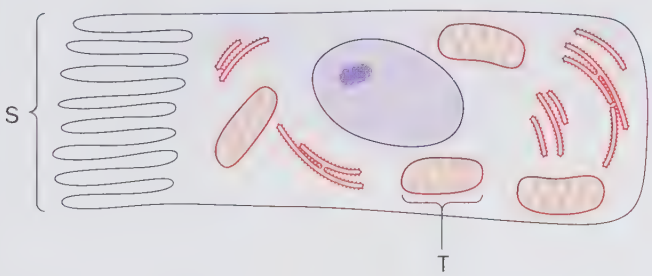
ii) State **three** consequences of constricting the diameter of blood vessel Y.

b) Much of the water in the filtrate is reabsorbed from the collecting duct.

i) Name the part of the nephron which provides the osmotic gradient for reabsorption.

ii) Suggest **one** way in which this section of the nephron might be modified in desert animals.

7 The diagram shows a cell from the proximal convoluted tubule of a nephron.



a) State **three** ways in which this cell may differ from a prokaryotic cell.

b) Explain fully the part played by S and T in the functioning of the proximal convoluted tubule.

8 a) i) Name the structure that detects changes in the water potential of the blood.

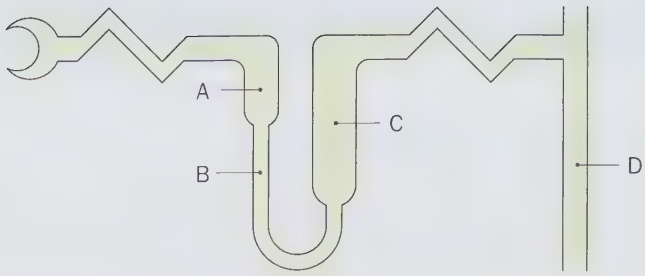
ii) Name the gland that secretes antidiuretic hormone (ADH) into the blood.

b) The diagram represents the parts of a nephron. Which letters A, B, C or D show the region where

i) the epithelium is relatively impermeable to water,

ii) the epithelium is freely permeable to water,

iii) ADH increases the permeability of the epithelium to water.



9 The table shows the volumes of fluid filtered by the glomerulus and the urine excreted under three different conditions. The total amount of solute excreted is the same in each case.

a) i) In A, calculate the percentage of water reabsorbed.

ii) State whether A, B or C leads to the most concentrated urine.

iii) Which condition, A, B or C, would you expect to find in a person who has taken strenuous exercise on a hot day?

iv) What effect would ADH have on the water potential of plasma?

b) i) Name the structure found in *Amoeba* which regulates the water potential of the cytoplasm.

ii) Suggest why the structure is absent from

1. marine and parasitic Protozoa,
2. freshwater algae.

Condition	Glomerular filtration rate ($dm^3\,day^{-1}$)	Volume of urine ($dm^3\,day^{-1}$)
A urine isotonic to plasma	180	2.4
B ADH increased	180	0.5
C ADH absent	180	23.3

10 The diagram shows part of the nephron and collecting duct of a mammal.

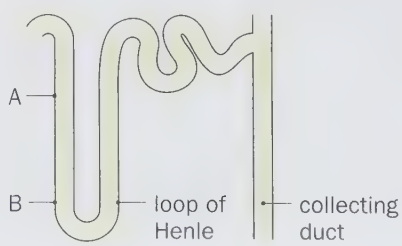
a) Why does the solute concentration of the fluid in the loop of Henle increase between points A and B?

b) Why do the cells in the ascending limb of the loop of Henle have many mitochondria?

c) Small desert mammals are able to exist in arid conditions. Explain the advantage to these mammals of

i) high levels of ADH in their blood,

ii) nephrons with very long loops of Henle.



20 Control and coordination

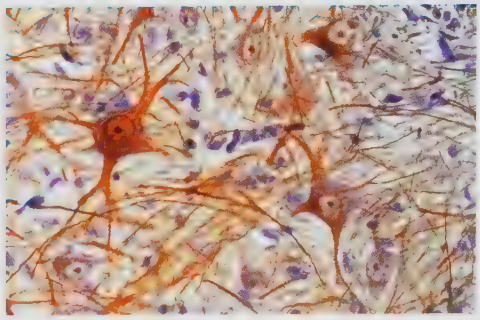
Brain transplants

Once damaged, brain cells, unlike most cells, never regenerate. But a new transplant operation pioneered in the United States could change all that.

Don Fitch suffered a stroke when a tiny blood clot in an artery leading to the brain cut off the blood supply. Don's left arm was paralysed and the whole of the left side of his body was severely weakened.

At the University of Pittsburgh Medical Centre, Douglas Kondziolka led the team of neurosurgeons – Don would be only the fourth person in the world to have the operation.
The surgery was fast, taking just 15 minutes, and done under local anaesthetic. Dr Kondziolka and his team drilled a small hole into Don's skull and then, using a syringe, injected about 2 million new human brain cells into the damaged area. It is hoped that these cells will link up with the live brain tissue around them. The brain cells transplanted into Don's skull were taken from a tumour in a 22-year-old man. Researchers discovered that immature cells from the brain tumour could be treated to develop into mature nerve cells suitable for transplanting into the brain.

It will take time to see if the implanted brain cells survive and 'wire up' into Don's existing brain circuitry.
If the brain transplants are successful, the technique could be used to treat many forms of brain damage, including Parkinson's disease and spinal cord injuries.



Human brain cell of the type injected into Don Fitch

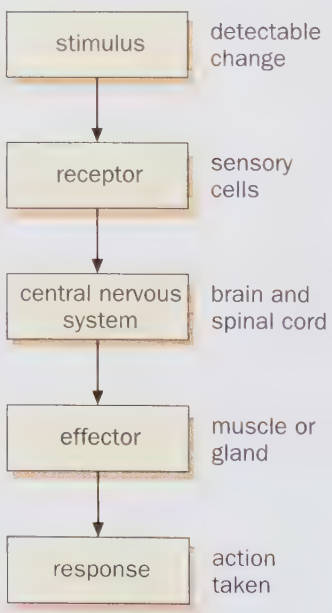
The nervous system

The nervous system controls and coordinates our actions by

- detecting changes (**stimuli**) inside and outside our bodies,
- processing the information received about these stimuli and deciding what to do, often as a result of previous experience,
- initiating responses to these stimuli by coordinating the body's actions.

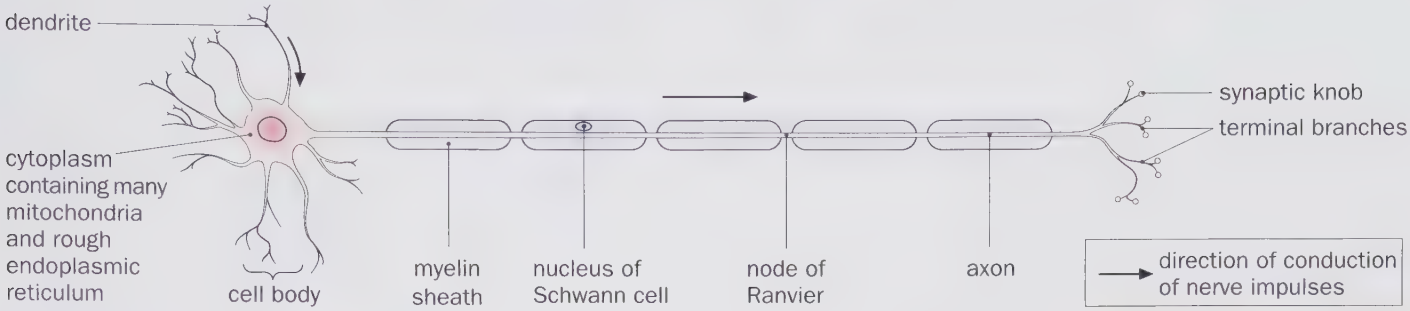
The information about our internal and external environment is detected by **receptors**, for example eyes and ears.
Responses are brought about by organs called **effectors**.
Effectors are usually muscles or glands.

Processing sensory information, deciding what to do and initiating a response is the role of the **central nervous system** (**CNS**).
The CNS is made up of the **brain** and the **spinal cord**.
Many nerves are paired, joined to the brain or spinal cord, bringing information to the CNS and taking other information away.
These nerves make up the **peripheral nervous system** (**PNS**).
The nerves of the PNS can be classified according to their function into the **somatic nervous system**, which controls voluntary actions, and the **autonomic nervous system** (see page 169) which controls involuntary actions and enables internal organs to function properly.



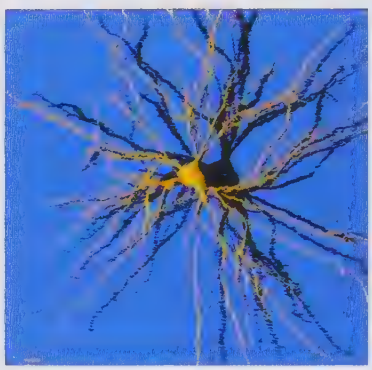
Neurones are the basic functional unit of the nervous system.
Neurones are highly specialised cells that are able to generate and transmit nerve impulses.
They link up to form nervous pathways around the body.

There are different types of neurone, but basically their structure is the same. The diagram shows a **motor neurone**, which carries nerve impulses from the brain or spinal cord to a muscle or a gland (the effector).



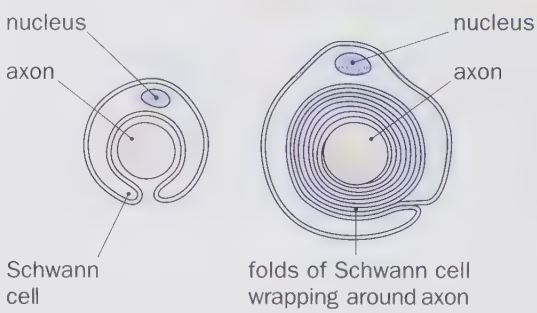
Motor neurone

The motor neurone consists of a **cell body**, which contains a nucleus, a nucleolus and other organelles such as mitochondria and ribosomes.
Many thin cytoplasmic extensions carry impulses ***towards*** the cell body.
These **dendrites** are relatively short and are able to communicate with other neurones.
One particularly long extension carries impulses ***away*** from the cell body.
An **axon** can travel from your spinal cord all the way to your big toe.
The axons of motor neurones form connections with a muscle or a gland at structures called **motor end plates**.
Mitochondria are particularly numerous in the branched ends of the axon where they are involved in the formation of transmitter substances.



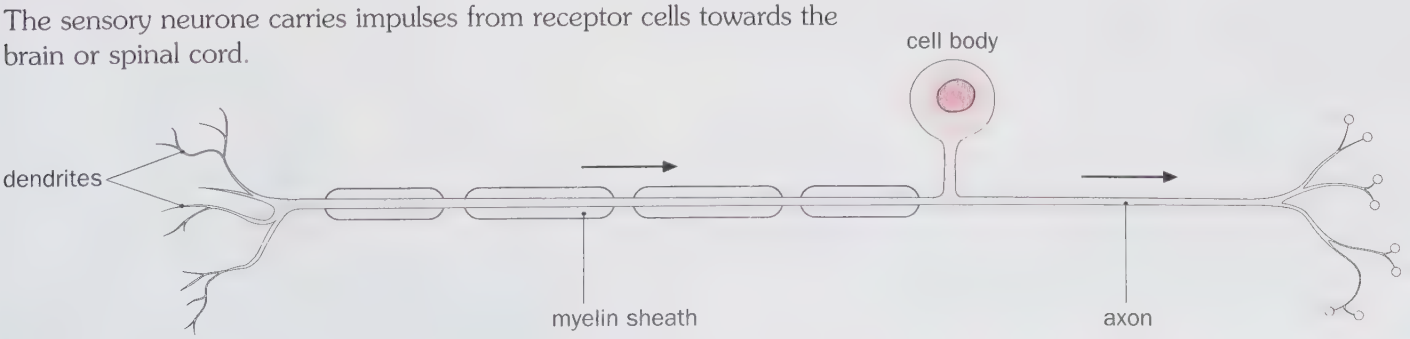
A multipolar neurone

The axon may have a fatty sheath of **myelin** wrapped around it.
If so, it is said to be a **myelinated** axon.
The sheath is formed when **Schwann cells** wrap themselves around the axon along its length.
This results in several layers of fatty myelin surrounding the axon.
Between adjacent Schwann cells are small gaps called **nodes of Ranvier**.
At these points the axon is exposed as there is no myelin present.
The myelin sheath has the effect of increasing the rate of transmission of impulses along the axon.



TS of an axon showing myelin sheath formation

The structure of a **sensory neurone** is similar to that of a motor neurone, the main difference being that a sensory neurone has one long dendrite bringing information to the cell body rather than a long axon taking information away.
The sensory neurone carries impulses from receptor cells towards the brain or spinal cord.



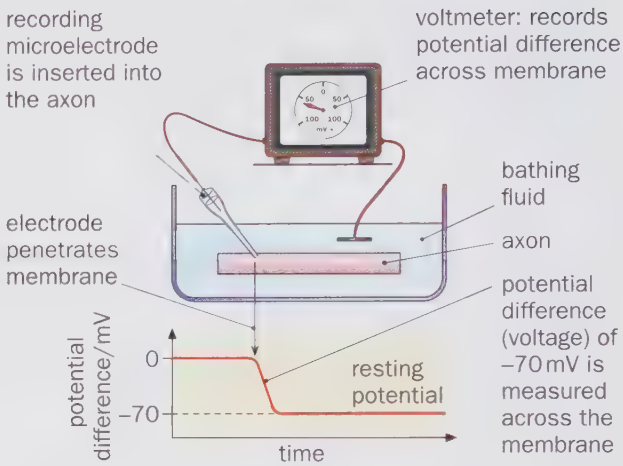
Sensory neurone

Transmission of nerve impulses

Neurones transmit impulses as a series of electrical signals.
These electrical signals pass rapidly along the cell-surface membrane surrounding the axon as a nerve impulse.
This is not the same as an electric current passing along a wire, which is a great deal faster.
Nerve conduction is a specialised development of the excitability that is common to all animal cells.
This mechanism is the same throughout the animal kingdom.

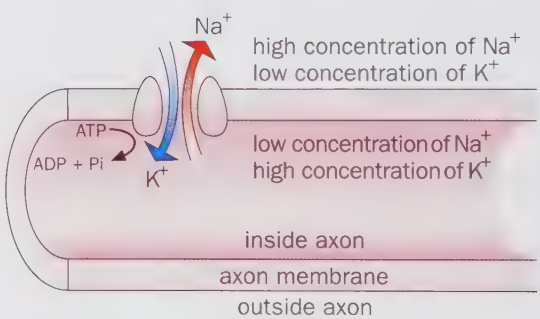
Resting potential

Experiments have been carried out using squid axons.
These are large enough to have microelectrodes inserted into them to measure changes in electrical charge.
The piece of squid axon is placed into a bath of saline solution and two microelectrodes are connected to a voltmeter.
One microelectrode is placed on the outside of the cell-surface membrane of the axon and one is inserted into the axon.
This demonstrates that, in a resting axon, the inside of the membrane has a negative electrical potential compared with the outside. The difference between the two potentials is called the **resting potential** and is about −70 mV.
So the electrical potential on the inside of the axon is 70 mV lower than that on the outside.
In this resting state the axon is said to be **polarised**.



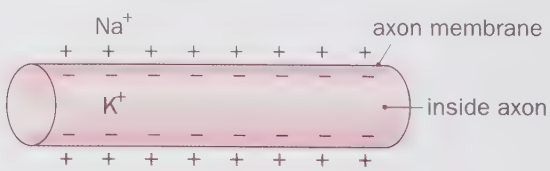
How is the resting potential produced and maintained?
As with most cells, the neurone can maintain an internal composition which is different from the outside.
In the case of neurones, sodium ions (Na^+) and potassium ions (K^+) are transported across the membrane against their concentration gradients by **active transport**.

Carrier proteins in the membrane pick-up Na^+ ions and transport them to the outside.
At the same time, K^+ ions are picked up from the outside and brought across the membrane into the cytoplasm of the axon.
This is known as the **sodium–potassium pump** and, like every active transport system, it relies upon ATP from respiration.



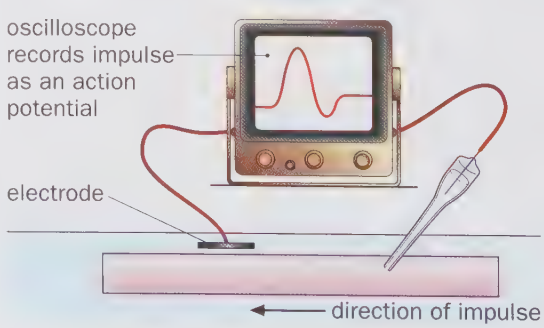
How the resting potential is maintained

But how does the outside of the membrane end up being positive compared with the inside?
The Na^+ ions are passed out faster than the K^+ ions are brought in.
Approximately three Na^+ ions leave for every two K^+ that enter.
Also, K^+ is able to diffuse back out quicker than Na^+ can diffuse back in.
So the net result is that the outside of the membrane is positive compared with the inside.
The resting potential is established and the axon is polarised.



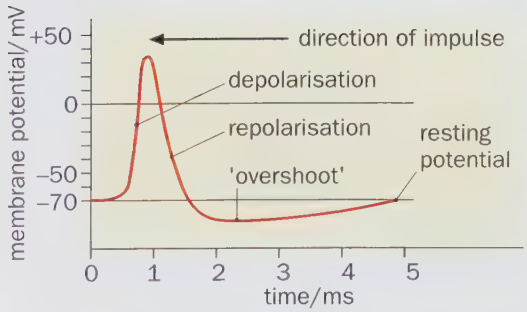
Part of the axon of a resting neurone

If the electrodes from the experiment above are connected to a cathode ray oscilloscope, then it is possible to see the action potential as a peak, before the resting potential is returned.
The action potential is discussed further opposite.



The action potential

A nerve impulse can be initiated in a neurone by mechanical, chemical, thermal or electrical stimulation.
Experiments have tended to use electrical stimuli since their strength, duration and frequency can be controlled and the axons are not damaged.

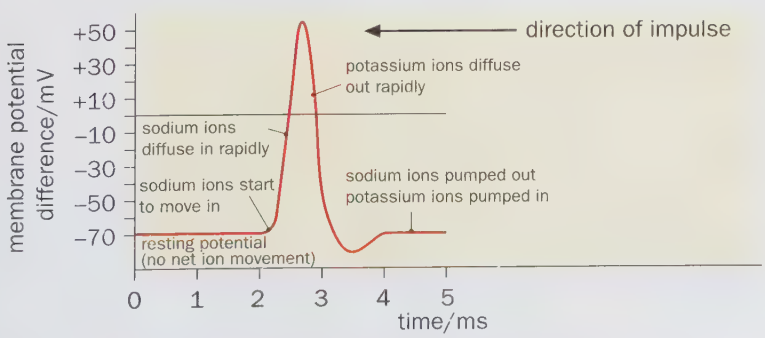


When a small electrical current is applied to the axon, the resting potential changes.
It switches from −70 mV inside the membrane to +40 mV.
So, for a very brief period, the inside of the axon becomes positive and the outside negative.
This change in potential is called the **action potential** and lasts about 3 milliseconds.
When an action potential occurs, the axon is said to be **depolarised**.
When the resting potential is re-established, the axon membrane is said to be **repolarised**.

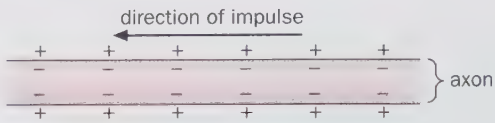
Depolarisation

So what happens to the membrane when it becomes depolarised?
Changes occur in the permeability of the axon membrane to both Na^+ ions and K^+ ions.
When the axon is stimulated, channels open in its cell-surface membrane which allow Na^+ ions to pass through.
Since there is a higher concentration of Na^+ ions outside the axon membrane, they flood in by diffusion.
The Na^+ ions create a positive charge of +40 mV inside the membrane, reversing the resting potential and causing the action potential.

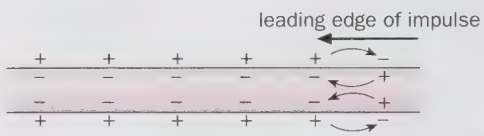
Potassium channels open in the membrane and K^+ ions diffuse out along a concentration gradient, starting off **repolarisation**.
At the same time, sodium channels in the membrane close, preventing any further influx of Na^+ ions.
This re-establishes the resting potential, since the outside of the membrane will become positive again compared with the inside.
We say that the membrane is **repolarised**.
In fact, so many K^+ ions leave that the charge on the inside of the membrane becomes more negative than it was originally.
This shows up as an 'over-shoot' on the oscilloscope.
The potassium channels close and the sodium–potassium pump starts again, restoring the normal concentration of Na^+ and K^+ ions either side of the membrane.
This re-establishes the resting potential.



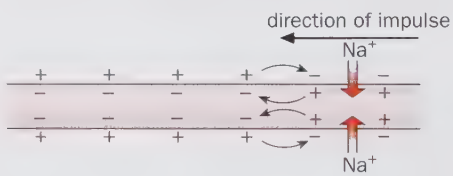
Ion movements during passage of an action potential



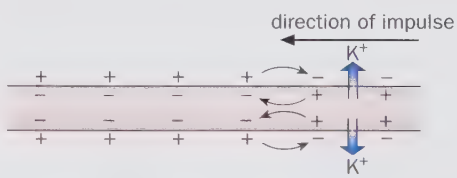
a) In the resting axon, there is a high concentration of Na^+ ions outside and a high concentration of K^+ ions inside. But the net effect is that the outside is positive compared with the inside giving the resting potential.



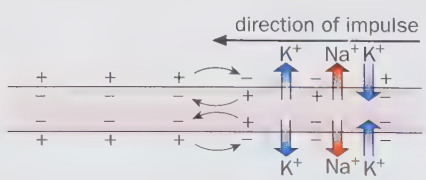
b) The axon is stimulated producing an action potential, setting up local circuits on the axon membrane.



c) Na^+ ions rush into the axon along a diffusion gradient depolarising the membrane and causing an action potential.



d) As the action potential passes along the axon K^+ ions diffuse out along a concentration gradient, starting off the process of repolarisation.



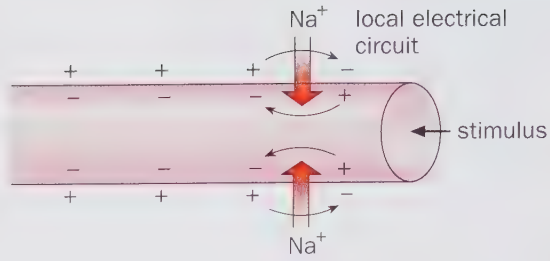
e) The sodium-potassium pump is re-established, fully repolarising the membrane.

Transmission of an impulse

Progress of an impulse

You have seen how an action potential can be started.
But how does it travel ***along*** an axon from one region to the next?

As you have seen, when a nerve impulse reaches any point on the axon, an action potential is generated.
Small local circuits occur at the leading edge of the action potential.
Na^+ ions move across the membrane towards negatively charged regions.
This excites the next part of the axon and so the action potential progresses along its length.
The local circuits effectively change the potential of the axon membrane, creating a 'new' action potential ***ahead*** of the impulse.

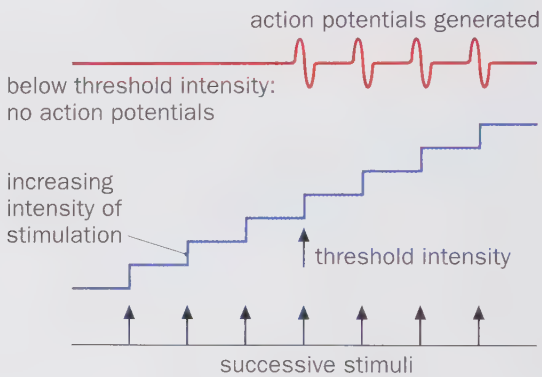


The passage of an impulse

The all or nothing law

One of the properties of neurones is that an action potential can only be generated if the stimulus reaches a certain **threshold intensity**.
Below this threshold, no action potential can be created.
Once the threshold level is reached, the size of an impulse is independent of the intensity of the stimulus.
So a more intense stimulus will not give a greater action potential.

So how is it that we can distinguish weak and strong stimuli?
The answer is that a strong stimulus produces a greater ***frequency*** of action potentials.
So more action potentials are fired off as the intensity of stimulation increases.
For a weak stimulus, fewer action potentials would be generated.
Also, a strong stimulus is likely to result in action potentials occurring in more neurones than a weak stimulus.



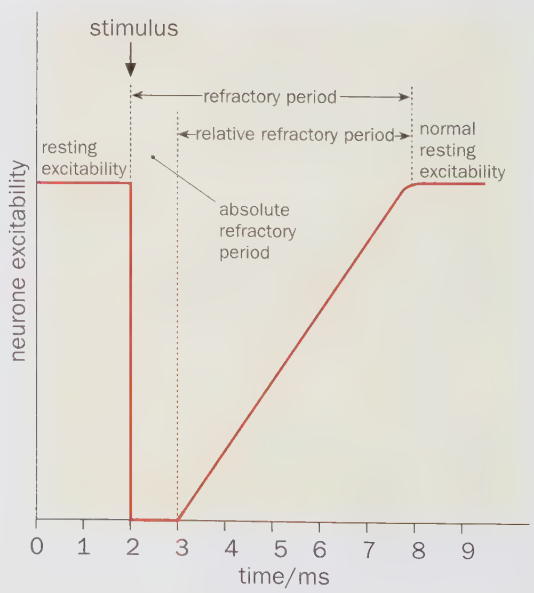
The refractory period

Following the passage of one action potential, there is a time delay before the next one can pass.
This is called the **refractory period** and it lasts a few milliseconds.
During this time the sodium channels in the membrane are closed, preventing the inward movement of Na^+ ions.
This is known as the **absolute refractory period** and another impulse cannot be conducted, no matter how intense the stimulus.
The absolute refractory period lasts about 1 millisecond.

After this, the membrane starts to recover as potassium channels open.
Even though it is not fully repolarised, an action potential can occur if the stimulus is more intense than the usual threshold level.
This time of reduced excitability can last a further 5 milliseconds and is known as the **relative refractory period**.

The importance of the refractory period is that it ensures that

- impulses can only flow in one direction along an axon, since the region of axon behind the impulse cannot be depolarised,
- it limits the frequency at which successive impulses can pass along an axon.



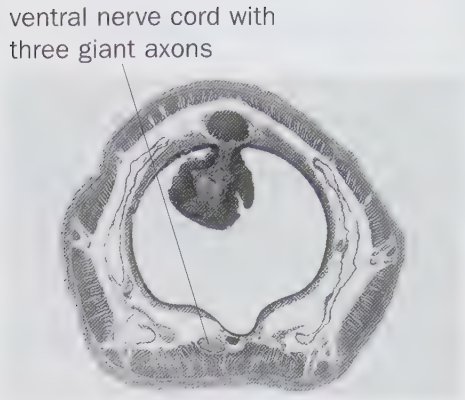
Speed of transmission

Myelinated neurones are able to transmit action potentials at a speed of up to $100\,m\,s^{-1}$.
In unmyelinated neurones, the transmission speed is much slower at $1–3\,m\,s^{-1}$.

The speed of transmission of an impulse depends upon the axon diameter and the myelin sheath.

- **Axon diameter**

The thicker the axon, the faster the rate of transmission of an impulse.
This is thought to be due to the greater surface area of axon membrane over which exchange of ions can occur.
Giant axons are found in a number of invertebrates, including earthworms, marine annelids and crustaceans.
They are thought to be associated with rapid escape responses, since rapid transmission of impulses between receptors and muscles helps to withdraw the animal from danger.



TS of an earthworm to show ventral nerve cord

- **The myelin sheath**

Myelin speeds up the rate of transmission by insulating the axon.
Myelin is a fatty substance which does not allow Na^+ ions or K^+ ions to pass through it.

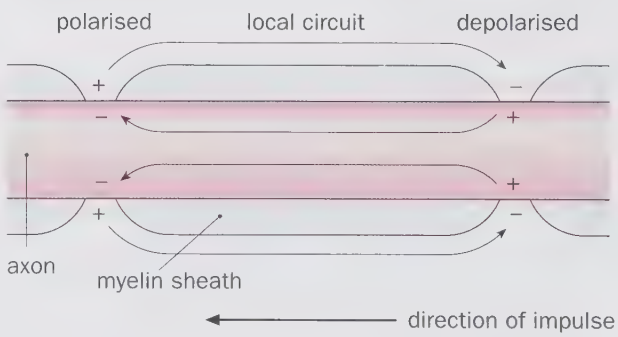
So depolarisation and action potentials cannot occur in those areas of the axon that are myelinated.
They can, however, occur at the **nodes of Ranvier**, so in myelinated axons, the action potential 'jumps' from one node to the next.
This can increase the speed of transmission by up to 100 times.

This is known as **saltatory conduction** and is only found in the myelinated axons of vertebrates.
Saltatory comes from the Latin *saltare*, which means to leap.

False colour TEM of myelinated nerve fibre

Saltatory conduction has the advantages of

- increasing the speed of impulse transmission (human myelinated axons transmit impulses over a 100 times faster than unmyelinated axons),
- myelinated axons also have the effect of conserving energy. As the sodium–potassium pump operates only at the nodes, fewer ions have to be transported across the membrane to restore the resting potential.



Saltatory conduction along a myelinated axon

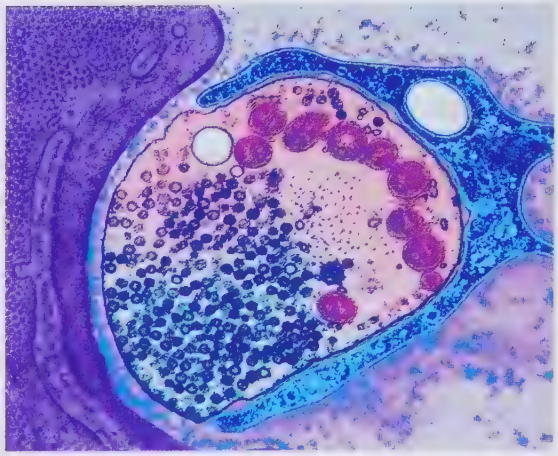
Metabolic poisons have been used in experiments on nerve axons.
Dinitrophenol inhibits the sodium–potassium pump and prevents transmission of an impulse along a nerve.
However, after washing, the poisoned axons transmit impulses if treated with ATP.
This is clear evidence that restoring the resting potential is an energy-requiring process, relying upon ATP from respiration.
Anything that affects the rate of respiration, such as temperature, will affect the transmission rate in a nerve.

The synapse

A **synapse** is where two neurones ***functionally*** meet.
When two neurones meet, they do not touch.
There is a small gap, about 20 nm wide.
The gap is called the **synaptic cleft**.
The neurone that carries the impulse to the synapse is called the **presynaptic** neurone.
The neurone that carries the impulse away from the synapse is called the **postsynaptic** neurone.

So how is information transferred across the synapse from one neurone to the next?
Chemicals known as **neurotransmitters** are released by the presynaptic cell and diffuse across the synaptic cleft to trigger an action potential in the postsynaptic cell.

Motor neurones have specialised synapses with muscles called **neuromuscular junctions**.

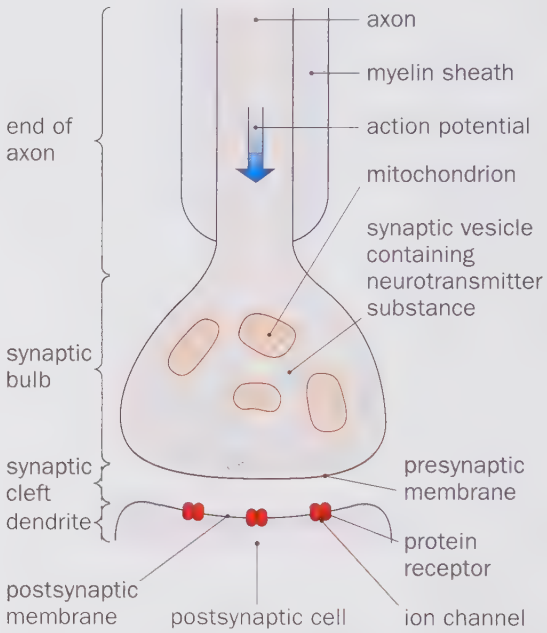


TEM of a synapse showing the synaptic cleft and synaptic vesicles

Structure of the synapse

The axons of neurones end in swellings called **axon terminals** or **synaptic bulbs**.
The surface of the synaptic bulb is called the **presynaptic membrane**.
It is separated by the synaptic cleft from the **postsynaptic membrane** of the cell body or dendrite of the next neurone.
The postsynaptic membrane contains many channels through which specific ions can pass.
The postsynaptic membrane has a number of large protein molecules on its surface, which act as receptor sites for the neurotransmitter substance.

If you look at the diagram showing the structure of a synapse, you will see that a number of mitochondria are present in the synaptic bulb.
This should suggest to you that active transport is involved in synaptic transmission.
Also present in the synaptic bulb are a number of **synaptic vesicles**.
These vesicles contain the neurotransmitter substance, which is released into the synaptic cleft on the arrival of an impulse.



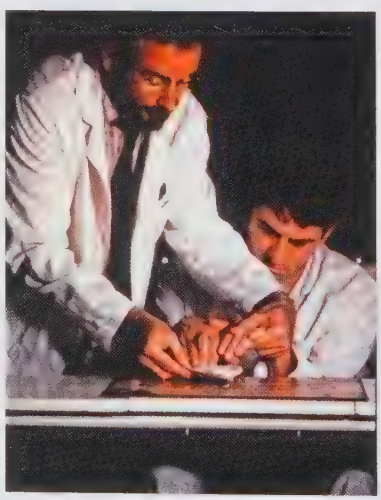
The structure of a synapse

A number of different neurotransmitters are produced by the nervous system.

Dopamine and **serotonin** are neurotransmitters that are active in the brain.
We need only be concerned with the main two neurotransmitters that occur in the body, **acetylcholine** and **noradrenaline**.
Neurones that release acetylcholine are said to have **cholinergic** synapses.
Neurones releasing noradrenaline have **adrenergic** synapses.

Whilst looking at the mechanism of synaptic transmission, we will concentrate on cholinergic synapses and the transmitter acetylcholine.
You can find out the effects that different chemicals have on synaptic transmission on page 362.

In 1969, Dr Oliver Sacks used a dopamine-derived drug to successfully treat post-encephalitis patie s. Unfortunately the effects were only temporary.
The story provided the for the film 'Awakenings'.

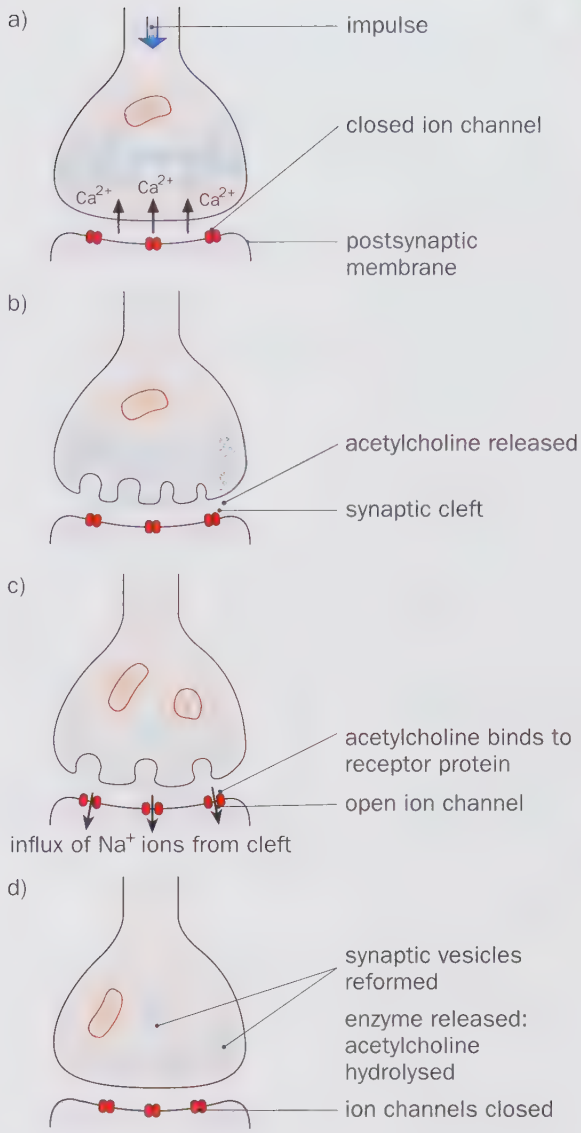


Robin Williams and Robert De Niro in the film 'Awakenings'

Synaptic transmission

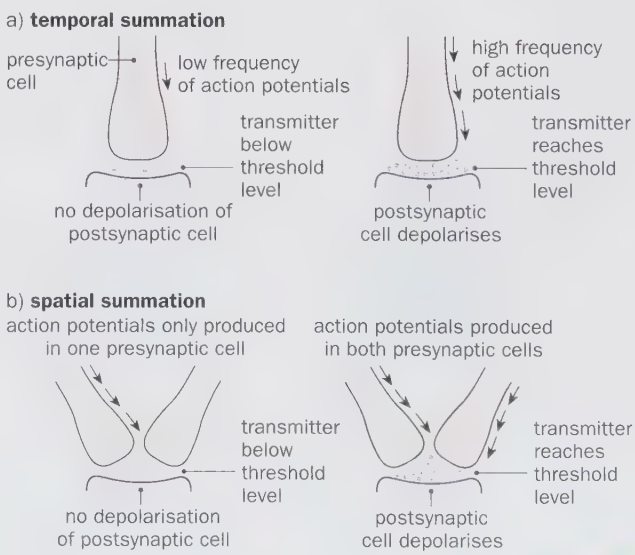
The following description shows how acetylcholine brings about transmission at a synapse.

- When an action potential arrives at a synaptic bulb it causes calcium channels to open in the presynaptic membrane. Since the concentration of calcium ions (Ca^{2+}) is many times greater in the synaptic cleft than inside the synaptic bulb, the Ca^{2+} ions rush in.
- This influx of Ca^{2+} ions causes vesicles containing acetylcholine to move towards the presynaptic membrane. The vesicles fuse with the presynaptic membrane, releasing the neurotransmitter into the synaptic cleft.
- The released acetylcholine diffuses across the synaptic cleft and attaches to specific receptor sites on the postsynaptic membrane. These protein receptor sites have a complementary shape to that of acetylcholine but the binding is only temporary.
- The binding of the neurotransmitter to the receptor sites opens up sodium channels in the postsynaptic membrane. Na^+ ions flood in, depolarising the membrane and creating an action potential.
- If acetylcholine stayed bound to the receptor sites on the postsynaptic membrane, then the sodium channels would remain open, continually producing action potentials. This is prevented from happening by the enzyme **acetylcholinesterase**, which is present in the synaptic cleft. This splits acetylcholine into acetate and choline. The choline is taken up by the presynaptic cell and combined with acetyl coenzyme A to reform acetylcholine. Hence the presence of mitochondria in the synaptic bulb.



Functions of the synapse

- Each action potential that arrives at the presynaptic membrane will cause a number of vesicles to release their transmitter. A number of action potentials are required before there is enough transmitter (**the threshold level**) to initiate an action potential in the postsynaptic cell. This is called **temporal summation**.
- A number of presynaptic neurones may form synapses with one postsynaptic neurone. Action potentials arriving in each presynaptic neurone will release transmitter, which builds up to the threshold level and triggers a postsynaptic impulse. This is called **spatial summation**.
- Since synaptic vesicles are present only in the synaptic bulb of the presynaptic neurone, then impulses can only pass across a synapse in ***one*** direction.
- The events at the cholinergic synapse described above show what happens at an **excitatory synapse** in a nervous response. But some synapses respond to the neurotransmitter by opening potassium channels and keeping sodium channels closed. Potassium diffuses out and this makes it more difficult for the postsynaptic membrane to be depolarised. These are called **inhibitory synapses**. Inhibitory synapses are involved in the stretch reflex. In this case, motor neurones controlling muscles that are antagonistic to the one being stimulated are inhibited.



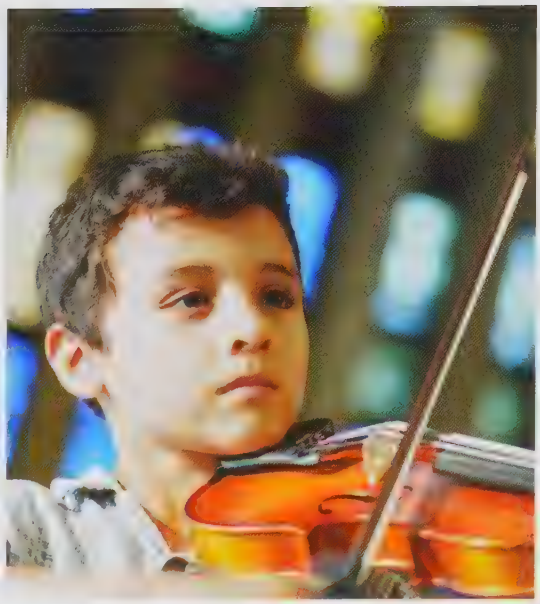
Receptors

Animals are able to detect internal and external changes.
They have specialised cells that are sensitive to particular stimuli.
These **receptor** cells are able to convert stimuli into electrical impulses in nerve cells.
This process of converting one form of energy, such as light or sound, into the electrochemical energy of an action potential is known as **transduction**.

There are five main categories of receptor, depending on the nature of the stimulus:

- **chemoreceptors** detect chemical stimuli when we taste or smell a particular substance,
- **photoreceptors** detect light rays,
- **thermoreceptors** detect changes in temperature,
- **mechanoreceptors** detect pressure, movements and vibrations,
- **electroreceptors** detect electrical fields and occur mainly in fish.

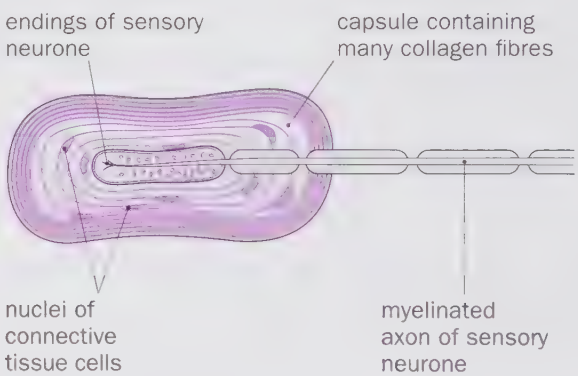
So receptors are only able to respond to ***specific*** stimuli.
Let's look at an example of a mechanoreceptor and a photoreceptor.



Which receptors are being stimulated here?

The Pacinian corpuscle

The Pacinian corpuscle is a mechanoreceptor found in the dermis of the skin.
It consists of the ending of a single sensory neurone, surrounded by several layers of connective tissue that make up the **capsule**.
When pressure is exerted on the capsule, it becomes squeezed out of shape.
This deforms the sensory nerve ending inside it, causing sodium channels in the membrane to open up.
An influx of Na^+ ions into the sensory nerve ending causes its membrane to depolarise and create a **generator potential**.



A Pacinian corpuscle

The greater the pressure applied to the Pacinian corpuscle, the greater is the deformation and the more stretch-mediated sodium channels open up and the greater the depolarisation.

Once the generator potential reaches a certain **threshold level**, an action potential is generated and transmitted along the axon.

Below the threshold level, only local depolarisation occurs and this is insufficient to create an action potential.
This has the effect of cutting out minor mechanical stimuli.
The threshold level is exceeded when summation of generator potentials occurs and this triggers an action potential.
Greater pressure upon the Pacinian corpuscle results in an increase in the frequency of the action potentials produced.

Exceeding the threshold level!

The eye

The human eye is a complex organ containing photoreceptors. It is able to

- control the amount of light that enters it,
- refract (bend) light rays in order to focus them,
- transduce light energy into action potentials.

Structure of the eye

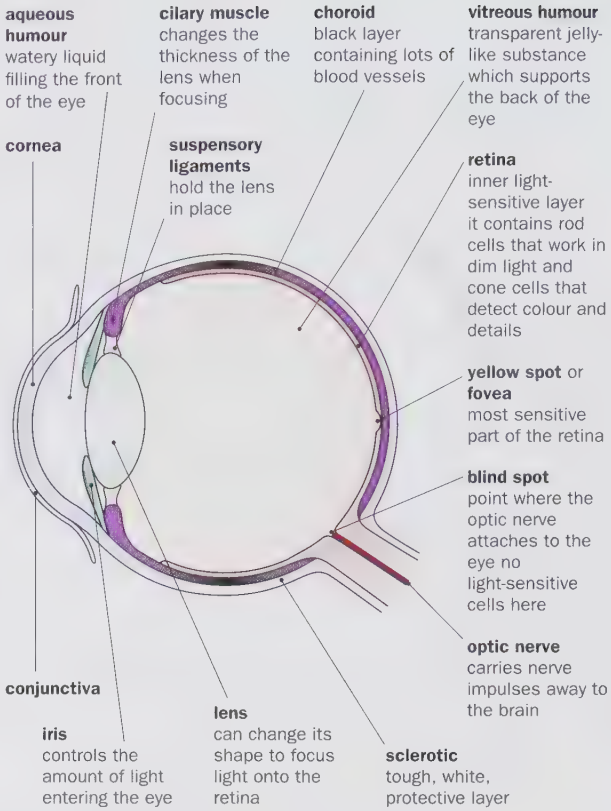
The eye is a spherical structure held in a protective, bony socket in the skull called the **orbit**.
It can be rotated in the orbit by two pairs of **rectus muscles** and one pair of **oblique muscles**.
These muscles are attached to the tough, opaque outer layer of the eye, the **sclerotic**.
The main role of the sclerotic is protection and to keep the eye in shape, under the pressure of its fluid contents.
At the front of the eye it forms the transparent **cornea**, which refracts the light rays entering.
Over the surface of the cornea is the thin, transparent **conjunctiva**.
This is continuous with the eyelids and helps to protect the cornea.
Tears produced by the **lachrymal gland** lubricate these two layers.

Inside the sclerotic is the highly pigmented **choroid**, which prevents internal reflection of light.
The choroid has a rich blood supply to nourish the layer of light-sensitive cells of the retina, which is attached to it.
At the front of the eye, the choroid forms the **iris**.
This contains involuntary muscle with both radial and circular fibres.
If you look at the diagram, you can see how the antagonistic action of these two sets of fibres controls the size of the **pupil** and so regulates the amount of light entering the eye.

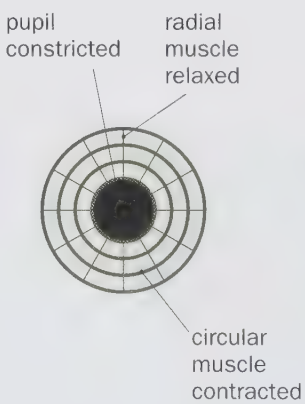
Behind the pupil is the **lens**, which is made out of transparent protein enclosed in a capsule attached to the **suspensory ligaments**.
The **ciliary muscles** control the tension on the suspensory ligaments and so are able to change the shape of the elastic lens.
This focuses rays of light onto the light-sensitive **retina**.

The retina is the innermost layer of the eye.
It contains the light-sensitive **rods** and **cones**, which convert light rays into nerve impulses that leave the eye along the **optic nerve**.
The **fovea** or yellow spot is a focal point on the retina which contains only cones.
The **blind spot** is the point where the optic nerve leaves the eye and there are no light-sensitive cells here.

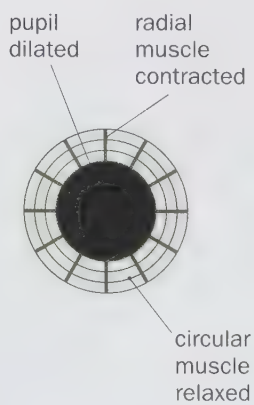
The eye is divided into two chambers. The one in front of the lens contains a transparent liquid called **aqueous humour**.
The second chamber lies behind the lens and is filled with transparent, jelly-like **vitreous humour**, which helps to maintain the eye's shape.



Vertical section (VS) through the human eye

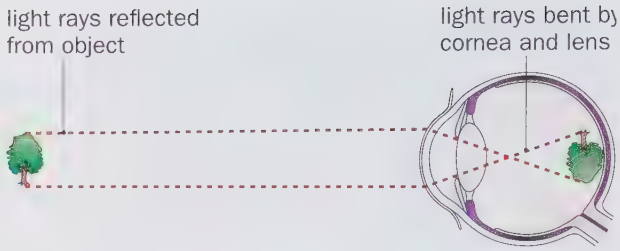


Front view of iris and pupil in bright light



Front view of iris and pupil in dim light

Focusing light onto the retina

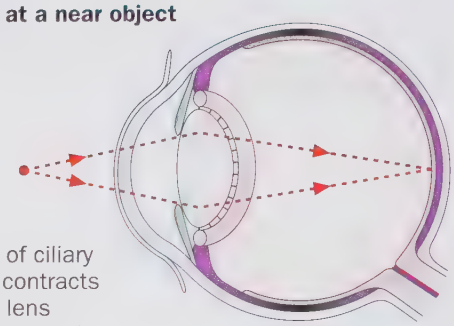


If you don't focus your camera correctly, you end up with a blurred photograph.
Your eye also has to focus incoming light rays if it is to produce a clear image on the retina.
As light rays enter the eye, they are **refracted** (bent) to focus them onto the retina. Most of the refraction is carried out by the cornea.
But the amount of refraction needed varies depending on how far away the object is from the eye.
Light rays from an object close to the eye need to be refracted more than distant objects.
The elastic lens changes its shape to focus close objects onto the retina. This process is called **accommodation**.

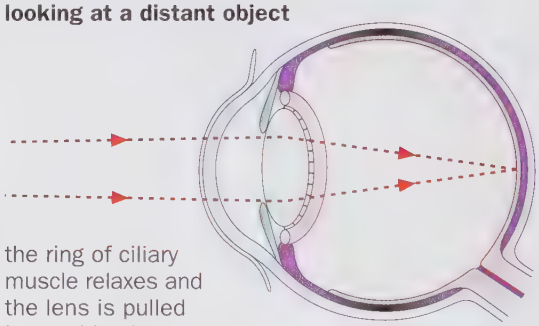
When viewing close objects, the lens becomes more biconvex and bulges. This is because the ring of ciliary muscle contracts to close the aperture around the lens.
The suspensory ligaments anchoring the lens to the ciliary muscle slacken, so the lens assumes a more spherical shape under its own elasticity. This shape of lens focuses close objects onto the retina and the eye is said to be **accommodated**.

When viewing distant objects, the lens is thin (elliptical).
In this condition, the ring of ciliary muscle relaxes, widening the aperture around the lens.
This tightens the suspensory ligaments, which pull the lens out into a thinner shape.
A thin lens focuses distant objects clearly onto the retina and the eye is said to be **unaccommodated**.

looking at a near object



looking at a distant object



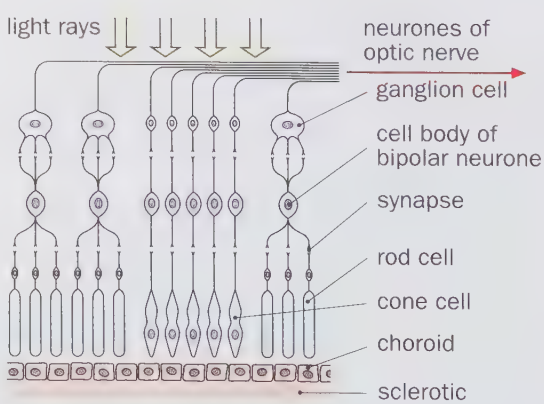
The retina

This delicate inner layer of the eye contains the **photoreceptor** cells.
There are two types: the **rods** and the **cones**.

Both transduce light energy into chemical energy.
Each contains a photochemical pigment in its outer segment.
The cones are high light intensity colour receptors that are located mainly in the yellow spot.
There are no rods here and the fact that the cones are so closely packed together (about $125\,000\,mm^{-3}$) gives them high definition.

If you look at the diagram, you will see that each cone has its own single connection with the optic nerve.
So for each cone stimulated, an impulse passes to the brain.
This gives cones **high visual acuity**.
On the other hand, you will see that many rods connect with a single bipolar neurone. A bipolar neurone has ***two*** extensions coming out of the cell body. One is a dendrite and the other is an axon.
So impulses from a number of rods summate before triggering an impulse in the bipolar neurone, giving **low visual acuity**.
This characteristic of rods is called **retinal convergence**.
Rods are distributed more or less evenly over the rest of the retina, though they are highly concentrated in the periphery.

Notice that light rays have to pass through the nerve network before reaching the photoreceptors, producing an **inverted retina**.



Structure of the retina

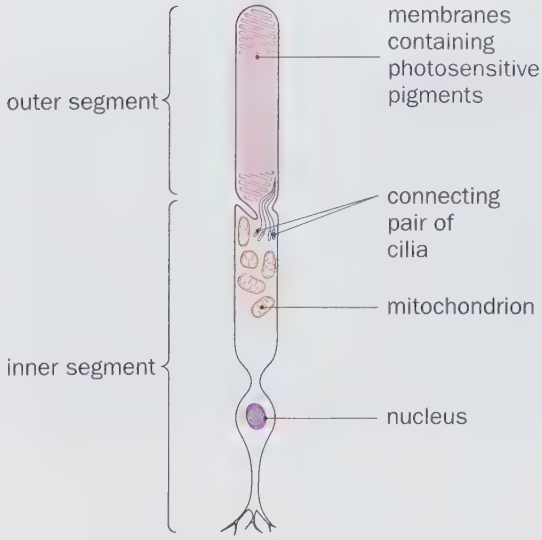
Photoreception

Rods and cones are composed of an inner and an outer segment.
The outer segment is made up of numerous membranes stacked on top of each other, rather like the thylakoids in the granum of a chloroplast.
These membranes carry a photosensitive pigment: **rhodopsin** in the case of rods and **iodopsin** in cones.
The inner segment of each photoreceptor cell contains the cell's nucleus and many mitochondria.

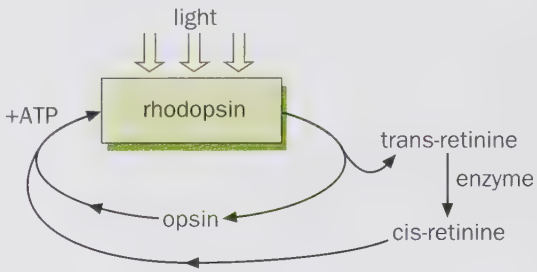
Rods are sensitive to low light intensity.
Their photochemical pigment rhodopsin, or **visual purple**, is made up of a protein, **opsin**, and a light-absorbing component, **retinine**.
Retinine is derived from vitamin A.
When light strikes a molecule of rhodopsin, it splits into opsin and trans-retinine.
This process is called **bleaching** and results in depolarisation of the membrane of the rod.
A generator potential is created and an impulse passes to the brain.

Before it can be stimulated again, a rod has to resynthesise rhodopsin.
The mitochondria provide energy in the form of ATP to resynthesise rhodopsin from opsin and cis–retinine.

Cones are high light intensity colour receptors.
Their visual pigment, iodopsin, is less easily broken down and takes longer to be resynthesised.
In bright light, most of the rhodopsin in the rods is bleached and the eye is said to be **light-adapted**.
Vision is poor if the retina is exposed to dim light, as it takes time for the rods to resynthesise rhodopsin and regain their response.
This is why your eyes need time to adjust if you enter a cinema on a sunny day.
Once your rods have resynthesised rhodopsin, your retina is said to be **dark-adapted**.



The structure of a rod

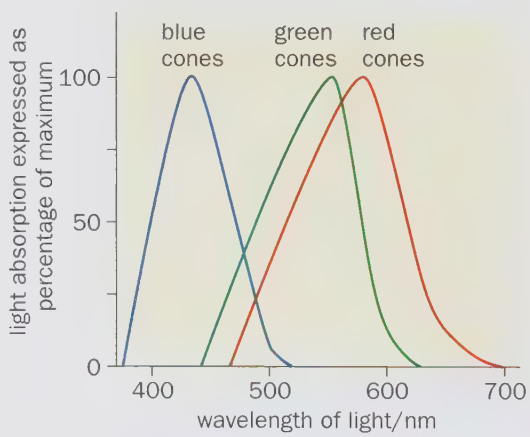


The breakdown and regeneration of rhodopsin

Colour vision

The **trichromatic theory** of colour vision suggests that there are three different types of iodopsin located in three different types of cone.
Each distinct type of cone responds to one of blue, green or red light.
The graph shows the extent of stimulation of each type of cone at different wavelengths of light.
Can you see that the graphs overlap? So light with a wavelength between blue, green and red stimulates a combination of cones.
For instance, yellow light stimulates equal numbers of red and green cones and the brain interprets the impulses as yellow.
Orange light stimulates more red than green cones and the brain interprets these impulses as orange.

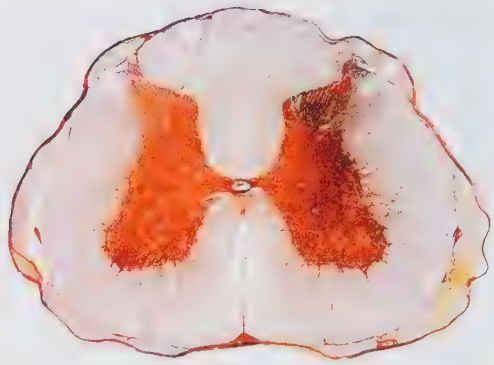
Deficiency of one or more cone types produces **colour blindness**.
Because the graphs overlap, absence of red cones means that it is still possible for green cones to detect green, yellow, orange and red.
But the brain cannot distinguish between these colours properly as there are no impulses from red cones with which to contrast impulses from green cones.
This **red–green colour blindness** is relatively common.



Can you see these numbers ...?

The spinal cord

The **spinal cord** is a hollow tube running from the base of the brain to the end of the spine.
Together with the brain it makes up the central nervous system (CNS).
The spinal cord is protected by the vertebrae which make up the backbone.
Pairs of **spinal nerves** branch off the spinal cord in the gaps between neighbouring vertebrae.
Each pair of spinal nerves contain sensory neurones bringing impulses to the CNS and motor neurones carrying impulses away.



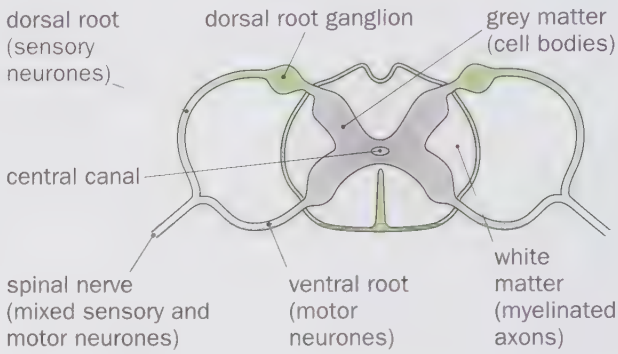
Photomicrograph of a TS of the spinal cord

When viewed in section, the spinal cord can be seen to consist of two distinct areas.

- The central **grey matter** containing the cell bodies of relay and motor neurones.
- The outer **white matter** containing myelinated axons, which run up and down the spinal cord to and from the brain.

In the centre of the grey matter is the **spinal canal**, through which the nutritive **cerebrospinal fluid** circulates.

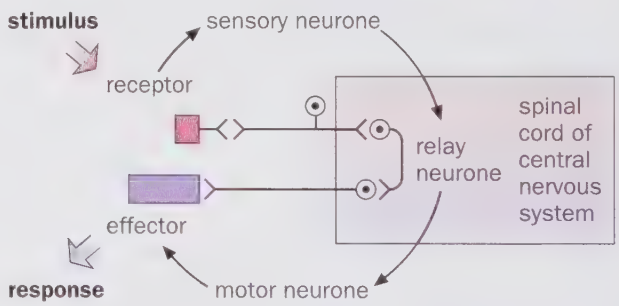
Spinal nerves join and leave the spinal cord through the dorsal and ventral roots.
Sensory neurones enter the spinal cord via the dorsal root and the concentration of their cell bodies forms a swelling called the **dorsal root ganglion**.
Motor neurones leave the spinal cord via the ventral root.



TS of the spinal cord

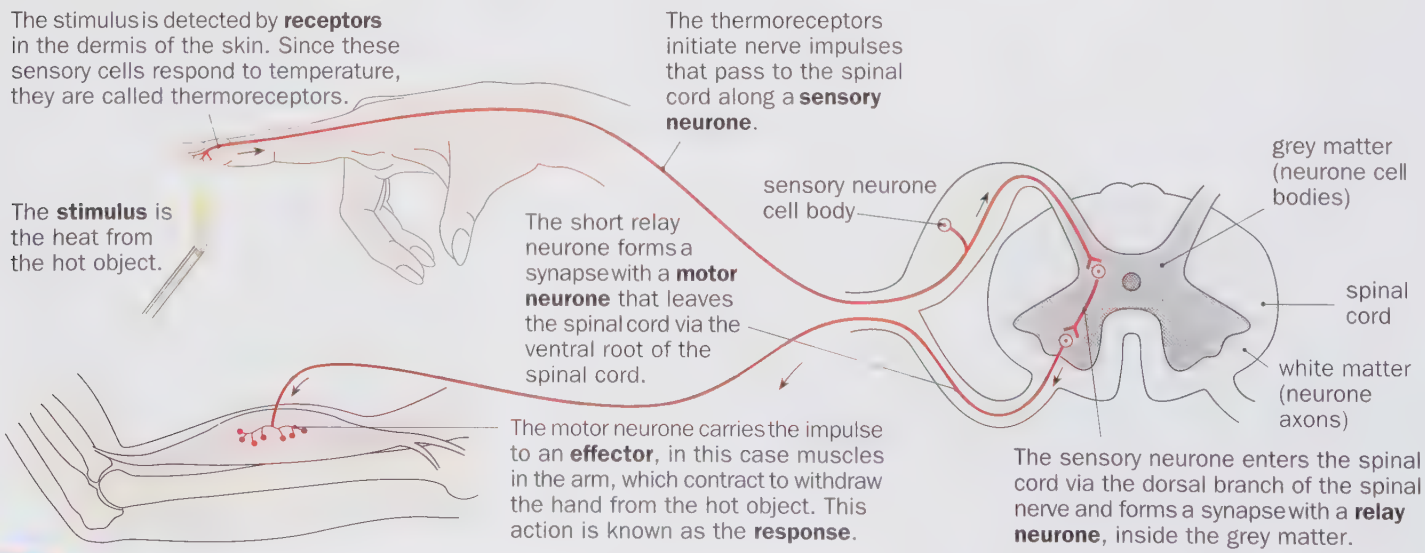
Spinal reflexes

A **reflex** is an automatic, rapid response to an adverse stimulus.
For instance, when you remove your hand from a hot object.
The action is involuntary in that the brain is not involved in the actual response, though it may be informed of the event.
Many reflexes are protective, but as you have seen in earlier chapters, complex actions such as swallowing, coughing and blinking are also coordinated by reflexes.
The neurones that are involved in a reflex make up a **reflex arc**.



A simplified reflex arc

Let's look at a withdrawal reflex arc.

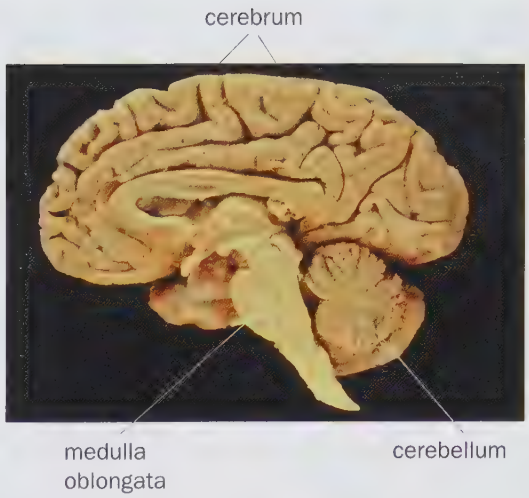


The brain

Like the spinal cord, the brain is made up of grey and white matter. In this case, the white matter is on the inside and the grey matter, made up of cell bodies, is located in the outer **cortex**.
The brain has spaces continuous with that in the spinal cord. These cavities are called **ventricles** and contain cerebrospinal fluid.

During the development of the CNS, the brain forms as a swelling at the end of the spinal cord.
This swelling soon forms three distinct regions: the **forebrain**, the **midbrain** and the **hindbrain**.
It is not easy to see this division in humans because the roof of the forebrain (the **cerebrum**) has grown massively to completely cover the midbrain and the hindbrain.

The brain is surrounded by three protective membranes called **meninges**, and is of course encased by the skull.



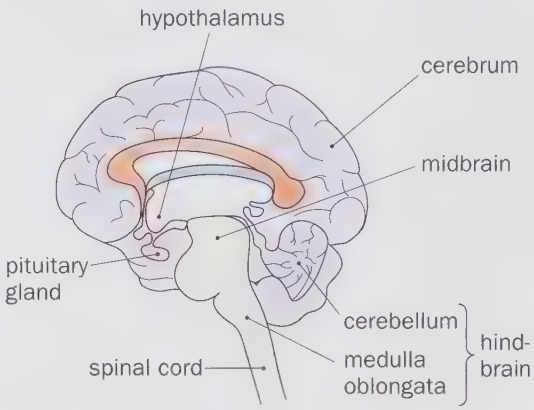
VS through the human brain

Functions of the brain

The brain is a complex organ to study, and we will only look at the major parts and their associated functions.

The **medulla oblongata** (or just **medulla**) is part of the hindbrain that controls many reflex actions of the body.
The **vagus nerve** leaves the medulla, carrying impulses that affect heart rate (see Chapter 10), breathing rate (see Chapter 8), blood pressure and peristalsis of the gut.
The medulla also controls reflexes such as swallowing, coughing and the secretion of saliva.

The **cerebellum** is also part of the hindbrain. It receives sensory information from muscles and the ears.
It is concerned with posture, body movement and balance.
The cerebellum coordinates smooth body movements such as walking, dancing and riding a bike.

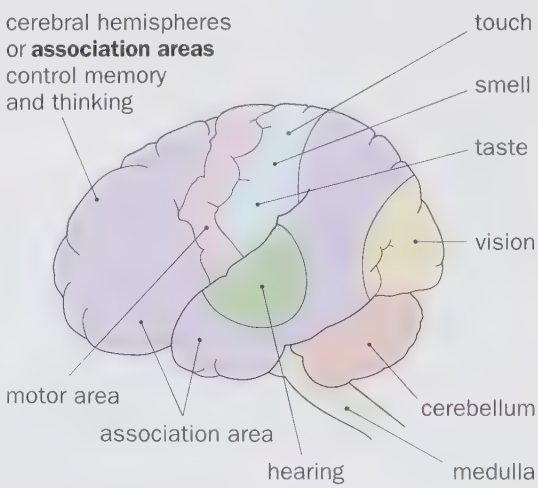


VS through the centre of the human brain

The **hypothalamus** is found at the base of the forebrain.
It is the main controlling centre for the autonomic nervous system.
As you have seen, the hypothalamus monitors the blood passing through it and so regulates body temperature and blood composition.
The hypothalamus also regulates the activities of the pituitary gland, which itself influences the actions of other endocrine glands.

The hypothalamus therefore provides an important link between the nervous system and the endocrine system.

The **cerebral hemispheres** form the cerebrum, or roof, of the forebrain.
The cerebral hemispheres receive sensory information, interpret it in terms of previous experience and send out motor information to bring about the appropriate responses.
In this way, all the voluntary actions of the body are coordinated.
The cerebral hemispheres are the site of such complex faculties as learning, reasoning, intelligence, personality and memory.
War injuries and operations on brain tumours have enabled scientists to map out many of the sensory areas and motor areas of the cerebral cortex.



Different parts of the brain have different jobs to do

The endocrine system

Endocrine glands are tissues and organs that secrete hormones into the blood.
Endocrine means that the gland produces hormones that are secreted directly into the bloodstream rather than into a duct.
Hormones travel throughout the body but only certain organs or tissues recognise each hormone and respond to it.
These are referred to as **target organs**.
Each hormone alters the activity of its target organs.

The adrenal glands

The adrenal glands are located immediately on top of the kidneys.
Each consists of two parts, the outer **cortex** and an inner medulla.
Their hormones are chemically and functionally quite different.

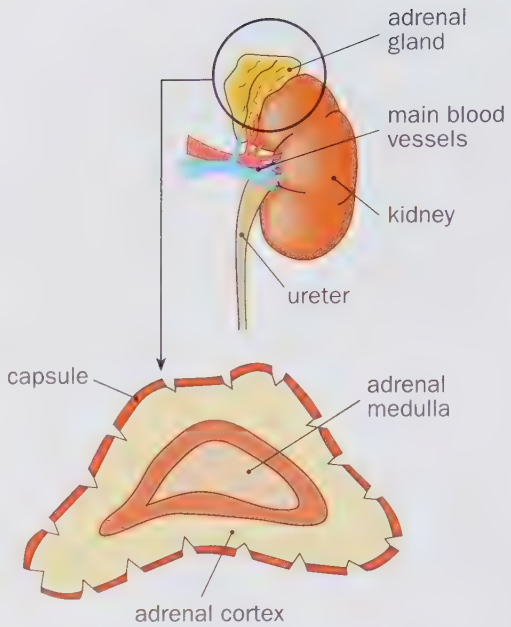
Adrenal cortex

The adrenal cortex secretes hormones that are called **corticoids**.
They are steroids and are formed from cholesterol.
Being lipid-based, corticoids are able to pass directly through cell-surface membranes.

- **Glucocorticoids**
  These are involved in the metabolism of glucose and amino acids.
  The most important is **cortisol** (hydrocortisone) which is produced in response to stress.
  It is essential if the body is to cope with almost any kind of stress such as injury, shock, pain, exposure to cold and infection.
  The production of cortisol, along with other glucocorticoids, is triggered by the hypothalamus which stimulates the anterior pituitary to secrete **adrenocorticotrophic hormone (ACTH)**.
  This in turn, causes the adrenal cortex to increase the release of glucocorticoids which:
  - raise blood glucose level, by inhibiting insulin and by the formation of glucose from fats and protein (gluconeogenesis),
  - promotes glycogenolysis, which also raises blood sugar by the breakdown of glycogen in the liver and muscles,
  - suppresses the immune system.

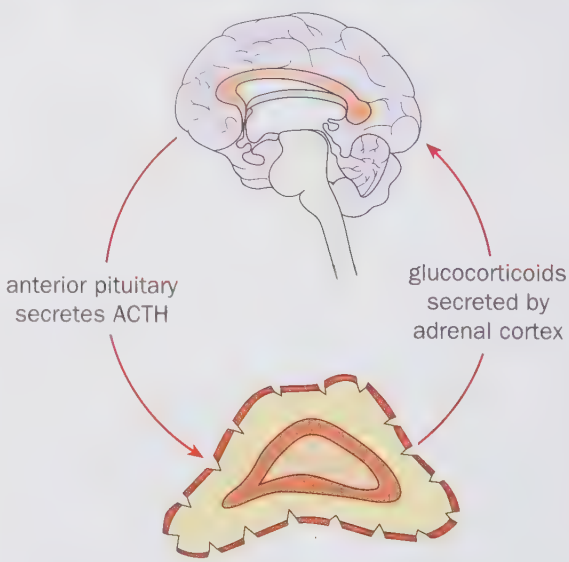
Almost every cell in the body has receptors on its surface for cortisol.

- **Mineralocorticoids**
  This is a group of hormones that includes **aldosterone**.
  This influences water retention in the body by regulating the distribution of sodium and other mineral ions in the tissues.
  Aldosterone targets the distal convoluted tubules and collecting ducts of the kidney, where it causes increased reabsorption of Na^+ ions and increased excretion of K^+ ions and chloride ions.
  So Na^+ ions are retained and K^+ ions are excreted in the urine.
  Aldosterone acts by causing the kidney to conserve both Na^+ ions and water.



Section of the adrenal gland to show cortex and medulla

Cortisone injections can be given to athletes to provide short-term pain relief



The hypothalamus is able to monitor the level of glucocorticoids in the blood and adjust the level of ACTH produced (negative feedback loop)

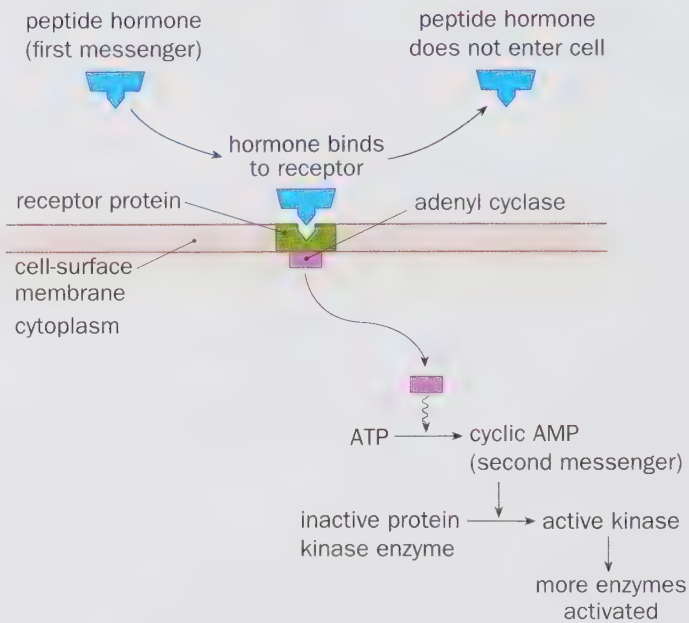
Adrenal medulla

The adrenal medulla is the central part of the adrenal gland.
It has evolved from part of the sympathetic nervous system and it is under direct control of the brain.
It secretes two hormones, **adrenaline** and noradrenaline (see page 169), and both are important in preparing the body for action.

Adrenaline is released during times of excitement fear or stress.
Often called the 'flight or fight' hormone, adrenaline helps the body to prepare for action in the following ways.

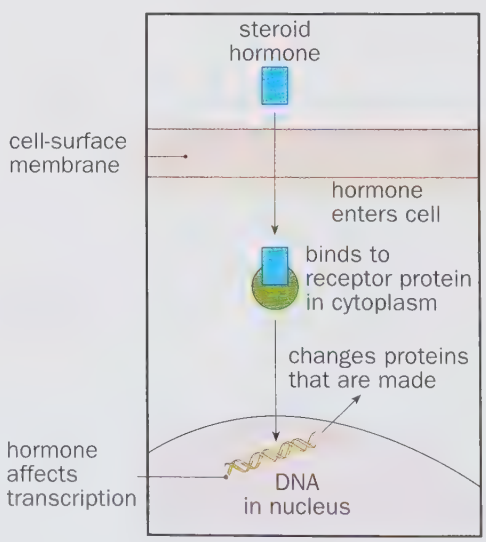
- The liver cells convert glycogen to glucose, which diffuses into the blood. This makes more glucose reach the muscles as a source of energy for the rapid muscle contractions needed for sudden action.
- The heart rate and the volume of blood pumped out with each beat increases, so that more glucose and oxygen are delivered to the muscles for energy release.
- Dilation (widening) of the bronchioles ensures that more air reaches the alveoli in the lungs for quicker gas exchange.
- Vasodilation of the arterioles in the brain and muscles delivers more glucose and oxygen to these organs.
- Vasoconstriction of the arterioles in the gut and other organs allows blood to be diverted to the muscles.
- Hairs are raised ('goose pimples' in humans). This makes furry animals look larger to deter attackers.
- The pupils of the eyes are dilated to increase the range of vision and bring about greater perception of visual stimuli.

The production of adrenaline has evolved to protect us and other animals from danger.
If too much adrenaline is produced, as a result of prolonged stress, constant high blood pressure and heart disease may result.
Beta-blockers are drugs that have been developed to combine with adrenaline and so reduce its effects.



Action of a peptide hormone (first messenger → second messenger)

Hormones fall into two groups: **peptide hormones** (small proteins), for example adrenaline and glucagon, and **steroid hormones**, for example sex hormones like oestrogen, progesterone and testosterone.
These two groups have different mechanisms of action within target cells.
Peptide hormones are not lipid-soluble and cannot get into cells through the lipid cell-surface membrane.
Instead they act as **first messengers** and bind to the target receptors on the outside of the membrane.
This binding activates an enzyme, **adenyl cyclase**, which is found on the inside surface of the membrane.
This activated enzyme converts ATP into **cyclic AMP**, which is the **second messenger** that moves into the cytoplasm activating existing enzymes.
Steroid hormones, being lipid-soluble, move through the membrane to bind with **target receptors** inside the cytoplasm.
The hormone-receptor complex enters the nucleus and binds directly to the DNA affecting transcription, and modifying the mRNA that is formed.
A steroid hormone is capable of either switching on or switching off protein synthesis of particular genes.



Action of a steroid hormone

The effectors: muscles

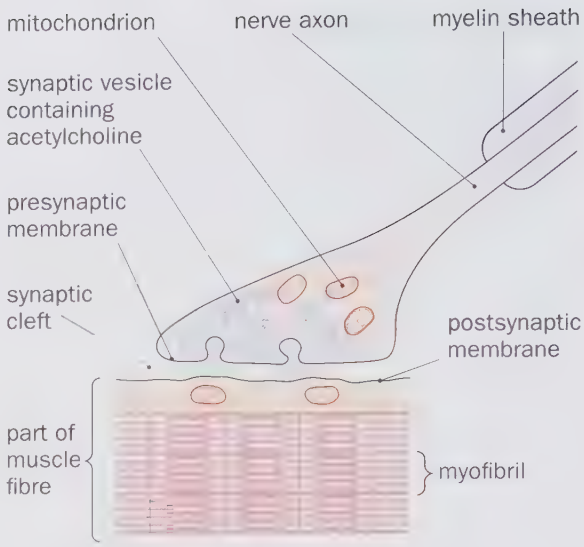
There are three types of muscle.

Cardiac muscle is found only in the heart. As you saw in Chapter 10, it is ***myogenic*** – that is, it initiates its own contractions. (See page 166 for the structure of cardiac muscle.)

Smooth muscle is often called **involuntary muscle**, because it is not under conscious control.
It is under the control of the autonomic nervous system and is found in the walls of the gut (see page 151), blood vessels, ureters, bladder, urethra and uterus.

Skeletal muscle is often called **voluntary muscle**, since it is under conscious control, or **striated muscle**, because of its appearance.
It is this type of muscle that we shall be looking at in detail.

As its name suggests, skeletal muscle is attached to the skeleton by non-elastic tendons.
Skeletal muscles occur in antagonistic pairs.
Contraction of skeletal muscle enables us to carry out movements.
See page 261 for slow-twitch and fast-twitch skeletal muscles.



A neuromuscular junction

Muscle structure

An individual skeletal muscle is made up of hundreds of **muscle fibres**.
Within each muscle fibre are numerous **myofibrils**, which are thin threads that run the length of a muscle fibre and have a characteristic striped appearance which is visible using an electron microscope.

Each myofibril is made up of alternating light and dark bands, because each myofibril is composed of overlapping strands of contractile protein called **myosin**, and smaller protein strands called **actin**.

Each contractile unit within the myofibril is called a **sarcomere**.
It is the way in which the myosin and actin filaments overlap that gives each sarcomere its banded appearance.

If you look at the diagrams you will see that there is a region where the actin and myosin filaments overlap, giving a dark **A band**.

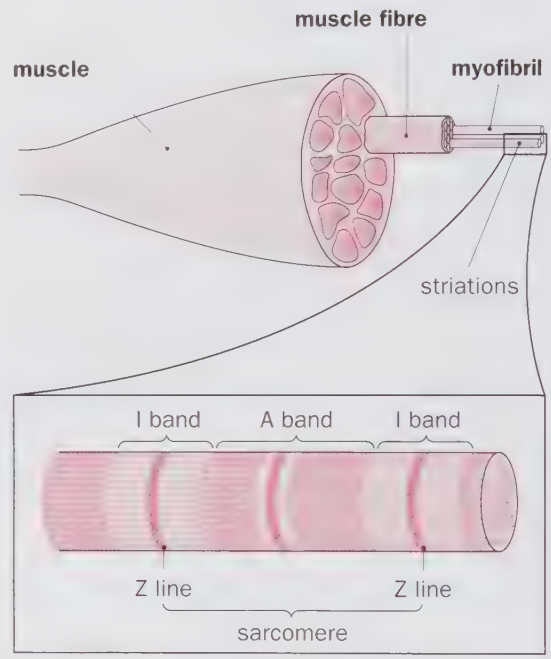
Between the dark bands are lighter bands where only actin is present.
This region is called the **I band**.

Within each dark A band you can see that there is a region made up of only myosin filaments. This is called the **H zone**.

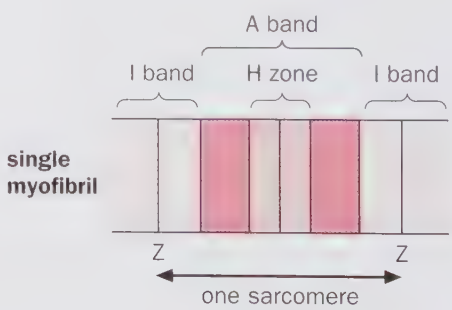
In the centre of each I band is the **Z line**.
The Z line marks the end of one sarcomere and the beginning of the next. The sarcomere is the basic unit of muscle contraction.

Around each myofibril is a network of small tubes containing Ca^{2+} ions.
These have a major part to play in the contraction of skeletal muscle.

Actin filaments also contain two other proteins that are involved in the mechanism of muscle contraction.
These are called **troponin** and **tropomyosin**.



The detailed structure of a muscle

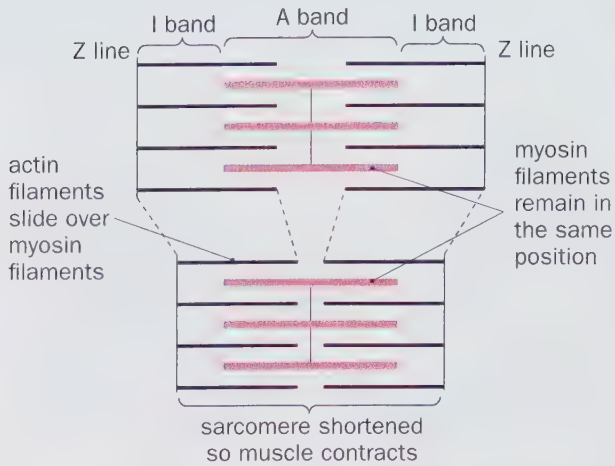


detail of one sarcomere

How do muscles contract?

When a muscle contracts, the actin filaments slide over the myosin filaments.
This brings about the following changes that can be observed in a sarcomere under the electron microscope:

- the I bands become shorter,
- the A band does not change in length,
- the Z lines become closer together, so the sarcomere shortens,
- the H zone becomes narrower.



Changes in a sarcomere during muscle contraction

The sliding filament theory of muscle contraction

So how do the actin filaments slide over the myosin filaments when a muscle contracts?

- A nerve impulse reaches the neuromuscular junction.
- Synaptic vesicles are released (in the same way as in a synapse), and the transmitter (acetylcholine) diffuses across and depolarises the membrane of the muscle fibre, producing an action potential.

- Ca^{2+} ions are released from the system of tubes into the muscle fibres.
- The Ca^{2+} ions bind with troponin and alter its shape.
- Troponin is now able to displace tropomyosin, which has been blocking its binding site on the actin filaments.

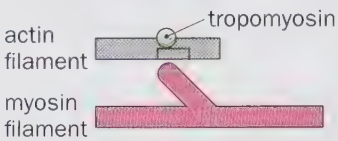
- This enables the myosin heads to attach to the actin filaments, using energy provided by ATP.
- As the myosin heads attach to the actin binding sites, they tilt, causing the actin filaments to slide past the myosin filaments.

- As the actin filaments move, the myosin heads become detached, and then attach to the next binding site on the actin filaments.
- Ca^{2+} ions are actively absorbed back into the system of tubes.

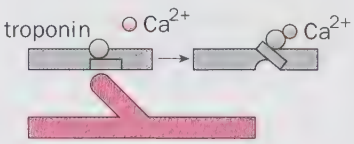
- Troponin changes back into its original shape and so allows tropomyosin to once again block the binding sites on the actin filaments.
- The way in which the muscle contracts is known as the **ratchet mechanism**.

The roles of ATP and phosphocreatine in providing energy for muscle contractions can be found on page 260.

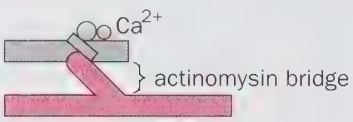
1. When the muscle is relaxed, the binding sites on the actin are covered by tropomyosin.



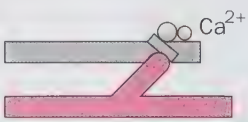
2. When the membrane of the muscle is depolarised Ca^{2+} ions are released from the tubes and bind with the troponin which displaces tropomyosin from the binding site.



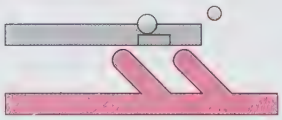
3. The myosin head binds to the actin, using energy from ATP and forming an actinomysin bridge.



4. As the myosin heads attach to the actin filaments they tilt causing the actin filaments to slide past.



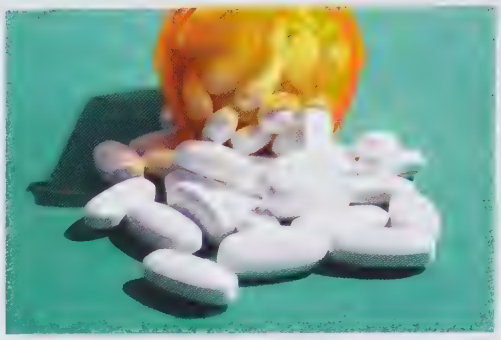
5. As the actin filaments move past, the myosin heads become detached and attach to the next binding site. Troponin reverts to its original shape and once again tropomyosin blocks the binding site on the actin filaments.



Biology at work: The effect of drugs on synaptic transmission

Most drugs that affect the nervous system do so by influencing the transmission of nerve impulses across synapses. In some cases (for example, amphetamines) the drug affects the release of the neurotransmitters. Other drugs (for example, beta-blockers) modify the effects that the neurotransmitters have on the postsynaptic membrane.

Drugs affecting the nervous system can also be categorised as either ***excitatory*** or ***inhibitory*** in their effects. Excitatory drugs amplify synaptic transmission whilst inhibitory drugs decrease the process.



Excitatory drugs

Amphetamines are a group of excitatory drugs which also suppress the appetite.
Their original medical use was in treating obesity but they are much less widely prescribed now because patients may develop a dependency.
Amphetamines stimulate the release of neurotransmitters such as noradrenaline.
This has the effect of increasing brain activity, making a person more alert.
Due to this stimulant effect, amphetamines are liable to abuse and their prescription is controlled by legislation.
Caffeine also leads to the release of more noradrenaline.

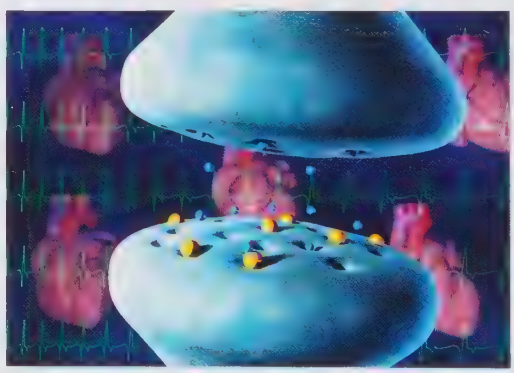
MDMA more commonly known as Ecstasy

Inhibitory drugs

Beta blockers are an example of inhibitory drugs.
They are principally used to treat heart disease, although they can also be used to reduce the physical symptoms of anxiety, such as palpitations and tremors.
They work by blocking the so-called **beta receptors**.
These are specific sites where neurotransmitters bind.
The beta-1 receptors are found in heart muscle and the beta-2 receptors in tissues such as the lungs and blood vessels.

By blocking these receptors, beta blockers inhibit the binding of neurotransmitters such as adrenaline and noradrenaline.
The normal effect of these chemicals is to increase cardiac output, increase airflow in the lungs and dilate blood vessels.

Drugs such as beta blockers are known as **antagonists** because they interfere with the normal action of a transmitter.
Drugs that produce a similar effect to a transmitter are known as **agonists**.



A computer generated image of beta blockers (yellow) blocking the receptors (dimples) for the hormone adrenaline (blue)

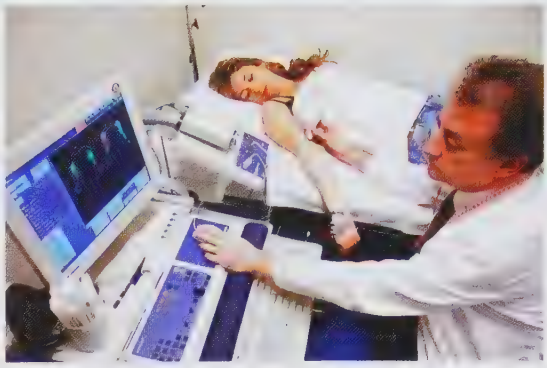
Heroin is an antagonist, with a chemical structure similar to **endorphins**.
Endorphins are made naturally in the brain and provide relief when the body experiences pain or stress.
Endorphins work by flooding the synapses in the brain and preventing neurones from transmitting impulses from pain receptors.
When a person takes heroin, the heroin molecules bind to the endorphin-receptor sites on the postsynaptic membrane of the synapse, blocking nerve transmission. This mimics the function of natural endorphins.

Biology at work: Motor neurone disease

Motor neurone disease (MND) occurs when motor neurones in the brain and spinal cord degenerate.
MND is characterised by the following progressive symptoms:

- twitching and cramping of muscles in the hands and feet,
- weakness in the muscles of the arms and feet,
- slurred speech (dysarthria),
- increasing body paralysis,
- shortness of breath and difficulty in swallowing which eventually leads to death.

Intellect and memory are usually not affected, nor are the senses, which are dependent on **sensory neurones** to relay impulses.
Diagnosis of the disease is difficult and requires a range of tests to eliminate other conditions.
Often an **electromyograph** (**EMG**) is used, in which a needle is inserted into various muscles to measure electrical activity.

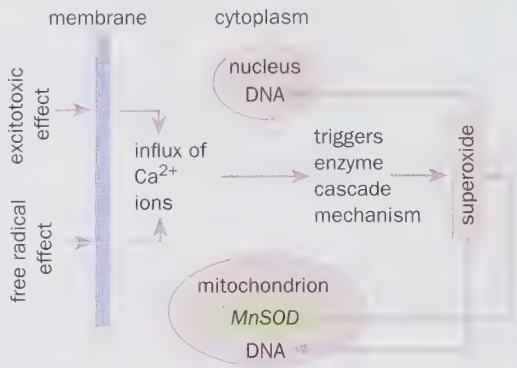


A small needle is inserted through the skin into the muscle. The electrical activity detected by this needle is displayed on a computer

MND affects about 2 in every 100 000 people each year in the UK, and about 50% of patients die within 3 years of their first symptom. Some people may live for up to 10 years and others even longer.

The cause of MND is unclear but research has suggested two possible mechanisms:

- free radical effects,
- excitotoxic effects.



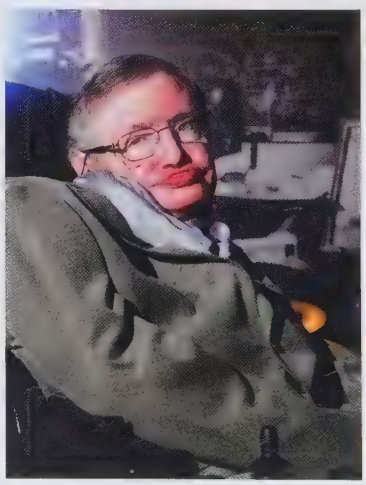
The effects of free radicals and excitotoxicity on the membranes of motor neurones are linked

Free radicals are highly unstable chemicals containing an unpaired electron.
They react with and damage key biological molecules such as nucleic acids.
One possible cause of MND is a mutation in the gene coding for the enzyme **superoxide dismutase** (**SOD**).
This is a detoxifying enzyme found in high concentrations in motor neurone cells.
When working normally it will remove free radicals, but it is thought that the mutated gene reduces the activity of SOD by up to 50%.

Excitotoxicity is the process by which nerve cells are damaged and killed by excessive stimulation by neurotransmitters.
One example of an excitotoxic neurotransmitter is the amino acid **glutamate** and this is found in increased concentrations in the cerebrospinal fluid of MND sufferers.

It is possible that both mechanisms may be linked in bringing about MND.

There is no cure for MND and the only drug that has demonstrated a survival benefit for sufferers is **riluzole**.
This drug (licensed in 1996) slows down the progression of the disease by reducing the sensitivity of motor neurone cells to glutamate.
After 18 months of treatment it may increase survival by 2–4 months on average, but it will not reverse any nerve damage already present.



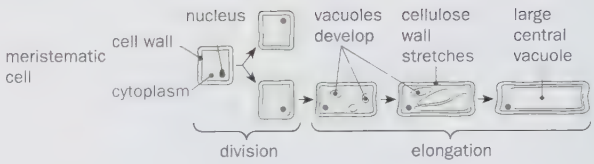
Professor Stephen Hawking, the world famous physicist, has had MND since he was 21-years-old

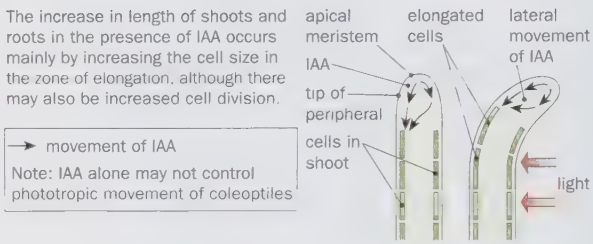
Plant growth substances

Growth in plants is coordinated by **plant growth substances** (**PGS**). These are produced in certain areas of the plant and transported to other parts where they can affect cell division, cell elongation and cell differentiation.
Plant growth substances are not specific and can affect different tissues and organs in contrasting ways.
Different PGS may interact to increase each other's effects, in which case they are said to be **synergic**.
Alternatively PGS may act to decrease each other's effects, in which case they are said to be **antagonistic**.

Auxin

Auxin (**IAA**) was the first growth-promoting substance to be isolated. As long ago as 1880, Charles Darwin noticed that grass **coleoptiles** grew towards the direction of a light source.
A coleoptile covers and protects the embryo leaves and stem apex.
The response of part of a plant to light is called **phototropism**.
Shoots respond by growing towards the light, so are **positively phototropic**.
Roots respond by growing away from the light, so are **negatively phototropic**.



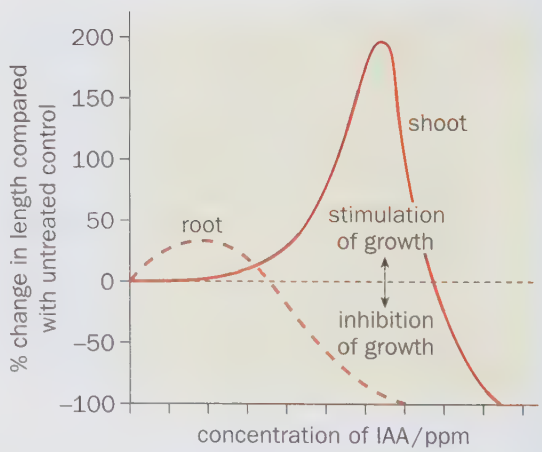


Regions of actively dividing cells are known as **meristems**.
Apical meristems are found at the tips of shoots and roots.
Experiments have shown that when shoots are exposed to light from one direction, IAA is transported to the shaded side.
Here it stimulates cell elongation and the elongated cells cause the shoot to bend towards the light.

Gravitropism (also referred to as geotropism) is the response of a shoot or root to gravity.

If a shoot or root is placed in a horizontal position, then IAA tends to accumulate on the lower side.
In shoots, the IAA stimulates more cell elongation on the lower side and as a result, the shoot bends upwards. It is **negatively gravitropic**.
However in roots, high concentrations of IAA inhibit cell elongation.
So when IAA accumulates on the lower side of a root it slows down the degree of cell elongation compared with the upper side of the root.

The result is that there is greater cell elongation on the upper surface and so the root bends downwards. It is **positively gravitropic**.

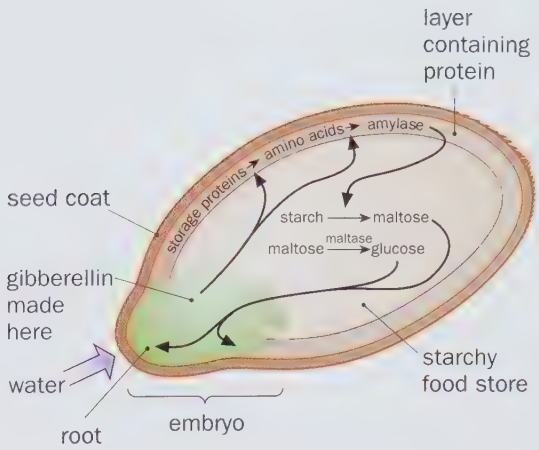


At high concentrations, IAA prevents the growth of lateral shoots.
Many gardeners remove the shoot tips of a plant to make it grow bushier. This is because the uppermost (apical) bud produces IAA, which is transported down the stem and inhibits the growth of lateral buds.
This is known as **apical dominance**.
Normally the IAA produced by the apical bud, passes down the stem and stimulates elongation in the cells just behind the apical meristem, but inhibits the growth of lateral buds. Removal of the apical bud means that the growth of lateral buds is no longer inhibited and so a bushier plant is produced. This effect occurs every time a hedge is clipped.

Gibberellins

Gibberellins were first discovered by Japanese scientists studying a plant disease caused by a fungus.
The active growth substances were isolated from the fungus *Gibberella fujikuroi* in 1935 and called gibberellins after the fungus.

Gibberellins are produced in seeds, young leaves and young apical tissues, particularly in root apices. In cereal grains, gibberellins stimulate the production of the enzyme α-amylase.
After water has been absorbed by the seed, gibberellins diffuse from the seed embryo to a layer where α-amylase is made.
The enzyme hydrolyses the seed's food store, mobilising food that the embryo uses to grow.
Dormancy, a period of low metabolic activity, is broken and germination begins.



The role of gibberellin in seed germination

Gibberellins also affect stem elongation in dwarf plants.
Normal growth is prevented because the gene controlling gibberellin production is switched off.
But if gibberellins are sprayed onto the surface of dwarf plants they grow to their normal height.

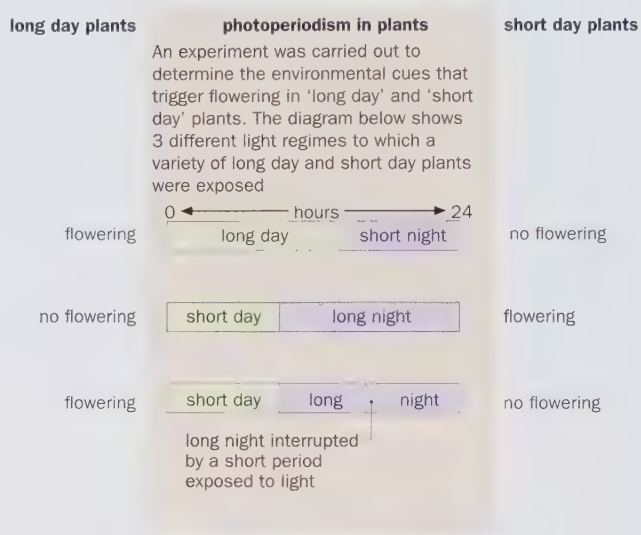
Photoperiodism

Photoperiodism is the response of a plant to the relative lengths of daylight and darkness.
Flowering is affected by the period of illumination (**photoperiod**).
Flowering plants can be classified according to the photoperiod in which they flower.

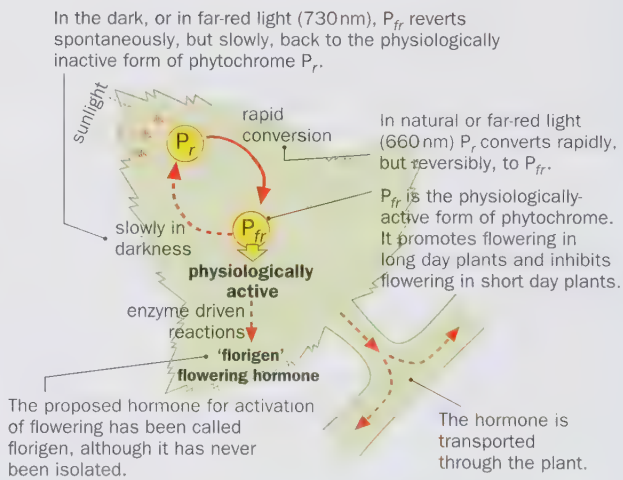
Day neutral plants, such as snapdragons, flower whenever they have grown sufficiently. The photoperiod has no effect.

Long day plants, such as spinach and poppy, only flower if the number of hours of light to which they have been exposed is ***above*** a certain critical level (or alternatively, the number of hours of darkness is ***below*** a critical level).

Short day plants, such as chrysanthemums and strawberries, only flower when the number of hours of light to which they are exposed is ***below*** a certain critical level (or alternatively, the number of hours of darkness is ***above*** a critical level).



The photoreceptor involved in photoperiodism is the light-sensitive pigment called **phytochrome**, which exists in two forms:
phytochrome 660 (P_r) absorbs red light, and
phytochrome 730 (P_{fr}) absorbs far-red light.
On absorbing light of a particular wavelength, each form of phytochrome is converted to the other form.
Sunlight contains much more light of wavelength 660 nm than 730 nm, so during daylight P_r is converted to P_{fr} which accumulates.
P_{fr} is unstable and, during the hours of darkness, slowly reverts back to P_r which accumulates.
The plant measures day length (or rather night length) by the amount of phytochrome existing in each of the two forms. In daylight, P_{fr} predominates.
Flowering in plants is thought to be initiated by a hormone 'florigen'.
In long day plants, a high concentration of P_{fr} is needed for the release of florigen.
In short day plants, a high concentration of P_r elicits the release of florigen.



Biology at work: Commercial applications of plant hormones

Plant hormones are commonly referred to as **plant growth substances** because they are chemicals that modify plant growth.
They can affect cell division, cell elongation or cell differentiation or indeed any combination of these stages of growth.

Plant growth substances, or more commonly their synthetic derivatives called **plant growth regulators**, are widely used in horticulture and agriculture.
They are used to increase both the yield and quality of a crop, as well as to increase the efficiency of harvesting.

Synthetic auxins such as 2,4-D are very effective as **selective weed killers**.
Important cereal crops are narrow leaved (monocotyledonous), whereas most of their competing weeds are broad leaved (dicotyledonous).
Concentrations of IAAs that have a significant effect on broad leaved plants have little effect on the narrow leaved crops.
The IAA increases the growth rate of the affected plants, probably through interfering with protein production.
The plants cannot sustain this rate of growth and they soon die.

Synthetic IAAs are also used in rooting powders because they stimulate the growth of side roots from cut stems.

Ethene is another plant growth substance with important commercial applications.
Its main effect is the stimulation of flowering and the ripening of fruit.
It is used with crops such as pineapples to synchronise fruiting. This makes harvesting a much more efficient process.
It is also widely used to induce ripening of fruit prior to its appearance on supermarket shelves.

Other plant growth substances also have commercial applications.
Cytokinins, for example, are used to extend the life of leafy crops like lettuce.
They achieve this by delaying leaf senescence (ageing).

Some of these chemicals can be used in combination with each other (synergistically).
Cytokinin together with IAA and gibberellin can be used to promote **parthenocarpy**.
This is fruit formation without fertilisation.
The seedless fruits it produces are very popular with consumers.

The yield of a crop can be significantly reduced by competing species

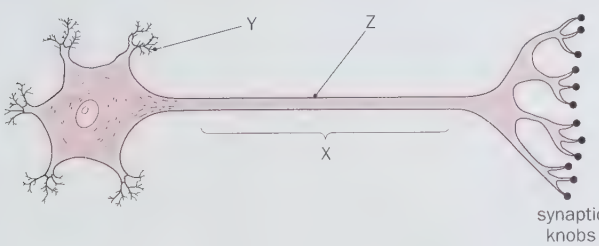
Ethene can induce ripening of bananas

Summary

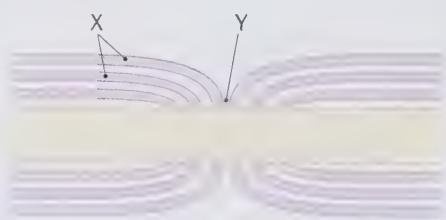
- The nervous system controls and coordinates our actions by detecting stimuli, processing the information and initiating responses.
- The stimuli are detected by receptors and the responses are brought about by effectors.
- Neurones are the basic functional units of the nervous system. Sensory neurones carry impulses to the brain and spinal cord, passing their impulses on to relay neurones, which in turn pass their impulses on to motor neurones.
- At rest, the nerve axon is polarised. The sodium-potassium pump makes the outside of the axon positive and the inside of the axon negative.
- An action potential results in the inside of the axon becoming positive and the outside negative.
- Changes in the permeability of the axon membrane result in an influx of sodium ions and depolarisation before the sodium-potassium pump is re-established and the axon is repolarised.
- An action potential can only be generated if the stimulus reaches a certain threshold intensity.
- Following the passage of an impulse, there is a time delay, or refractory period, before another action potential can be created.
- The speed of transmission of an impulse is affected by the axon diameter and the presence of a myelin sheath.
- A synapse is where two neurones functionally meet. The transfer of information from one neurone to the next relies on the secretion of neurotransmitters such as acetylcholine and noradrenaline.
- The Pacinian corpuscle is a mechanoreceptor found in the dermis of the skin.
- The human eye is a complex organ containing photoreceptors, and able to control the amount of light that enters it, refract light rays in order to focus them, and transduce light energy into action potentials.
- The trichromatic theory of colour vision suggests that there are three distinct types of iodopsin located in three different types of cone.
- A spinal reflex consists of a receptor, a sensory neurone, a relay neurone, a motor neurone and an effector.
- The sliding filament theory provides the basic mechanism for muscle contraction.
- Plant growth substances have various commercial applications, including rooting powders, selective weed killers and stimulating the ripening of fruit.

Questions

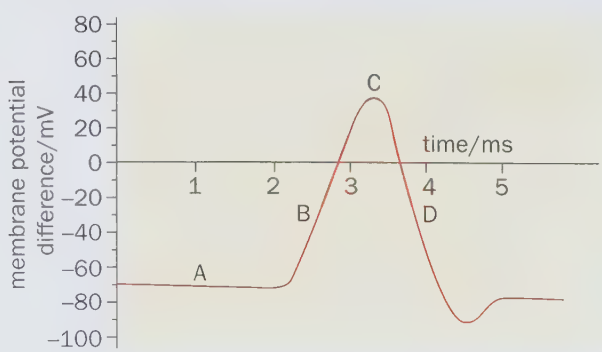
1 a) Name the type of neurone shown in the diagram.
b) Name the structures labelled X and Y.
c) A nerve impulse can be initiated by stimulation with a microelectrode.
What would be the effect of stimulation at point Z?
d) The synaptic bulbs release a chemical transmitter, acetylcholine. Nerve gases prevent the breakdown of this chemical. From this information suggest
i) one early symptom of nerve gas poisoning,
ii) one reason for this observed symptom.



2 a) Name the regions X and Y on the diagram of the nerve axon.
b) What effect does X have on the part of the axon that it covers.
c) What is the main chemical present in the region labelled X?
d) What effect does the distribution of this chemical have on the transmission of impulses along the neurone?



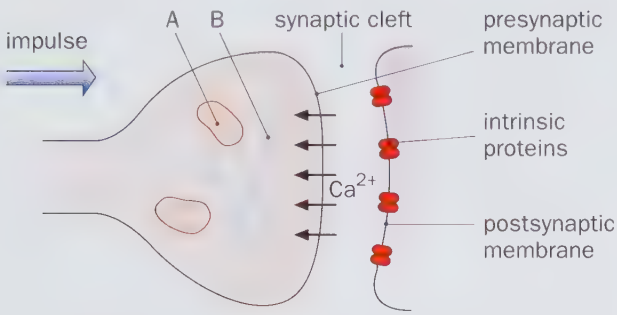
3 The graph shows the change in potential difference at a point in a neurone during the propagation of a nerve impulse.



a) From the graph give the value of
   i) the resting potential of the neurone,
   ii) the maximum change which occurs in the potential difference across the membrane.

b) Explain, in terms of ion movements, the change in potential difference that takes place between
   i) points A and B,
   ii) points C and D.

4 a) Name the following structure in the synapse:
   i) structure A,
   ii) structure B,
   iii) the contents of structure B.



b) The arrival of an impulse changes the permeability of the presynaptic membrane, allowing calcium ions (Ca^{2+}) to rapidly diffuse in as shown by the arrows on the diagram.
Describe the effect caused by this influx of ions.

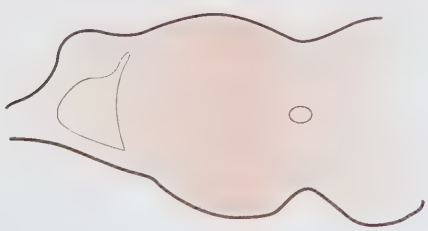
c) Sodium ions (Na^+) also play a vital part in this process.
   i) Use the diagram to name the parts of the synapse that can act as a channel for Na^+ ions.
   ii) Suggest how these channels are opened.

d) i) Describe the effect that their opening has on the postsynaptic membrane.
   ii) Explain fully why structure A is found abundantly in the presynaptic region.

5 When the back of the hand accidentally touches a hot surface, the biceps muscle contracts and the hand is rapidly removed. This is an example of a reflex action involving three neurones.

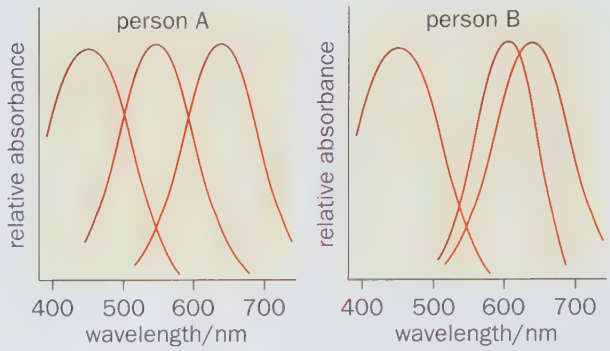
a) i) Explain what is meant by a reflex action.
   ii) Name the effector involved in the above reflex action.

b) The diagram shows a cross-section through the spinal cord.
Copy and complete the diagram, labelling the neurones involved in the reflex action.



6 a) Describe the trichromatic theory of colour vision.

b) It is possible to measure the light-absorbing properties of the pigments in the colour-sensitive cells of the retina. The graphs show the results obtained from two people.
Suggest and explain how the colour vision of these two people would be different.

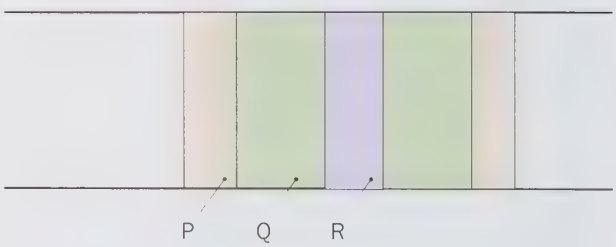


7 The diagram shows the appearance of a sarcomere from a relaxed muscle myofibril, as seen with a microscope.

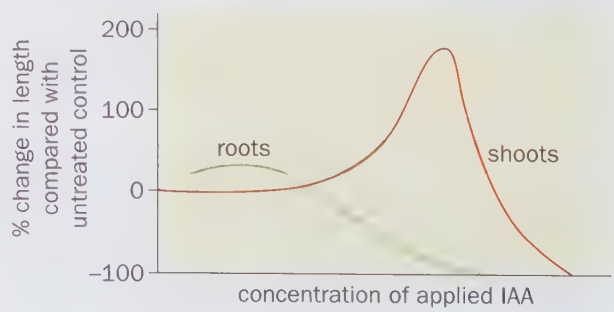
a) Use your knowledge of the sliding filament theory to explain the appearance of each of the bands P, Q and R.

b) Draw a similar diagram to show the appearance of the sarcomere when the myofibril is contracted.

c) Explain what has happened to cause the effect shown in your diagram.

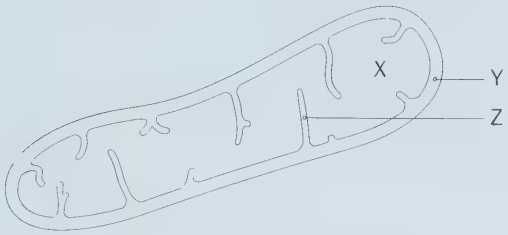


8 The graph shows the effect of applying different concentrations of auxin (IAA) to the roots and shoots of a plant. Use the graph to describe three ways in which the response of the roots to IAA differs from the response of the shoots.



Respiration

1 Aerobic respiration occurs in the cytoplasm and mitochondria of eukaryotic cells.
The diagram below shows a mitochondrion.

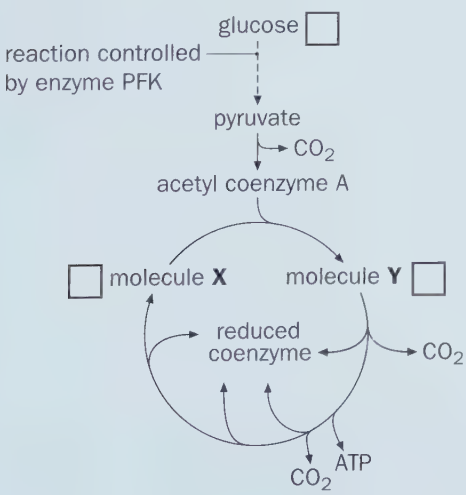


a) Which part of the mitochondrion (A–D) is labelled X?
A stroma
B crista
C cytoplasm
D matrix [1]

b) Parts Y and Z are involved in production of ATP in the mitochondrion. The process is known as:
A decarboxylation
B oxidative phosphorylation
C photophosphorylation
D dehydrogenation [1]

c) Red blood cells do not contain mitochondria. Which of the following processes can take place in red blood cells?
A glycolysis
B Krebs cycle
C link reaction
D production of reduced FAD [1]
[3]

2 The diagram below shows part of the respiratory pathway. The broken arrows represent intermediate stages.



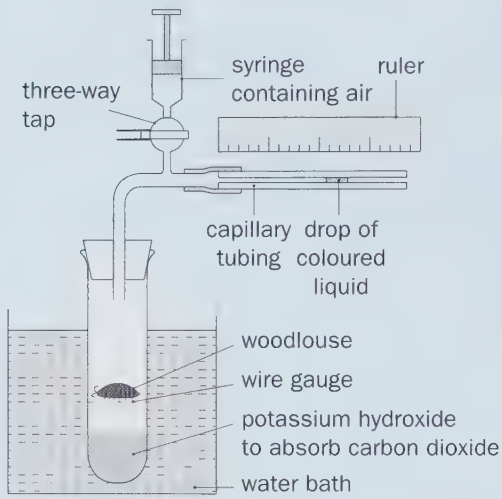
a) Copy the diagram and in each of the boxes write the number of carbon atoms in the named molecules. [1]

b) Give *two* different chemical processes which are involved in the conversion of molecule Y to molecule X in the Krebs cycle. [2]

c) Describe how the reduced coenzyme produced from the Krebs cycle may be used to produce ATP. [3]

d) ATP regulates its own production in respiration by affecting the activity of the enzyme PFK. Suggest how this regulation may be achieved through negative feedback. [2]
AQA (formerly NEAB) **[8]**

3 A student used the apparatus shown below to measure the rate of aerobic respiration of a woodlouse at different temperatures.



a) Initially the student removed the syringe and opened the three-way tap so that air could enter the boiling tube. The water bath was kept at 30 °C.
The student left the apparatus for 5 minutes before closing the tap. Explain why. [2]

b) The student closed the tap. After 30 minutes the drop of coloured liquid had moved towards the boiling tube.
Explain why the drop of coloured liquid moved towards the boiling tube. [3]

c) The student repeated the experiment three more times at 30 °C after leaving the tap open for 10 minutes between repeats. She then repeated the experiment four times with the water bath at 20 °C.
i) The syringe is used to reset the apparatus. Describe how. [1]
ii) The same woodlouse was used each time. Explain why. [1]

d) The student did not set up a control experiment.
   i) Suggest a suitable control experiment for this investigation. [1]
   ii) Explain why a control experiment is needed. [1]

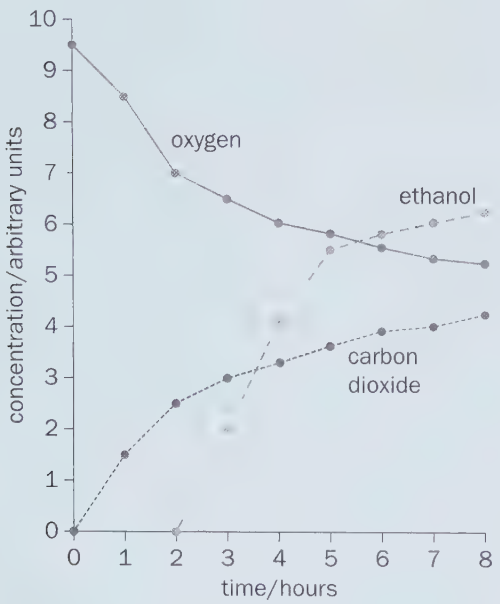
The table below shows the mean results of her investigation at each temperature.

	Temperature (°C)	
	30	20
Distance moved by bead of liquid in 30 minutes per mm	12	7

e) The cross-sectional area of the tubing is 1 mm^2. Calculate the rate of respiration at each temperature in $mm^3O_2\ min^{-1}$. [2]
f) Suggest and explain *one* reason for the difference in rate of respiration at the two temperatures. [2]

[13]

4 A scientist investigated the use of a new source of carbohydrate in the production of ethanol for biofuel. He wanted to find the optimum time to leave a mixture of yeast and this carbohydrate to produce ethanol. The scientist set up an airtight container containing yeast and this carbohydrate. He then measured the oxygen, carbon dioxide and ethanol concentrations over 8 hours.
The results of his investigation are shown in the graph below.

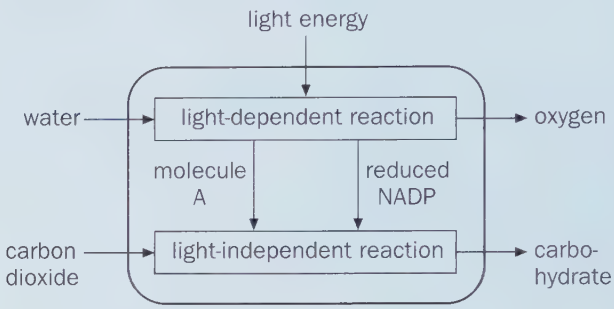


a) The scientist used a container that was airtight. Give *two* explanations for why the container had to be airtight. [4]
b) Explain the relationship between the concentration of oxygen and the concentration of carbon dioxide between 0 and 3 hours. [2]
c) The scientist concluded that yeast starts to respire anaerobically when the oxygen concentration falls below a certain concentration. What is the oxygen concentration when the yeast starts to respire anaerobically? Explain your answer. [2]
d) The scientist recommended that when ethanol is produced commercially as a biofuel, the reaction should be stopped at 6 hours. Suggest why. [2]

AQA **[10]**

Photosynthesis

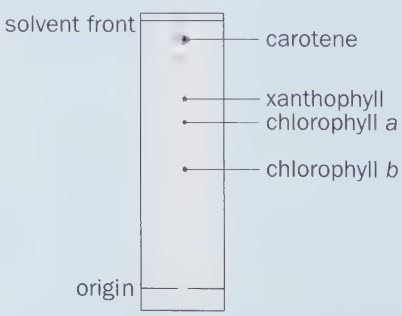
5 The diagram below summarises the biochemical pathways involved in photosynthesis.



a) Name molecule A. [1]
b) i) Describe how NADP is reduced in the light-dependent reaction. [2]
   ii) Describe the role of reduced NADP in the light-independent reaction. [2]
c) Suggest why, in a young plant, you would expect the rate of photosynthesis to be greater than the rate of respiration over a 24-hour period. [1]

AQA (formerly NEAB) **[6]**

6 A student carried out an experiment to separate the pigments found in spinach leaves by chromatography. The diagram below shows the resulting chromatogram.



a) The student was provided with the spinach leaves, chromatography paper and solvent. Describe how the student would have set up her chromatography experiment. [3]

b) Chromatography separates the different pigments. Explain how. [2]

c) The student calculated the Rf value for each of the pigments. What measurements would she have made to calculate the Rf value of chlorophyll *a*? [2]

[7]

7 Oxygen is produced in the light-dependent reaction of photosynthesis.

a) Name the part of a chloroplast where the light-dependent reaction occurs. [1]

b) Oxygen is produced during photolysis. Describe what happens during photolysis. [3]

c) A student conducted an investigation where light of different colours was shone on *Elodea* pondweed. The table shows the amounts of oxygen released.

	Number of bubbles of oxygen released per minute in five trials							
Colour of light	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean	SD	Range
violet	22	19	26	18	17	20.4	3.65	9
blue	38	25	33	26	37	31.8	6.06	13
green	21	12	12	11	7	12.6	5.13	14
yellow	14	13	13	11	12	12.6	1.14	3
red	20	17	18	14	15	16.8	2.39	6

i) Describe how the student calculated the range. [1]

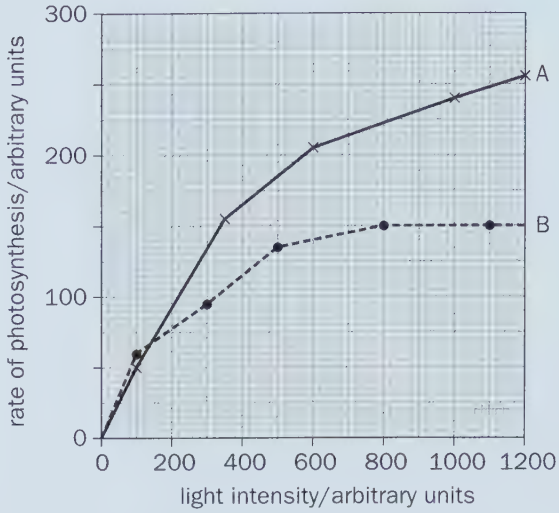
ii) The student decided it was better to use the mean and standard deviation (SD) rather than the range to describe variation in these results. Use the data to explain why. [2]

d) Scientists investigated photosynthesis in single-celled algae.
They did two experiments. In the first experiment, they supplied the algae with water molecules labelled with the isotope of oxygen, ^{18}O. The oxygen gas released by the algae contained ^{18}O. In the second experiment, they supplied the algae with carbon dioxide molecules labelled with ^{18}O. The oxygen gas released by the algae did ***not*** contain ^{18}O.
Use this information to explain why the following equation is ***not*** an accurate summary of photosynthesis. [2]

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$$

AQA **[9]**

8 The graph below shows the rate of photosynthesis in two crop plants, A and B, at different light intensities.



a) Describe the ways in which increasing light intensity has similar effects on the two crops. [2]

b) Suggest the most likely limiting factor for crop B at a light intensity of i) 100 arbitrary units and ii) 1000 arbitrary units [2]

c) From these data, suggest, with reasons, which crop is better suited for growth in tropical conditions. [2]

AQA (formerly NEAB) **[6]**

▶ Homeostasis

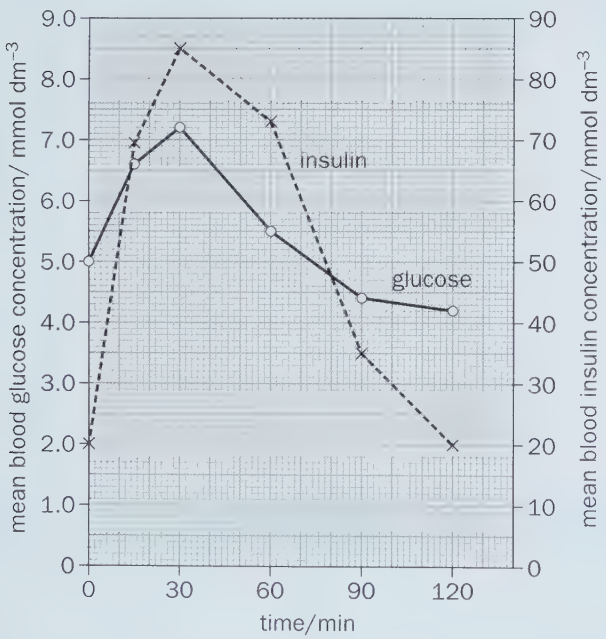
9 *Liolaemus* is a reptile which lives at a high altitude in the mountains of South America. In an investigation, the air temperature and the body temperature of this lizard were measured at intervals during one morning. The results are shown in the table below.

Time of day (24 hour clock)	Air temperature/°C	Body temperature of lizard/°C
07.10	−3.0	2.5
07.20	−1.0	10.0
07.30	−2.0	19.0
08.00	1.0	31.0
08.20	1.5	33.0
08.45	5.0	34.0
10.00	9.0	35.0
11.00	13.0	36.0

a) Describe the changes in air temperature and body temperature during the following time intervals.
   i) 07.10 to 08.00 [2]
   ii) 08.00 to 11.00 [2]
b) Suggest how the lizard increases its body temperature during the early morning. [2]
c) Suggest how the lizard controls its body temperature between the hours of 08.45 and 11.00. [2]
d) Explain why it may be an advantage for this lizard to be able to control its body temperature. [2]

Edexcel (formerly London) **[10]**

10 Research scientists carried out an investigation into the relationships between the concentrations of glucose and insulin in the blood of healthy people. At the start of the investigation, 34 volunteers each ingested a syrup containing 50 g of glucose. The concentrations of glucose and insulin were determined in blood samples at intervals over a period of 2 hours. The results shown in the graph below are mean values for the group of volunteers.



a) The volunteers were instructed not to eat or drink after midnight before taking part in the investigation at 8.00am in the morning. Explain why. [1]
b) Mean values were plotted but the data showed variation. Explain how the variation in these data could be shown. [1]
c) Describe the changes in mean blood glucose concentration during the following time intervals.
   i) 0–30 minutes. [1]
   ii) 30–120 minutes. [1]
d) Calculate the increase in mean blood insulin concentration between 0 and 30 minutes. [1]
e) Explain the relationships between concentrations of glucose and insulin as shown by this graph. [3]
f) Give the name of *one* hormone, other than insulin, which is involved with the regulation of blood glucose, and explain how it brings about a change in blood glucose concentration. [3]

Edexcel (formerly London) **[11]**

11 a) The kidney has a number of important functions which include excretion and homeostasis. Explain the meaning of the terms i) *excretion* and ii) *homeostasis*. [2]
b) The table below shows the composition of some fluids from the body of a healthy individual under normal conditions.

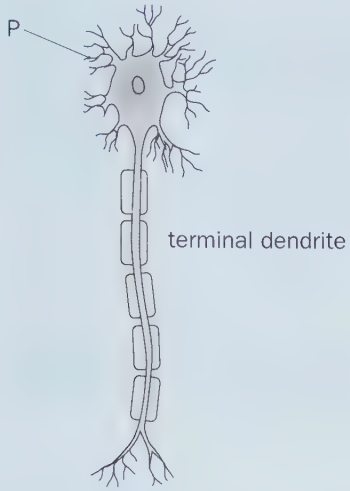
Fluid	Concentration as percentage of total volume		
	Protein	Glucose	Urea
Blood plasma	8.1	0.1	0.3
Region of nephron (kidney tubule)			
Renal capsule			0.03
End of first convoluted tubule			0.03
Urine			1.9

Copy and complete the table to show the values that you would expect for i) protein and ii) glucose. [2]
c) i) Which region of the nephron is responsible for establishing a gradient of ions across the medulla? [1]
   ii) Explain how this gradient of ions leads to the production of concentrated urine. [2]

AQA (formerly NEAB) **[7]**

Control and coordination

12 a) The diagram below shows a motor neurone.



i) Name the structure labelled P. [1]

ii) Draw an arrow to show the direction of the nerve impulse in the axon of this motor neurone. [1]

b) Eugenol is a drug that inhibits the movement of sodium ions through the cell-surface membranes of sensory neurones.
The table below shows the effect of eugenol concentration on the percentage inhibition of sodium ion movement.

Concentration of eugenol (mmol dm^{-3})	Percentage inhibition of sodium ion movement
0.2	30
0.4	50
0.6	65
1.0	80

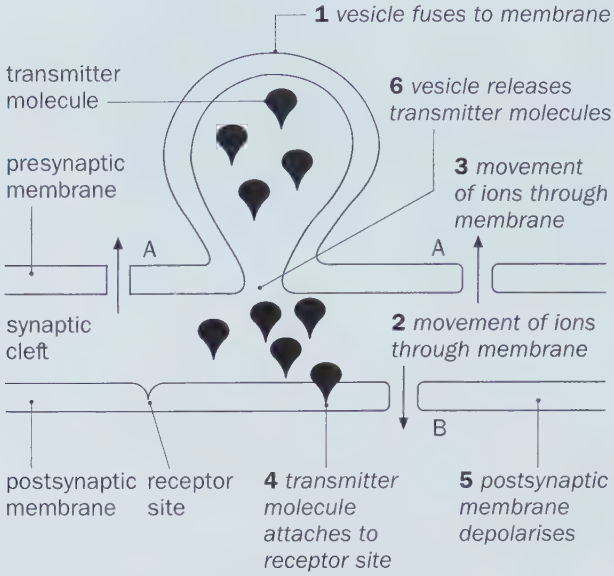
i) Describe the effect of eugenol on the percentage inhibition of sodium ion movement. [2]

ii) Describe how you could you use these data to estimate the percentage inhibition of sodium ion movement at 0.8 mmol dm^{-3}. [2]

c) Eugenol can be used to reduce pain.
Suggest and explain how eugenol affects the movement of sodium ions and reduces pain. [6]

Edexcel **[12]**

13 The diagram below shows some of the events which occur in a synapse after the arrival of an impulse at the presynaptic membrane.



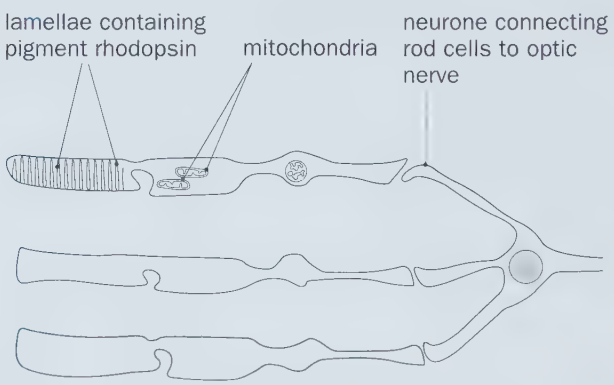
a) Put the events 1 to 6 on the diagram in the correct sequence. [1]

b) Name the ions labelled A and B. [2]

c) Name *one* transmitter molecule released by synaptic vesicles. [1]

AQA (formerly NEAB) **[4]**

14 The diagram below shows rod cells from the retina of a human eye.



a) Describe the effect of light on rhodopsin. [2]

b) i) What is the function of mitochondria? [1]

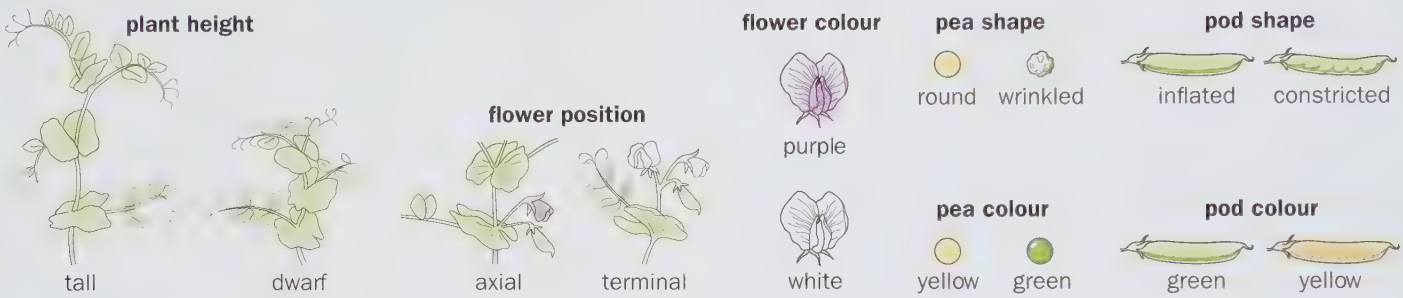
ii) Suggest why there are large numbers of mitochondria in a rod cell. [1]

c) Explain how the connections between the rod cells and the neurones in the optic nerve enable the eye to be sensitive to low light intensities. [2]

AQA (formerly NEAB) **[6]**

21 Genetics

Gregor Mendel (1822–84) was an Austrian monk and teacher.
He was the first person to work out the ways in which genes are inherited.
He worked with pea plants to study their patterns of inheritance.
He chose plants with easily observable features, such as plant height, flower colour and seed shape.
To some extent Mendel was lucky, since he chose characteristics that are controlled by single genes.
He was painstaking in his methods, meticulously collecting and recording his results. He built up sufficient amounts of accurate data to enable him to come to sound scientific conclusions.
The amazing thing about Mendel's work is that he worked out the underlying rules of inheritance before any knowledge of DNA, genes or chromosomes became available.



Alleles

As you saw in Chapter 6, nearly all organisms that reproduce sexually have homologous pairs of chromosomes: they are **diploid**. One of each pair comes from the father and one from the mother. At each particular position, or **locus**, along each chromosome pair, there will be equivalent copies of a gene.
Different versions of a gene can be found in a population and these are called **alleles**.

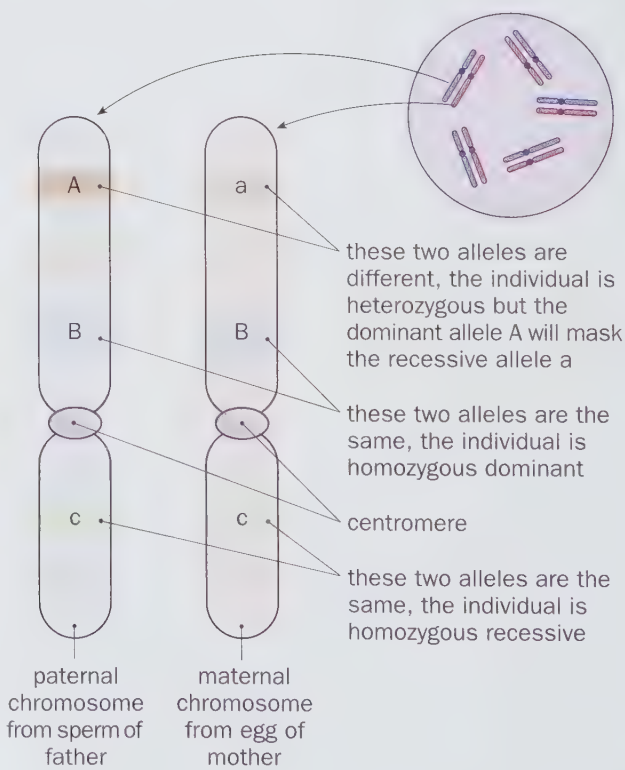
Some alleles are **dominant** and are able to mask the effect of other **recessive** alleles. For instance, the allele for tall plant, **T**, is dominant to the allele for dwarf plant, **t**, which is recessive.

If the two alleles are the same, we say they are **homozygous**.

They can be **homozygous dominant**, for example **TT**.
They can be **homozygous recessive**, for example **tt**.

We say that an individual is **heterozygous** if they have two alleles that are different, for example **Tt**.

The alleles that an individual has forms their **genotype**.
So the genotype of a tall plant could be **TT** or **Tt**.
The **phenotype** is the way the alleles are expressed in an individual.
For instance, the genotype **TT** or **Tt** would result in a tall phenotype and the genotype **tt** would result in a dwarf phenotype.
The phenotype is the result of the interaction of the genotype and the environment.



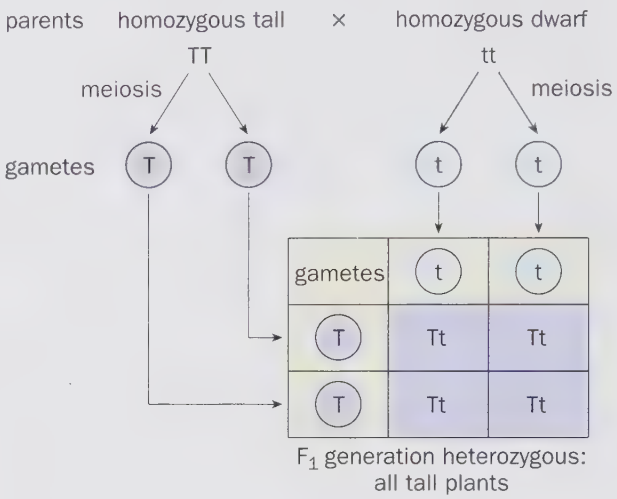
Monohybrid inheritance

Monohybrid inheritance concerns the inheritance of a ***single*** characteristic, such as plant height or flower colour.
It involves the inheritance of two alleles involving a single gene.
The best way to understand how genes are passed on is to use examples.
For instance, there is one gene for height in pea plants.
The gene for plant height has two alleles: tall (**T**) and dwarf (**t**).
Notice that we always show the dominant allele as a capital letter and the recessive allele as a small letter.
There will be three possible genotypes for plant height:

TT = homozygous tall
Tt = heterozygous tall
tt = homozygous dwarf

Variation in sweet pea plants

Let's cross a homozygous tall plant with a homozygous dwarf plant.
Can you see that, as a result of meiosis, all the gametes from the tall plant contain the dominant allele **T** and all the gametes from the dwarf plant contain the recessive allele **t**?

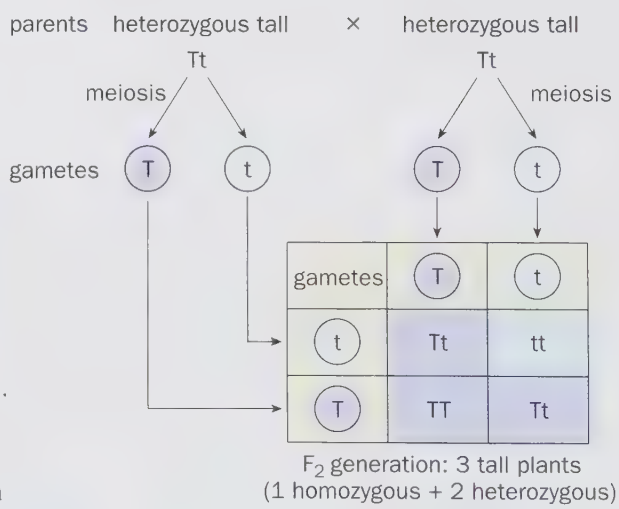


F_1 generation heterozygous: all tall plants

When fertilisation occurs, the new plant receives one dominant **T** allele and one recessive **t** allele.
So it will be heterozygous and have the genotype **Tt**.
Its phenotype will be tall, because the **T** allele is dominant to the recessive **t** allele.
The first generation is known as the $\mathbf{F_1}$, and in this particular genetic cross, all the F_1 are heterozygous tall (**Tt**).

What happens if we cross two of these F_1 plants?

This time half of the gametes of ***each*** plant will have the dominant (**T**) allele and half will have the recessive (**t**) allele.
This time when fertilisation occurs, there are three possible combinations of alleles in the $\mathbf{F_2}$, or second, generation:
TT (homozygous tall), **Tt** (heterozygous tall),
tt (homozygous dwarf).
So the ratio of phenotypes will be 3 tall : 1 dwarf because homozygous tall and heterozygous tall look the same.
This cross enabled Mendel to formulate his first law of inheritance.
The **law of segregation** states that:



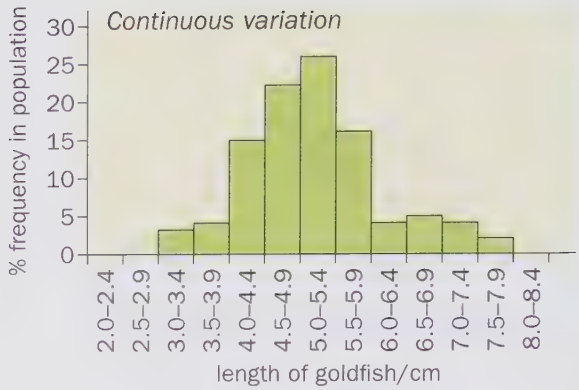
F_2 generation: 3 tall plants (1 homozygous + 2 heterozygous)
1 dwarf plant

The characteristics of an organism are determined by alleles which occur in pairs.
Only one of a pair of alleles can be present in a single gamete.

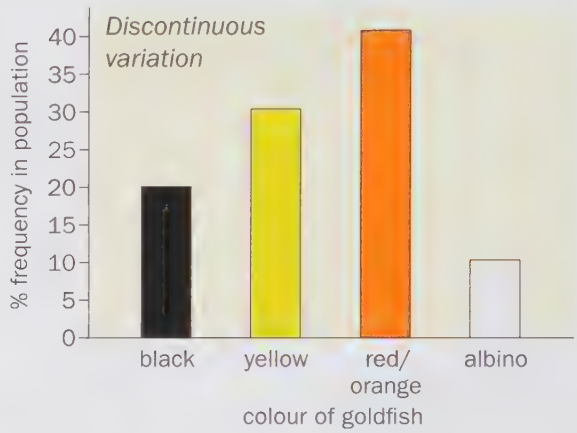
In Mendel's time, genes and chromosomes had not been discovered.
Instead of 'alleles' he used the term 'factors'.
Remember that ***pairs*** of chromosomes separate during meiosis.
If the alleles are present on the homologous chromosome pair, then they must also separate into different gametes.
We say that alleles **segregate** into different gametes during meiosis.
Look back to page 105 and read about how meiosis produces gametes with half the normal chromosome number and half the normal number of alleles.

Genetic crosses

As mentioned, Mendel was fortunate in his choice of characters, because he chose ones that are controlled by single genes.
Most characters are controlled by a number of genes, for instance height in humans.
There are not just tall and short people but a whole range of heights.
This is an example of **continuous variation**.
The differences are not clear-cut and often have to be measured to tell different phenotypes apart.

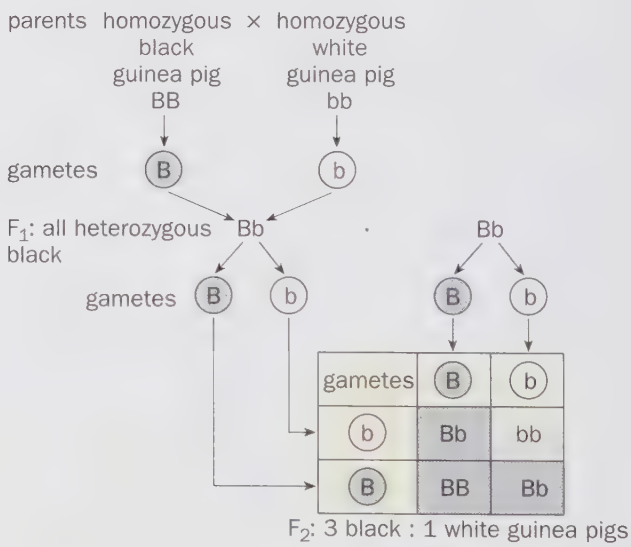


Mendel chose characters that were clear-cut and easy to tell apart, for instance flower colour.
These characters are controlled by a single gene.
Where these type of differences occur, we call it **discontinuous variation**.



Questions on single-gene inheritance do not often come up in advanced examinations, because they are fairly easy.
But you need to be able to understand them if you are to be able to do more demanding genetic crosses.
The best approach is to do as many examples as possible.
Here is a monohybrid cross involving animals.

Coat colour in guinea pigs is controlled by a single gene for which there are two alleles: black and white.
Since black is the dominant allele, we will give it the symbol **B**.
Since white is recessive, we will give it the symbol **b**.
You may not be given these symbols in an examination, in which case choose the first letter of the dominant character and make it a capital (as with **B** for black here).
Then choose the ***same*** letter for the recessive allele but make it small case (so white is **b** in our example ***not*** **w**).



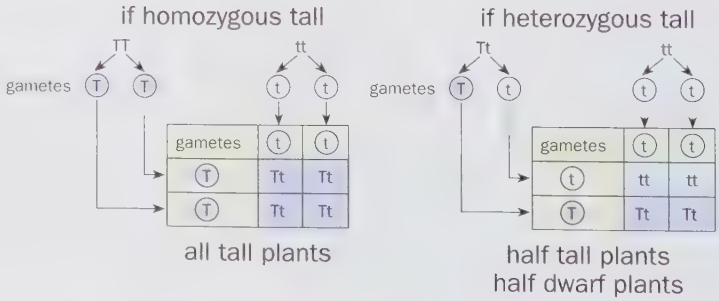
As you can see, if we cross a homozygous black guinea pig with a homozygous white one, all the F_1 are black.
Then crossing two of these animals from the F_1 gives us an expected ratio of 3 black to 1 white.
The 3 : 1 ratio is sometimes called a **Mendelian ratio** for a monohybrid cross.

Test cross

If you look at the last cross, you will see that the recessive alleles are hidden in the F_1 generation but reappear in the F_2.

But how can we tell if a black guinea pig is homozygous or heterozygous? They both look identical.
What we do is to take the individual and cross it with a homozygous recessive individual. This is called a **test cross**.

The diagram right explains what happens.



If our tall plant is homozygous (**TT**), then crossing it with a dwarf plant (**tt**) gives ***all*** tall plants (**Tt**).
If, however, our tall plant is heterozygous (**Tt**), then crossing it with a dwarf plant (**tt**) will give ***half*** tall plants (**Tt**) and ***half*** dwarf plants (**tt**).

Monohybrid inheritance in humans

Some 'faulty' alleles can be passed on from one generation to the next.

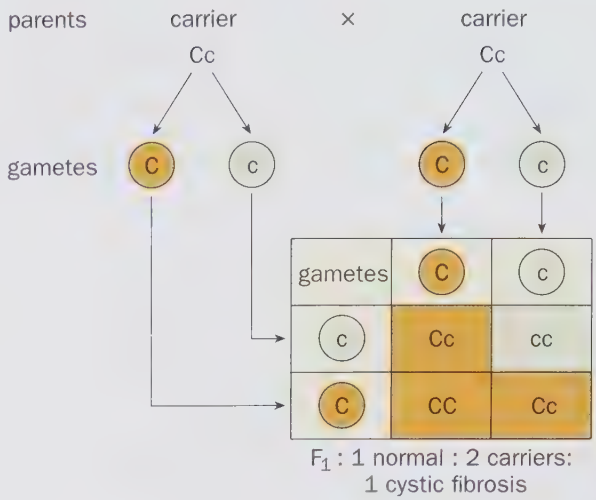
Cystic fibrosis

Cystic fibrosis (CF) is an inherited disease caused by a gene mutation.
In Britain it affects one child in every 2000.
The condition is caused by a recessive allele **c**.
So to have CF, a person must have ***two*** recessive alleles (**cc**).

Heterozygous individuals (**Cc**) do not suffer from the condition but are **carriers** and may pass the defective allele on to their children.
What would be the chances of a child inheriting CF if both parents were carriers (**Cc**)?

As you can see from the diagram, there would be a 1 in 4 chance of the child inheriting the disease.

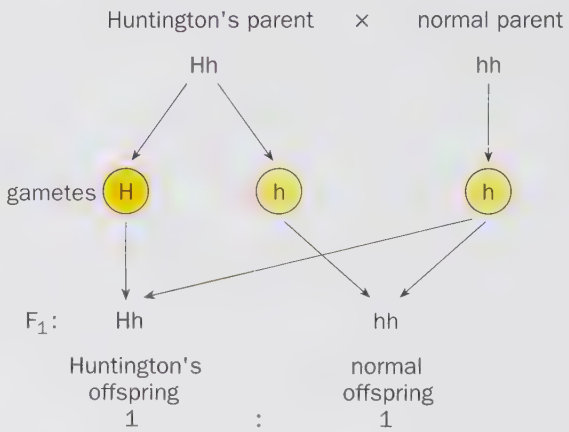
We will look at the possible future treatment of CF by gene therapy in Chapter 22.
Another disease caused by the inheritance of two recessive alleles is **thalassaemia,** which results in severe anaemia.



Huntington's disease

Huntington's is a rare inherited condition which affects about 1 in 20 000 people in Britain. The symptoms are particularly distressing. The cells of the brain degenerate and the patient's coordination is affected.
The patient becomes moody and depressed, memory becomes affected, they eventually become totally disabled and ultimately die.
The most sinister aspect of Huntington's is the fact that the symptoms only become apparent when patients are in their 30s or 40s.
By this time the person may well have had a family and so unintentionally passed the defective allele on to the next generation.

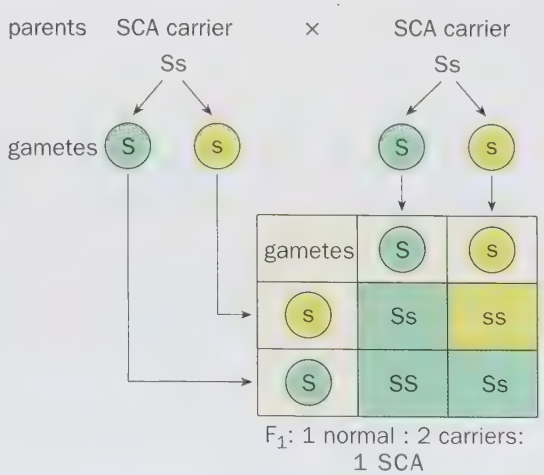
Huntington's disease is caused by a dominant allele.
So only ***one*** allele is necessary to give the disease.
So all heterozygous people are sufferers.



Sickle cell anaemia

Sickle cell anaemia (SCA) is a human blood disease caused by the inheritance of a recessive allele.
The red blood cells are malformed, taking on a crescent or sickle shape.
The sickle cells get stuck in capillaries and inhibit the circulation of oxygen to the tissues.
This can lead to death at an early age.

SCA is due to the recessive allele not coding for the correct form of haemoglobin.
A sufferer must have inherited two recessive alleles from its parents.
Carriers or heterozygous individuals have some normal red blood cells and some sickle cells, so they are not so badly affected.
You can find out more about SCA on page 213.



Dihybrid inheritance

It is unlikely that you have studied more than monohybrid inheritance.

Dihybrid inheritance involves the inheritance of ***two*** separate genes. In one experiment, Mendel looked at how seed colour and seed shape were inherited.

There are two alleles for seed shape: round (**R**) and wrinkled (**r**).
There are two alleles for seed colour: yellow (**Y**) and green (**y**).

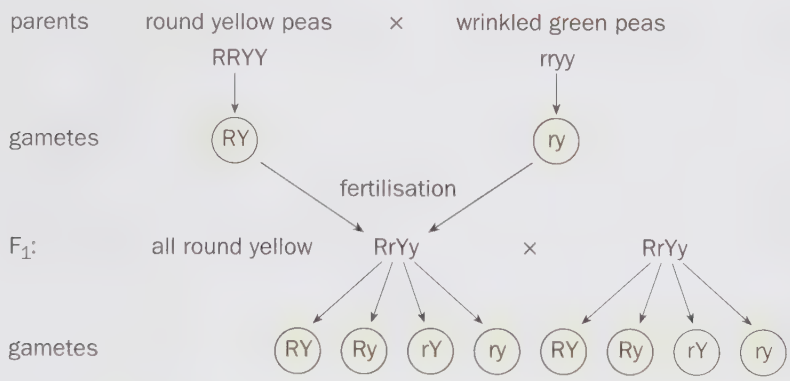
Mendel knew from doing monohybrid crosses that round seed shape was dominant to wrinkled, and that yellow seed colour was dominant to green.

Mendel decided to cross homozygous pea plants with the two dominant characters (round and yellow seeds) with homozygous plants with the two recessive characters (wrinkled and green).

He found that ***all*** the F_1 generation had round yellow seeds.
He then self-pollinated flowers from the F_1 (sometimes called '***selfing***').
When he collected and counted the seeds he found the following:

	round yellow	round green	wrinkled yellow	wrinkled green
	315	108	101	32
approx. ratio	9:	3:	3:	1

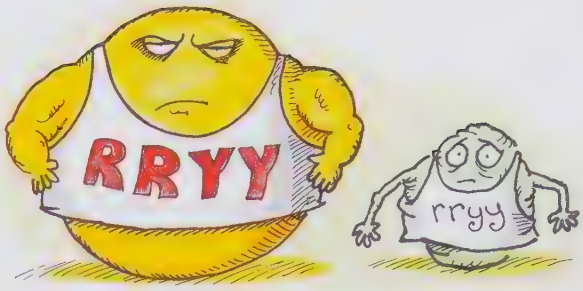
We can show the cross in a diagram.



gametes	RY	Ry	rY	ry
RY	RRYY	RRYy	RrYY	RrYy
Ry	RRYy	RRyy	RrYy	Rryy
rY	RrYY	RrYy	rrYY	rrYy
ry	RrYy	Rryy	rrYy	rryy

F_2 ratio: round yellow: 9
round green: 3
wrinkled yellow: 3
wrinkled [illegible]

Questions on dihybrid inheritance



Dihybrid crosses often appear in advanced examinations.
If you are not careful, especially with the layout of the cross, you can make mistakes.
Here are some useful tips.

- As you see in our example, there are ***two*** genes involved, each with ***two*** alleles.
  So this time we write ***four*** letters, one for each of the four alleles.
  For example, **RRYY** and **rryy**.
- Always put the alleles for the same characteristic together.
  For example, **RrYy** never **RYry**.
  This looks clearer and will help you to show segregation better.
- At segregation, remember Mendel's first law still applies, so only show ***one*** allele of each gene in the gamete.
  For example, **RY**, **Ry**, **rY** or **ry**.
  It is always a good idea to ***circle*** the gametes so you don't get them mixed up with the F_1 or the F_2 generations, and remember – an individual that is heterozygous for both characteristics can produce four different types of gametes.
- To keep things neat, use a **Punnett square**.
  If you are crossing two individuals that are heterozygous for both characteristics, you'll need to show $4 \times 4 = 16$ individuals in the F_2.
- Always write the gametes down in the same sequence on each axis of the Punnett square, starting with the two dominant alleles and ending with the two recessive alleles.
  If you do this, you can almost anticipate the position of each of the genotypes within the square.
- Try drawing out a Punnett square for a cross between **RrYY** and **RRYy.**

same sequence of gametes

gametes	RY	Ry	rY	ry
RY				
Ry				
rY				
ry				

same sequence of gametes

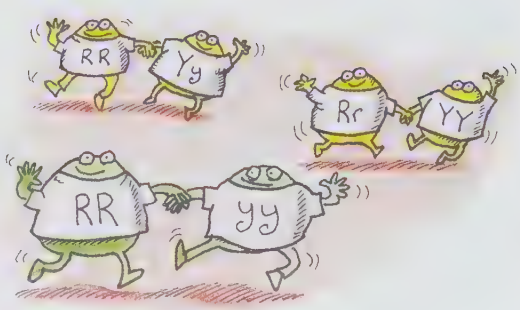
The results of the dihybrid cross led Mendel to formulate his second law of inheritance: the **law of independent assortment**.

Either of a pair of alleles may combine randomly with either of another pair.

So in our example, we don't just get **RR** and **YY** together, or **rr** and **yy** together.
We can get ***either*** of each pair of alleles combining to give any of the following:

RRYY, **RRYy**, **RrYy**, **RrYY**, **RRyy**, **Rryy**, **rrYY**, **rrYy** and **rryy.**

- Another important hint is to make sure that you convert the correct number of genotypes into the correct ratio of phenotypes if asked.
  Remember that there are 16 individuals in the Punnett square.
- In some examination questions, they give you the ratios or even the raw numbers of the phenotypes of the F_2 and expect you to work backwards to the correct genotypes of the F_1 and parents.
- Remember that practice makes perfect and that you can gain full marks for genetic crosses. They are ***always*** examined.



Either of a pair of alleles can combine with either of another pair

A dihybrid test cross

Mendel wanted to cross F_1 plants from his dihybrid cross.
He wanted to be sure that the heterozygous plants could produce four different types of gametes.
As with a monohybrid test cross, he crossed the F_1 plants with homozygous recessive plants that produced wrinkled green seeds.
These plants were **double recessive** because they were recessive for both characters. Here are Mendel's results:

gametes	(RY)	(Ry)	(rY)	(ry)
(ry)	RrYy	Rryy	rrYy	rryy

- round yellow 57
- round green 51
- wrinkled yellow 49
- wrinkled green 53

The results were close to the ratio of 1:1:1:1 that we would expect to get from the cross.

Chi-squared test (χ^2) of the dihybrid test cross

Our expected result for this test cross is 1:1:1:1 and Mendel's results (the observed results) seem pretty close to the expected.
We can use the chi-squared test to compare the observed results with those that we expected to get.
The test is a way of estimating the probability that differences between observed and expected results are due to chance.

Maths skills

Chi-squared is calculated as follows:

1 Calculate the expected values (E). In our example, this is the total number of seeds divided by the number of possible types $= \frac{210}{4} = 52.5$

2 Work out the differences between the observed (O) and expected results.

3 The differences are then squared, because some values will be negative.

4 Now work out the value of chi-squared using this formula:

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

In practice, it is best to use a table like the one below.

Phenotype	Observed (O)	Expected (E)	Difference ($O - E$)	Difference squared $(O - E)^2$	$\frac{(O - E)^2}{E}$
round yellow	57	52.5	4.5	20.25	0.38
round green	51	52.5	−1.5	2.25	0.043
wrinkled yellow	49	52.5	−3.5	12.25	0.233
wrinkled green	53	52.5	0.5	0.25	0.00048
					$\Sigma = 0.66$

Next we need to find the number of **degrees of freedom**. This is a measure of the ***spread*** of the data and is always one less than the number of classes of data. So in this case, it is four classes minus one, which equals three degrees of freedom.

Now look at the table of chi-squared values for three degrees of freedom.
Our result of 0.66 corresponds to a probability just below 0.9, so we can conclude that the deviation from the expected 1:1:1:1 ratio is not significant.

Degrees of freedom	Probability (p)						
	0.90	0.50	0.20	0.10	0.05	0.02	0.01
1	0.02	0.46	1.64	2.71	3.84	5.41	6.64
2	0.21	1.39	3.22	4.61	5.99	7.82	9.21
3	0.58	2.37	4.64	6.25	7.82	9.84	11.34
4	1.06	3.36	5.99	7.78	9.49	11.67	13.28

Linkage and recombination

Linkage occurs when two different genes are located on the ***same*** chromosome.
For this reason, they are usually inherited together, because they move together during meiosis and end up in the same gamete.
Under normal circumstances, the linked genes on the same chromosome pass into the gamete and then into the offspring ***together***.
This is contrary to Mendel's law of independent assortment.

If genes **A** and **B** had been on separate chromosomes, then there would have been more genetic variation in the gametes and in the offspring. Independent assortment would have allowed parent **AaBb** to produce four types of gametes,

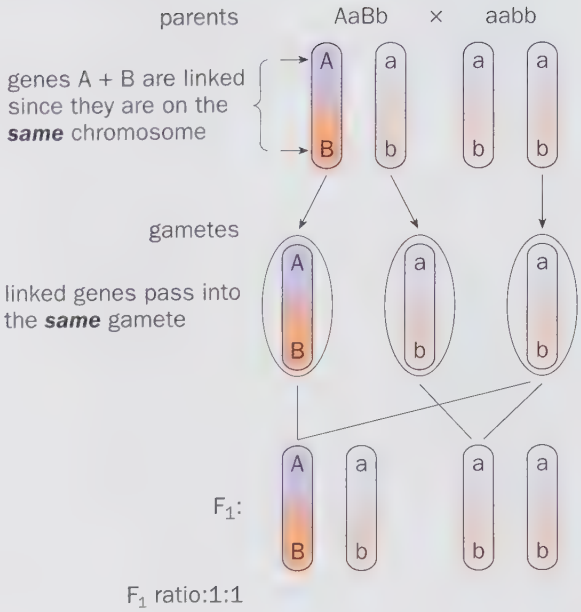
(AB) (Ab) (aB) (ab), instead of just two.

How many types of offspring could have been produced then? Draw a Punnett square to work it out.

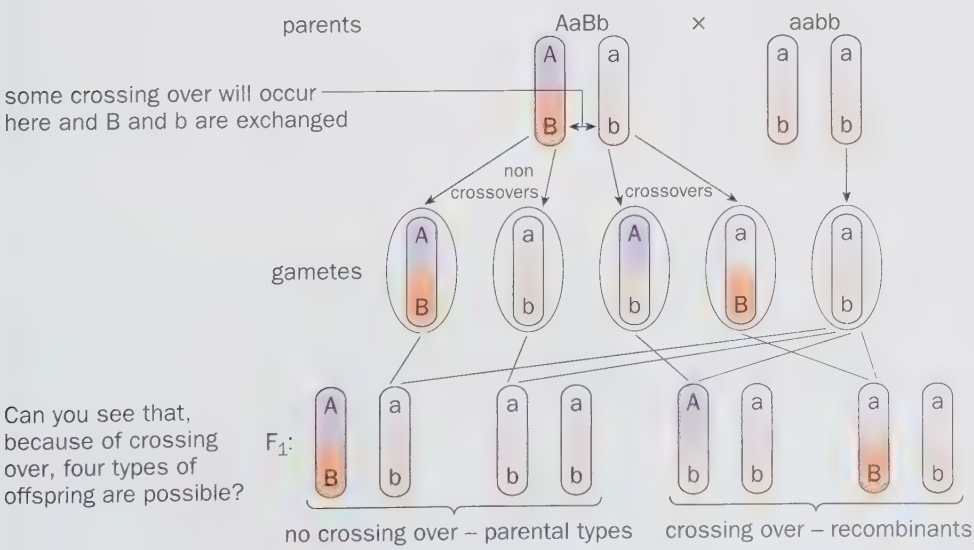
Recombination occurs when alleles are exchanged between homologous chromosomes as a result of ***crossing over*** (see page 108 to remind yourself).
If you look at the example below, you will see that, due to some crossing over, ***some*** alleles **A** and **B** end up on different chromosomes and so are no longer linked.
Recombination allows Mendel's second law of independent assortment to operate and so increases the genetic variation in the gametes and hence in the offspring.

The further apart two genes are on a chromosome, the more chance there is of crossing over occurring.
This has been used to determine where a gene **locus** (position) is on a chromosome and so produce **chromosome maps** (see more about the applications of chromosome mapping on page 398).

Linkage

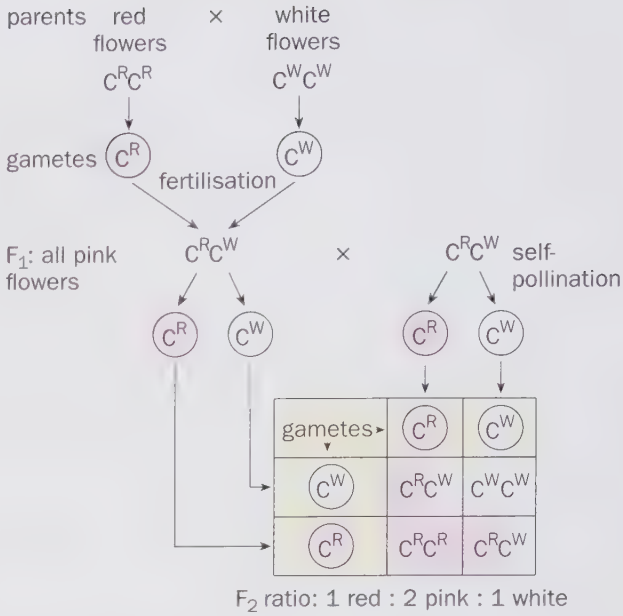


Recombination



Codominance

Up to now the examples that we have looked at involve alleles that are either dominant or recessive.
In these cases the effect of the recessive allele is masked by the dominant one in the heterozygous condition.
Sometimes, however, both alleles seem to be expressed and neither seems to dominate the other.
So the phenotype is often a mixture of the effects of each allele.
This condition is known as **codominance**.



Snapdragon plants can produce red flowers or white flowers. But if you cross the two homozygous plants, the offspring are all pink. The two parents produce heterozygous offspring that are intermediate between them.

What happens if we self-pollinate the pink plants of the F_1? You can see from the diagram that we end up with red, pink and white flowered plants in the F_2, in a ratio of 1:2:1.

Notice that the ratio of the phenotypes is the same as the ratio of the genotypes in a codominant cross.
Notice also that each allele is represented as a capital, so red flower = $\mathbf{C^R}$ and white flower = $\mathbf{C^W}$. This is to reflect that neither allele is recessive and that they both exert an equal effect on the phenotype.

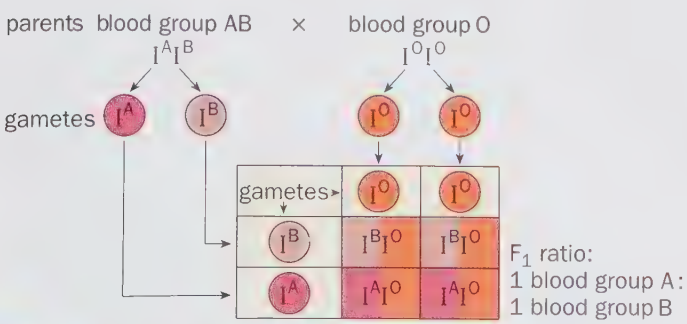
Multiple alleles

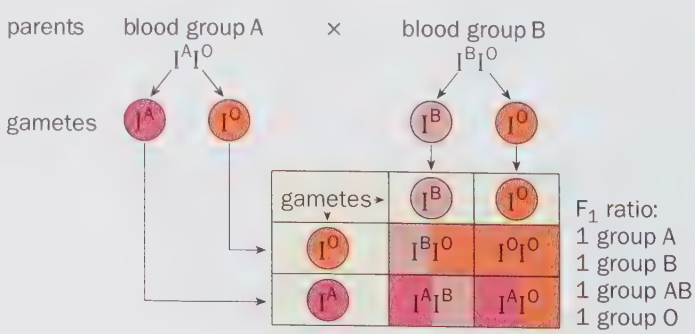
So far, we have looked at examples where a gene has only two alternative alleles. There are examples where a gene may have three or more alleles. For instance, the four common blood groups of the human ABO blood system are determined by one gene, **I**, with three different alleles. But remember, a gene can only be represented twice, with one allele on the locus of each homologous chromosome.

Genotype	Blood group
I^AI^A	A
I^AI^O	A
I^BI^B	B
I^BI^O	B
I^AI^B	AB
I^OI^O	O

The three alleles are $\mathbf{I^A}$, $\mathbf{I^B}$ and $\mathbf{I^O}$.
The alleles $\mathbf{I^A}$ and $\mathbf{I^B}$ are codominant and code for slightly different antigens on the surface of red blood cells.
The allele $\mathbf{I^O}$ is recessive to both $\mathbf{I^A}$ and $\mathbf{I^B}$ and codes for no antigens.
Look at the table. You can see the possible genotypes for each blood group.

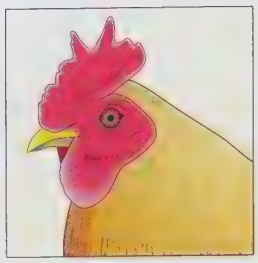
A cross between a blood group AB person and one with blood group O can produce offspring with neither of the parental blood groups.
A cross between a heterozygous blood group A person and a heterozygous blood group B person can give offspring of any of the four blood groups.

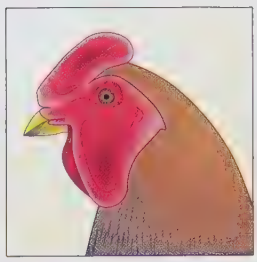


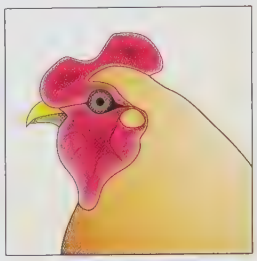


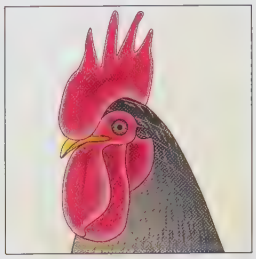
Gene interaction

The examples of dihybrid inheritance that we have looked at have involved genes that control different characters. Sometimes the alleles of more than one gene affect each other. This is known as **gene interaction**.


pea comb


walnut comb


rose comb


single comb

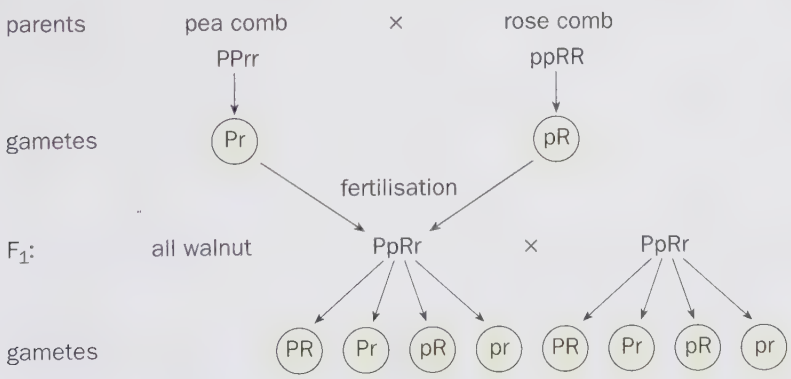
William Bateson and Reginald Punnett studied the interaction between two genes, found on different chromosomes, which affect comb shape in poultry. If you look at the diagram, you can see that there are different forms of comb.

Bateson and Punnett crossed homozygous 'pea' combed fowls with homozygous 'rose' combed fowls.
The F_1 individuals all had a different comb form called 'walnut'.

When the F_1 'walnut' combed birds were interbred, four types of comb occurred in the F_2: pea, rose, walnut and a new form of comb called 'single'.

Bateson and Punnett suggested that two genes were involved.
For one gene, the dominant allele **P** gave pea comb.
For the other gene, the dominant allele **R** gave rose comb.
But when both dominant alleles **P** and **R** appeared together, they interacted to produce a walnut comb phenotype.
And if neither of the two dominant alleles were present, then it gave a single comb.

The genotype of the homozygous pea comb parent = **PPrr**.
The genotype of the homozygous rose comb parent = **ppRR**.
The genotype of the F_1 heterozygous walnut comb birds = **PpRr**.
And the genotype of the F_2 single comb birds = **pprr**.
The results can be shown in the following diagram.



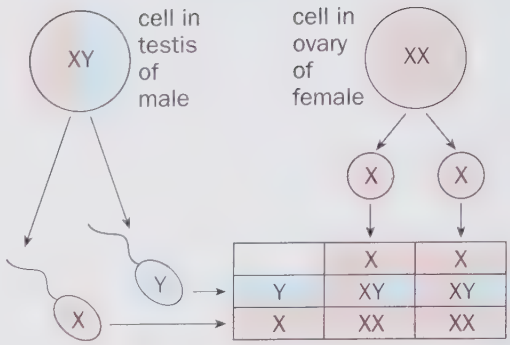
gametes	PR	Pr	pR	pr
PR	PPRR	PPRr	PpRR	PpRr
Pr	PPRr	PPrr	PpRr	Pprr
pR	PpRR	PpRr	ppRR	ppRr
pr	PpRr	Pprr	ppRr	pprr

F_2 ratio: walnut comb: 9
pea comb: 3
rose comb: 3

Sex linkage

You should remember from Chapter 6 that humans have 46 chromosomes that can be arranged into 23 pairs. The first 22 homologous pairs of chromosomes are referred to as **autosomes**. The last pair of chromosomes are the sex chromosomes. Females have two X chromosomes that look alike. Males have one X chromosome and one much shorter Y chromosome.

The diagram shows how sex is inherited.
All the female's eggs contain an X chromosome.
Half the male's sperm contain an X chromosome and the other half contain a Y chromosome.



At fertilisation, an egg may fuse with either an X sperm or a Y sperm.
So there is an equal chance of the child being a boy or a girl.
So it seems that the presence of a male-determining allele present on the Y sex chromosome confers maleness.

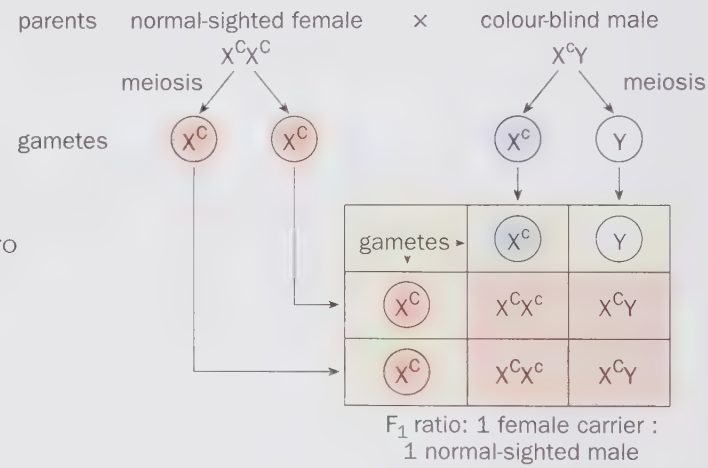
Linkage occurs when genes are located on the ***same*** chromosome.
Sex linkage occurs when genes are carried on the sex chromosomes.
These genes may have nothing to do with sex determination; they just happen to be carried on the X or Y chromosomes.

A Y chromosome is a bit like an X chromosome with a bit missing.
As the Y chromosome is smaller, it carries fewer alleles.

XY

This part is missing from the Y chromosome

The allele for colour vision is carried on the part of the X chro that is missing from the Y chromosome.
This means that a male will only have one allele for colour vision.
There is a defective, recessive allele of the colour vision gene which can lead to colour-blindness, particularly of red and green light.
Let **C** = the allele for normal colour vision
and **c** = the allele for colour-blindness.
What happens if we cross a normal-sighted female with a colour-blind male?

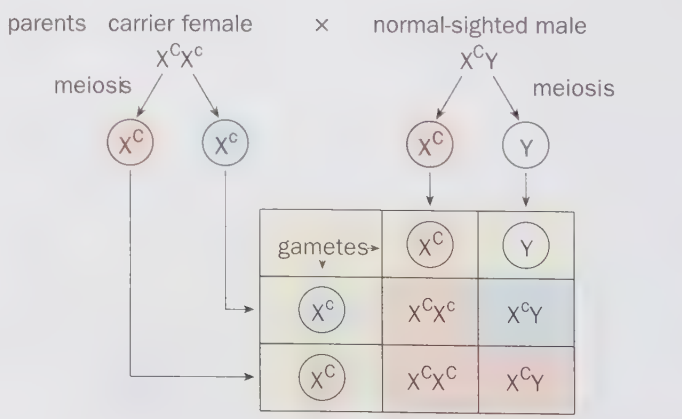


F_1 ratio: 1 female carrier : 1 normal-sighted male

Female with two alleles for colour vision X^CX^C

Male with one allele for colour vision X^CY

All the offspring are normal-sighted. The females have the allele for colour-blindness on one of their X chromosomes. We say that they are **carriers**. They have the colour-blind allele but do not show it in their phenotype.
Now let's cross a normal-sighted male with a carrier female.



F_1 ratio: 1 normal-sighted female : 1 carrier female
1 normal-sighted male : 1 colour-blind male

There is a 1 in 4 chance that the carrier female will pass on the colour-blind allele to one of her children and it will ***always*** be a boy.

Haemophilia

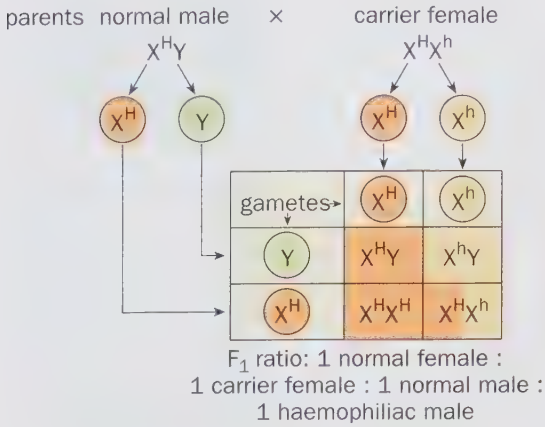
Haemophilia is a sex-linked disease, caused by a recessive allele on the X chromosome.
The gene that codes for factor VIII, an important protein involved in blood clotting, is a sex-linked gene found on the X chromosome.

A defective, recessive allele of the gene can lead to haemophilia. Haemophiliacs are unable to make factor VIII and can lose a lot of blood from even small injuries or bruises.
In the past this meant that most sufferers died in childhood, but now it can be treated by regular injections of factor VIII.

Here is a cross between a normal male and a carrier female.
Can you see that the male only has to have ***one*** recessive allele to get haemophilia?
This is because its effect cannot be masked by a dominant, normal allele on the Y chromosome.

Haemophiliac females occur very rarely.
Why do you think that the onset of menstruation at puberty can often prove fatal for female haemophiliacs?

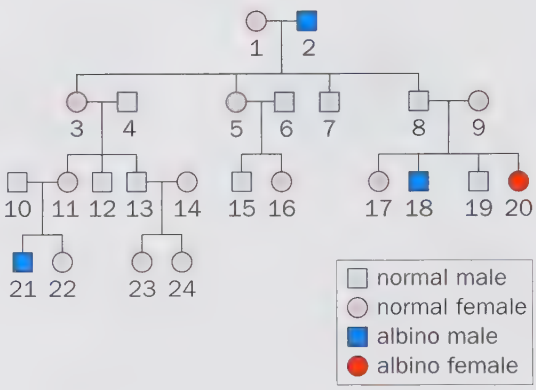
H = normal allele and h = haemophiliac allele



F_1 ratio: 1 normal female : 1 carrier female : 1 normal male : 1 haemophiliac male

Pedigree analysis

Pedigree diagrams can be used to show how a condition such as haemophilia has been passed on from generation to generation. They are like a family tree showing information that can be looked up in medical records.
The females are shown as circles and the males as squares.
A solid or coloured symbol is used to highlight the condition.
Here is a pedigree diagram showing the inheritance of albinism through four generations.

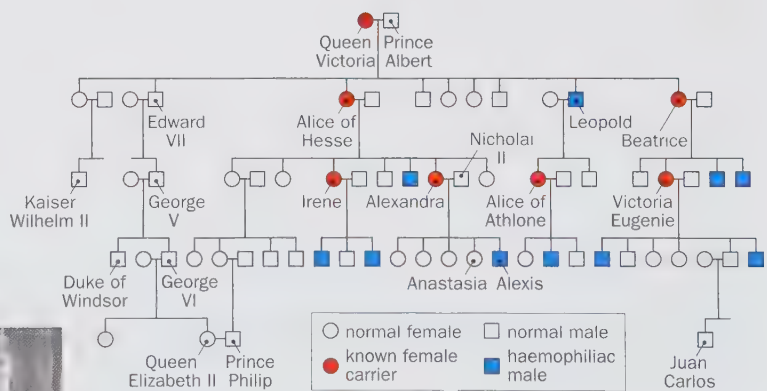


Other single gene conditions that can be illustrated using pedigree diagrams are thalassaemia, cystic fibrosis and Huntington's disease.

The inheritance of haemophilia in the British royal family can be traced from Queen Victoria, who was a carrier. Look at the pedigree diagram.

How many of Queen Victoria's children were haemophiliac and how many were carriers?
How many of her granddaughters were carriers?
How many of her great-grandsons were haemophiliacs?

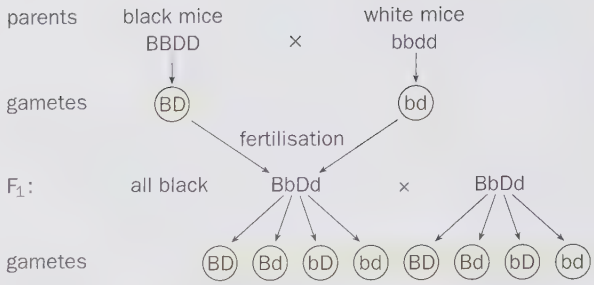
Explain why the allele for haemophilia has not been passed on to the present day royal family.



The transmission of haemophilia from Queen Victoria

Epistasis

In epistasis, two genes at different loci interact to control a single characteristic.
It occurs when an allele of one gene suppresses or masks the action of another.
Epistasis reduces the number of different phenotypes for the character, so instead of having ***four*** phenotypes for two genes, there will be ***three*** or ***two***.

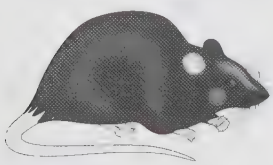


Examples of epistasis

- **Coat colour in mice**
  In mice one gene controls the production of coat pigment, and black pigment (B) is dominant to no pigment (b).
  Another gene controls the dilution of the pigment in the hairs, with dense pigment (D) being dominant to dilute pigment (d).
  The pigment gene (B) epistatic over the dilution gene (D) because the recessive allele of the pigment gene is a mutation that produces no pigment at all, so there is nothing for the dilution gene to affect.
  This gives three possible phenotypes.

gametes	BD	Bd	bD	bd
BD	BBDD	BBDd	BbDD	BbDd
Bd	BBDd	BBdd	BbDd	BBdd
bD	BbBD	BbDd	bbDD	bbDd
bd	BbDd	Bbdd	bbDd	bbdd

F_2 ratio: black (black dense): 9
brown (black dilute): 3
white (no pigment): 4


black (black dense)


brown (black dilute)


white (no pigment)

- **Flower colour in sweet peas**
  In a certain variety of sweet pea there are two flower colours (white and purple), but the F_2 ratio is 9 : 7.
  This is explained if the production of the purple pigment is controlled by two enzymes in a pathway, coded by genes at different loci.

Gene P is epistatic over gene Q because the recessive allele of gene P is a mutation that produces an inactive enzyme, so there is no compound B for enzyme Q to react with. This gives just two possible phenotypes.

Genotypes	Phenotype	F_2 ratio
PPQQ, PPQq, PpQQ, PpQq	purple	9
PPqq, Ppqq, ppQQ, ppQq, ppqq	white	7

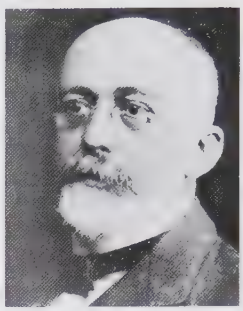
The recessive alleles do not code for functioning enzymes.
The purple pigment can only be produced when both dominant alleles are present in the genotype.

Population genetics

The gene pool is the sum of all the alleles of all the genes in a population. A population is an interbreeding group of organisms of the same species. So, in theory, the genes of any individual organism can combine with the genes of any other. The **Hardy-Weinberg principle** allows scientists to determine whether evolution has occurred. Any change in the allele frequencies in the population over time can be detected. If no evolution is occurring, then the frequencies of dominant and recessive alleles will remain constant over time. If this equilibrium of allele frequencies is to remain constant, ***five*** conditions must be met.

1 No mutations must occur, so no new alleles will be created.
2 No gene flow can occur (no immigration, so no new alleles are introduced, and no emigration, so no alleles are lost).
3 Random mating occurs, that is individuals must pair by chance.
4 The population must be large, so no genetic bottlenecks can cause the allele frequencies to change.
5 No selection can occur so that certain alleles are not selected for or against.

Obviously the Hardy-Weinberg equilibrium cannot exist in real life, since some or all of these types of forces act on living populations at certain times and evolution to some level will occur.



In 1908, two scientists, Godfrey H. Hardy, an English mathematician, and Wilhelm Weinberg, a German physician, independently worked out a mathematical relationship that related genotypes to allele frequencies

Maths skills

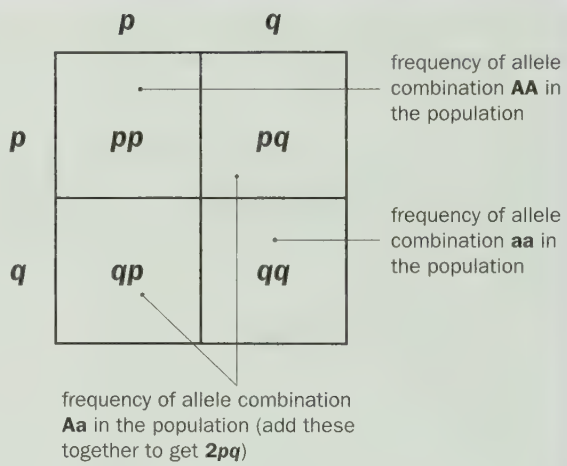
The Hardy-Weinberg equation

We can use the Hardy-Weinberg equation to calculate genotype and allele frequencies from observed phenotype frequencies:
p = the frequency of the dominant allele (represented here by A)
q = the frequency of the recessive allele (represented here by a)

For a population in equilibrium:

$$p^2 + 2pq + q^2 = 1$$

p^2 = the frequency of AA (homozygous dominant)
$2pq$ = the frequency of Aa (heterozygous)
q^2 = the frequency of aa (homozygous recessive)



Worked example

The ability to taste the chemical phenylthiocarbamide (PTC) is inherited as a single dominant, characteristic.
In a population of 1000 people, 400 are recessive and cannot taste PTC.
How can we find the number of individuals who are heterozygous and homozygous for the dominant tasting allele?
The phenotype frequency for tasting is 0.6 (600/1000), and for non-tasting is 0.4 (400/1000).
Since we know that non-tasting is a recessive allele, the non-tasters must be homozygous recessive,
so the frequency of genotype aa = 0.4
genotype aa has a frequency q^2, so $q^2 = 0.4$ $q = \sqrt{q^2} = \sqrt{0.4} = 0.6325$
$p + q = 1$, so $p = 1 - q = 1 - 0.6325 = 0.3675$

Now calculate the genotype frequencies:
frequency of AA = $p^2 = 0.3675^2 = 0.135$
frequency of Aa = $2pq = 2 \times 0.3675 \times 0.6325 = 0.465$
frequency of aa = $q^2 = 0.4$ (already found)
Make sure all of the frequencies add up to 1.
Now these frequencies can be converted into actual numbers for the given population, for example
heterozygous tasters = $0.465 \times 1000 = 465$

Regulation of gene expression

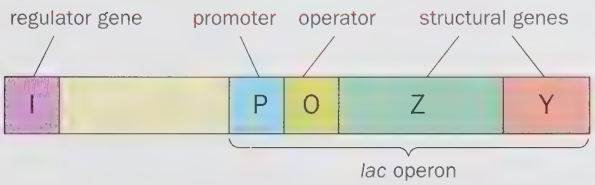
Every cell has many genes but only a few are expressed at any one time. You already know about protein synthesis, but it has to be highly regulated if the right protein is to be made in the correct amounts at the right time.

Gene expression can be regulated at any stage of protein synthesis by:

- control of transcription, when **transcription factors** control which genes are transcribed,
- control of post-transcriptional processing, when different splicing means different messenger RNAs (mRNAs) are made,
- control of translation, when mRNA can be destroyed by enzymes, or its translation by a ribosome blocked,
- control of post-translational modification, when the activity of a protein can be altered by other enzymes.

But the best known mechanisms occur *during* transcription.

The *lac* operon



The E. coli lac operon consists of the P and O sections, which control the expression of the structural genes Z and Y. They in turn code for the enzyme β-galactosidase and the protein lactose permease, respectively

Bacteria can adapt to synthesise the appropriate enzymes when a certain substrate becomes available.
If *Escherichia coli (E. coli)* that has been grown in a medium with no lactose (milk sugar) is transferred to a growing medium containing lactose initial growth is slow.
This is because two proteins are needed:

- the enzyme **β-galactosidase**, which hydrolyses lactose to glucose and galactose, and
- **lactose permease**, which carries lactose into the cell.

But very soon the bacterial cells are able to rapidly produce the two proteins, suggesting that the lactose acts as a trigger, called an **inducer**, for their production.

The ***lac* operon** is a section of bacterial DNA consisting of:

- the **structural genes** – gene Z codes for β-galactosidase and gene Y codes for lactose permease,
- the **operator** (O), a section of DNA immediately upstream from the Z gene, which is capable of switching the Z and Y genes on and off,
- the **promoter** (P), a section of DNA to which RNA polymerase binds in order to start the transcription of the structural genes Z and Y.

The regulator gene (I), is not part of the operon but produces a protein called the **repressor**.
So the *lac* operon acts as a gene switch, which ensures that the proteins are only produced when lactose is present.

The *lac* operon in action

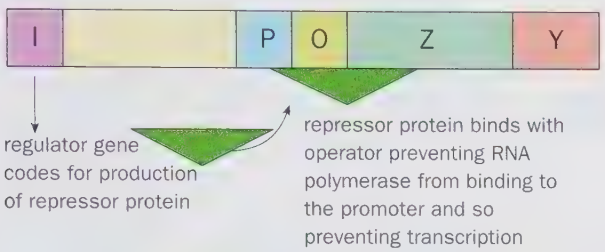
When lactose is absent

The regulator gene is transcribed and translated and a **repressor protein** is produced. This is an allosteric protein that has two binding sites, one where lactose can bind and one which binds to the operator section.

The repressor protein binds to the operator section and covers part of the promoter section. This means that RNA polymerase is unable to attach to its usual site on the promoter section.

Since RNA polymerase cannot bind to the promoter section, transcription of mRNA for the structural genes cannot take place.

Without the transcription of mRNA, translation of the structural genes cannot occur, and so β-galactosidase and lactose permease cannot be synthesised.



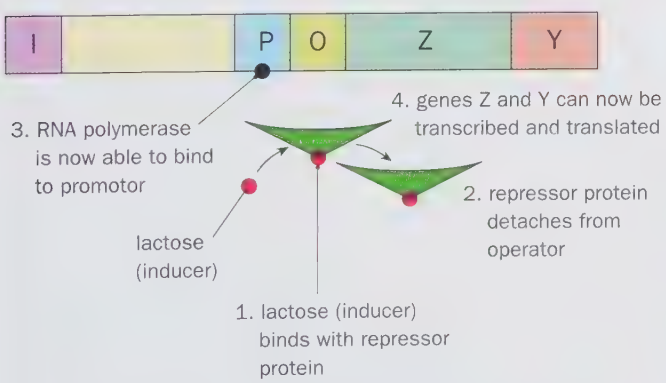
When lactose is present

Lactose acts as the inducer, and binds to the second site on the repressor protein. This causes the tertiary structure of the regulator protein to change. This change in shape affects the binding site with the operator section and the repressor protein is no longer able to engage with it.

The repressor protein breaks away from the operator section, this means that the promoter section is now free to bind with RNA polymerase and commence the transcription of mRNA for genes Z and Y.

So together, the inducer, repressor and operator act as a **genetic switch**. They initiate the transcription and as a result the translation of the structural genes Z and Y into the proteins β-galactosidase and lactose permease.

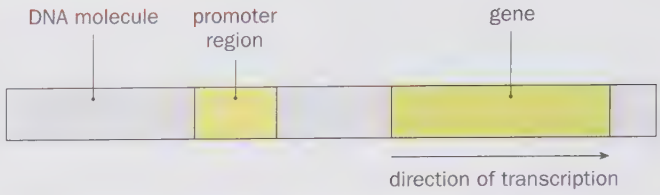
The lactose permease is used by *E. coli* to take up lactose from the surrounding medium and, subsequently, β-galactosidase is able to hydrolyse lactose into glucose and galactose.



Inducible enzymes, like β-galactosidase, are only produced in the presence of an inducer. Inducible enzymes are usually the raw materials for metabolic reactions such as respiration.

Control of transcription by transcription factors

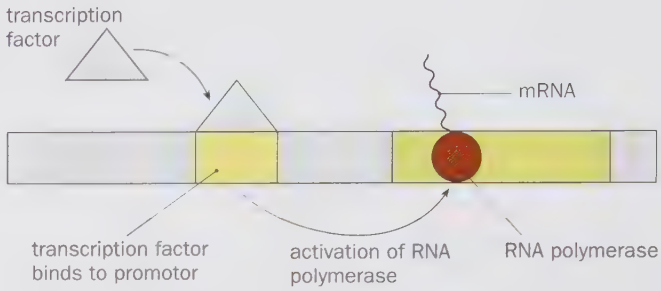
Transcription is the most important control of gene expression. This is because it is the earliest and most efficient point, so if mRNA is not produced, then it is not needed.
Every gene in eukaryotes is controlled by one or more **promoter regions**.
These are DNA sequences that lie 'upstream' of the gene that it controls, about 100 base pairs before the start of the gene.



Relationship between promoter region and functional gene on DNA molecule

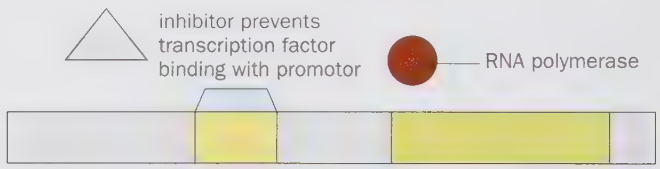
For transcription to start the gene needs to be stimulated by specific proteins called **transcription factors**.
These molecules have to move from the cytoplasm into the nucleus.
Each transcription factor has to bind to a particular promoter region on the DNA molecule.
This then enables RNA polymerase to attach to the DNA and start the process of transcription. mRNA is produced and the genetic code that it carries is translated into a polypeptide.

RNA polymerase can only begin to transcribe the gene if an appropriate transcription factor first attaches to its promoter region.



Transcription factor binds with promoter, RNA polymerase attaches to DNA, and the gene is expressed

When a gene is not expressed, that is it is switched off, the site on the transcription factor which binds to the promoter is blocked by an inhibitor molecule.
This inhibitor prevents the transcription factor from binding to the promoter so blocking transcription and inevitably protein synthesis.



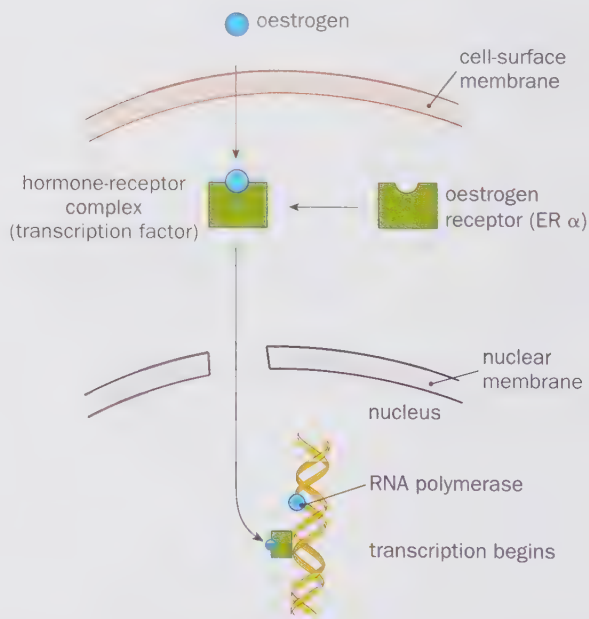
Inhibitor binds to promoter preventing transcription factor and RNA polymerase attaching to DNA – the gene is switched off

Oestrogen
Steroid hormones like oestrogen can control protein synthesis by switching on a gene to start transcription.
They do this by combining with the receptor protein called ER alpha (ER α) and forming a transcription factor.

Oestrogen, like all steroid hormones, is lipid-soluble, so the small molecules can diffuse easily through the phospholipid part of cell-surface membranes and enter the cytoplasm.
Once inside the cytoplasm, oestrogen binds to its receptor protein (ER α) forming a hormone-receptor complex, which is now a transcription factor.
The active transcription factor passes into the nucleus via a nuclear pore.
Once inside the nucleus the transcription factor binds to a DNA promoter (specific base sequence) upstream from RNA polymerase.

The combination of the transcription factor with the promoter on DNA stimulates RNA polymerase to transcribe genes and initiate protein synthesis.

Oestrogen stimulates the synthesis of many proteins in target tissues such as the uterus, hypothalamus and the breasts.

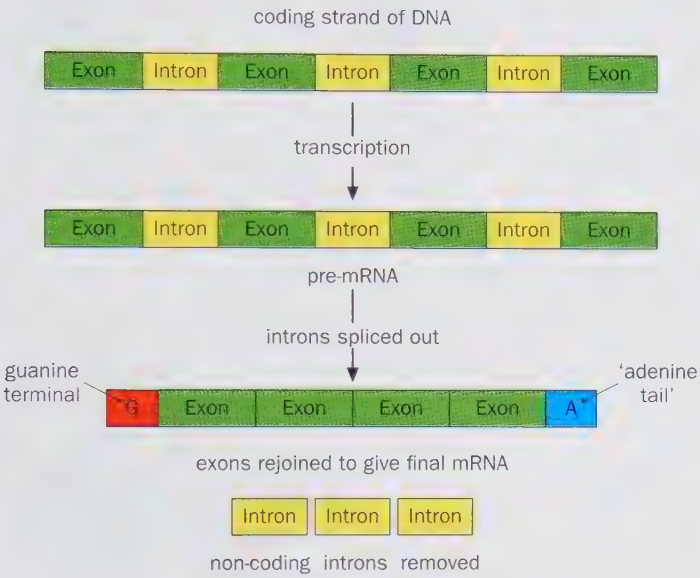


How oestrogen initiates transcription

On page 359 we looked at two mechanisms of hormonal action: the first being how protein hormones, such as insulin, operate by using a second messenger. Here we have an example of the second mechanism which is used by lipid-soluble hormones such as oestrogen.

Processing of mRNA

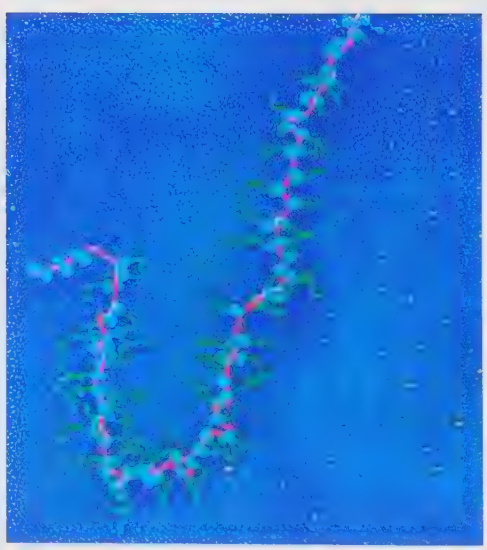
In eukaryotic cells much of the nuclear DNA does not, in fact, code for a polypeptide.
There are non-coding multiple repeats of base sequences between genes.
Even within a gene, only some base sequences, called **exons**, code for amino acid sequences.
Within the gene, these exons are separated by one or more non-coding base sequences called **introns**.
The mRNA produced at the end of transcription is called **pre-mRNA** and contains these intervening introns.
These introns have to be removed because they would interfere with the synthesis of a polypeptide.
A process called **splicing** removes the non-functional introns from the pre-mRNA.



Processing mRNA

After the introns have been cut out then removed from the pre-mRNA, the remaining exon sections can be spliced together to form the final mRNA.
A guanine nucleotide attaches to one end of the mRNA.
This is thought to initiate the process of translation when the mRNA reaches a ribosome.
About 100 adenine nucleotides are attached to the other end of the mRNA.
This 'tail' prevents the breakdown of mRNA by nucleases in the cytoplasm.
Final RNA molecules are mobile templates of the coding DNA strand.
They are too large to diffuse out of the nucleus so pass out through the nuclear pores into the cytoplasm where they associate with ribosomes to make polypeptides.
During the process of splicing when pre-mRNA forms final mRNA, the introns are removed, and the remaining exons can be rejoined in a variety of different combinations.
Different splicing patterns can lead to different RNA molecules, so a single section of DNA (gene) can code for more than one polypeptide chain.

Don't mix up exons with introns. A good way to remember the difference is that ***ex***ons are '***ex***pressed', whilst ***in***trons '***in***trude' in the coding of a gene.



TEM of translation in the midge (Chironomus). Ribosomes (blue) can be seen attached to an mRNA strand (pink). A protein (green) grows from each ribosome

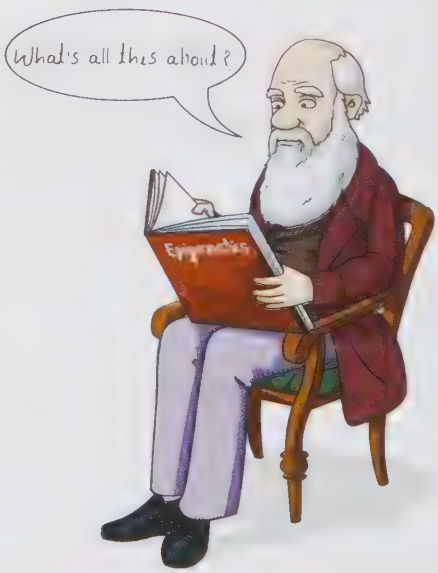
Epigenetics

We have previously learnt that the nucleotide sequence (or ***triple code***) of DNA determines the sequence of amino acids in a protein.
This involves the processes of transcription and translation.
You have seen how alleles are inherited from an individual's parents.
This is why genes are described as units of heredity, and why the sequence of the human genome has been called the 'instruction manual' or 'blueprint' for humans.

However, an organism's phenotype is determined by its genotype ***and*** its environment.
The environment can influence the expression of genes.
But, until recently, it was not thought that changes to the phenotype resulting from the environment could be passed on to the offspring.
Recent studies, however, indicate that environmental factors can cause inherited changes in the expression of genes, without altering the base sequence of DNA.

Nature versus nurture?

As we have said the DNA sequence alone is not responsible for all the phenotypic variation we see between individuals. A good example of this is identical twins.
According to the genetic crosses that we have seen, two individuals sharing identical DNA should be phenotypically identical.
Many studies have shown that this is not the case, particularly where identical twins have been separated at birth.
So what is causing the differences between these individuals if they are genetically identical? It must be the environment.



We now know that **biochemical tags** added to the genetic material can change the expression of genes, regulating when and how much protein is made from each gene.
These tags can be added or removed in response to environmental factors such as diet, stress and disease.
This explains how genetically identical twins can be phenotypically different if they have experienced different environments. It is important to remember that the tags do not change the sequence of the DNA.

The study of these tags is called **epigenetics**. The unique pattern of epigenetic tags on an individual's genome is called the **epigenome**.
One of the fundamental differences between genetics and epigenetics is that changes in the biochemical tags can be reversed in an individual's lifetime, unlike DNA mutations.

Epigenetics has been shown to be important in a wide range of examples in biology. These include the development of the queen honey bee, and the incidence of diseases such as cancer. Interestingly, these tags appear to be passed from parents to offspring, and therefore represent a way in which changes acquired in an individual's lifetime can be inherited. We will look at some examples in a little more detail later, but first we need to understand how epigenetics works.

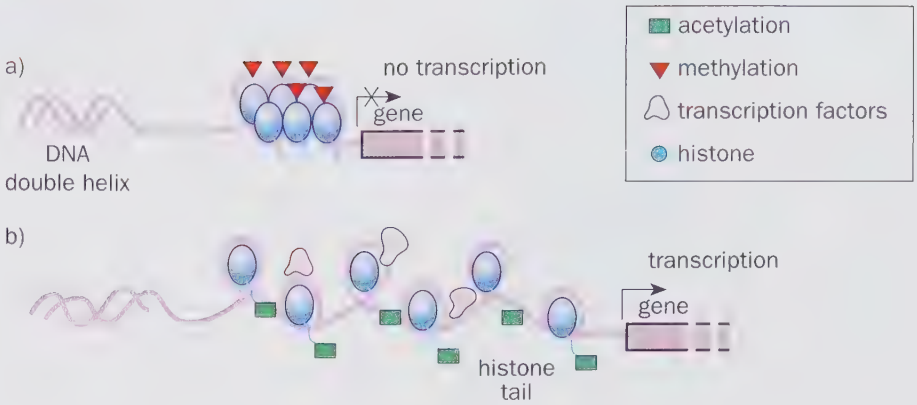
Despite being genetically identical, worker and queen bees are phenotypically very different. The queen bee, shown here in the middle, has been fed royal jelly, which has resulted in the development of large, active ovaries and a longer lifespan. This is one of the most well understood examples of how an environmental factor, such as diet, can have a significant effect on the phenotype of an individual by epigenetic changes.

Queen bee in centre surrounded by worker bees

Epigenetic mechanisms

Despite being important in a wide range of biological examples, the basic mechanism of epigenetic regulation is the same, and consists of chemical modifications to either the DNA itself or to the histone proteins around which the DNA is wound.

Methylation of DNA (the addition of a methyl group) is one form of epigenetic modification. Methylated DNA is usually in a 'closed' or 'condensed' structure. When this occurs in front of a gene, transcription factors cannot access the promoter regions of genes, and the gene is 'turned off' with little or no protein production. DNA methylation occurs on particular cytosine bases in the DNA, and is also responsible for recruiting proteins that condense the chromatin.



In a) the chromatin is in the 'closed' state. The associated DNA is methylated, and transcription factors cannot bind to the DNA to initiate transcription, so the gene is 'turned off' and no protein is made.
In b) the chromatin is an 'open' structure. The DNA is unmethylated, but the tails of the histone proteins are acetylated. The transcription factors can now bind to gene promoter regions, and the gene gets transcribed and translated into protein

Acetylation of histone proteins – conversely, the chromatin can be in an 'open' structure in which the DNA is not methylated. Instead the tails of the histone proteins are now **acetylated** (addition of an acetyl group). The transcription factors can now bind to the promoter regions, and the genes are transcribed and translated into proteins.

Inheritance of epigenetic information

One of the most interesting suggestions of recent epigenetic research is that the chemical modifications (in particular DNA methylation) can be passed on from parents to offspring.
This means that epigenetic tags laid down in response to your diet, and even stressful situations, may affect your offspring – in short you could be making choices for your grandchildren.

The exact details of how epigenetic tags can be inherited remain unclear. Yet there is increasing suggestion that this process is occurring. Some of the best evidence comes from studies on the inhabitants of the western Netherlands. During the Second World War, the inhabitants suffered an extreme famine called the Dutch Hongerwinter. Thousands of people died, and many babies were born severely underweight and malnourished. These infants were more susceptible to a range of diseases. But, incredibly, their offspring also were more likely to develop these diseases, even though they grew up with plenty of food. Some people now think that the epigenetic tags established in embryos and fetuses during the Hongerwinter have remained, and still affect the lives of individuals two generations on, although this explanation cannot be proven.

Epigenetic tags have also been shown to be involved in inheritance of behaviour.
A study with rats found that those mothers who cared well for their offspring and repeatedly groomed them had pups that were better able to cope with stressful situations in later life than pups who had little or no contact.

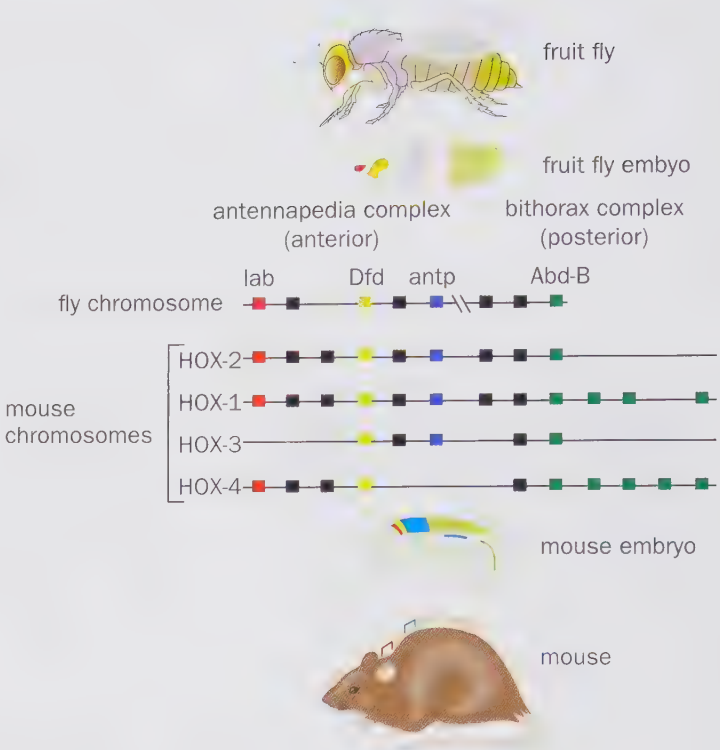
You are what your grandmother ate. Epigenetic tags established during development of your parents and grandparents could have an influence on your phenotype

Developmental genetics

As we have seen on page 388, the regulation of gene expression is an important biological process.
We have learnt that normal patterns of protein production are essential for maintaining a healthy individual.
So how are these "normal" patterns of gene expression established?

To answer this question we first need to understand that 'normal' depends on the type of cell, and differs depending on its developmental stage.
During the course of development, a single cell transforms itself into an adult organism, containing around 350 different types of cells in humans.
Cells change into different types of cell because some genes are activated (switched on), and some genes are inactivated (switched off).
As a result, the cell produces a specific set of proteins.
So, for example, a nerve cell produces only the proteins needed to make a nerve cell, and a muscle cell produces only the proteins needed to make a muscle cell.

Cells know which genes to switch on and off at different times using special genes that control the activity of other genes.
So, for example, **homeotic** or **homeobox** genes control whole sets of other genes to set out the basic body plan of the embryo, separating the front from the back, and producing the right body structure in the right place.



Related homeobox genes are found in different species, and their pattern of expression is important for determining the development of the overall body plan

In humans, there are an estimated 235 functional homeobox genes.
They all contain a particular DNA sequence that provides instructions for making a string of 60 amino acids known as the **homeodomain**.
Most homeodomain-containing proteins act as transcription factors, which means they bind to and control the activity of other genes.
The homeodomain is the part of the protein that attaches to specific regulatory regions of the target genes.
Homeobox genes are evolutionary ancient, and similar genes are found in animals, plants and fungi.

Genes in the homeobox family are involved in a wide range of critical activities during development.
These activities include directing the formation of limbs and organs along the anterior–posterior axis and regulating the process by which cells mature to carry out specific functions (differentiation).
Because homeobox genes have so many important functions, mutations in these genes are responsible for a variety of developmental disorders.
For example, mutations in the Hox group of homeobox genes typically cause limb malformations.

Mutations to Hox genes can put an entire leg where an antenna should sprout out and produce other equally grotesque transformations

Apoptosis

Apoptosis or programmed cell death occurs in all multicellular organisms. It should not be confused with **necrosis** which occurs when a cell is damaged by an external force such as an infection, poison or bodily injury. Necrosis involves the release of hydrolytic enzymes from lysosomes, the cell swells and bursts and inflammation occurs.
The process of apoptosis on the other hand is a controlled, predictable routine.
The human body replaces about 1 million cells per second.

In adult tissues, cell death exactly balances cell division.
If this was not the case, the tissue would grow or it would shrink.
If the rate of mitosis exceeds the rate of apoptosis then tumours can form.
If the reverse is the case, cell loss and degeneration can lead to strokes and degenerative diseases such as Alzheimer's and Parkinson's.
When programmed cell death is triggered, proteins called **caspases** (which exist in all cells as inactive precursors) go into action.
They break down the cell components and stimulate the production of other enzymes known as **DNAases**, which destroy DNA in the nucleus.

Sequence of events

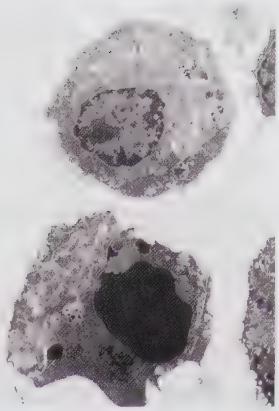
- The cell shrinks and becomes rounded because of the breakdown of the cytoskeleton by caspases.
- The cytoplasm appears dense with cell organelles tightly packed together.
- Chromatin condenses and the DNA becomes fragmented. The nuclear membrane breaks down.
- The cell-surface membrane shows irregular buds called **blebs**.
- The cell then breaks apart into vesicles.
- Macrophages ingest the vesicles and cell debris by **phagocytosis** without causing damage to any other cells and tissues.

Apoptosis progresses very quickly and the breakdown products are soon removed making it difficult to detect.

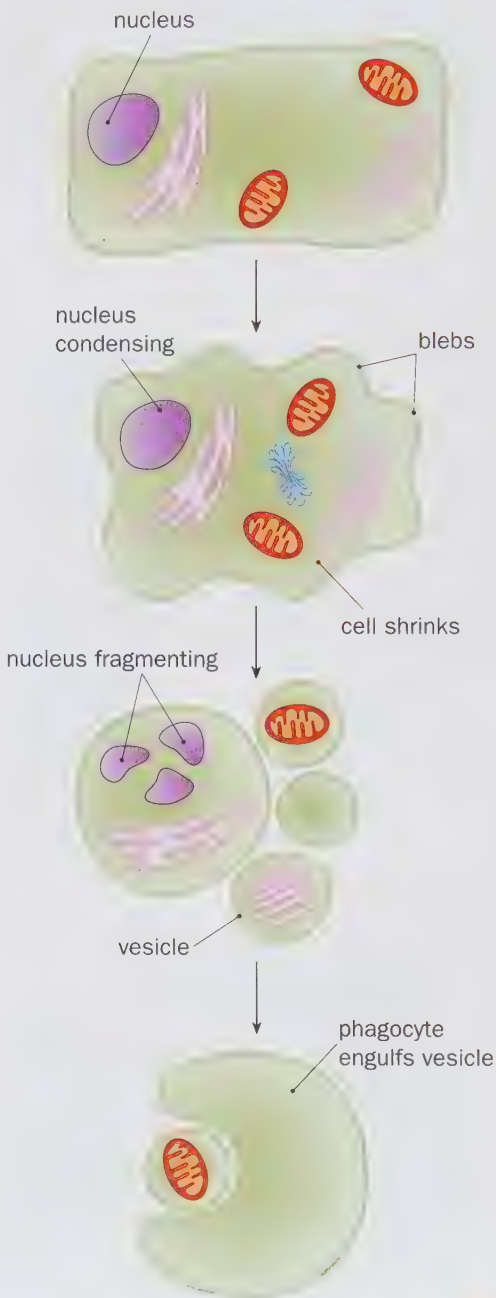
What triggers apoptosis?

Cells that go through apoptosis die in response to signals both inside and outside the cell. These signals may involve a number of chemicals such as cytokines, made by cells of the immune system, hormones and growth factors. When cells recognise viruses and cell mutations this may induce death to prevent the damage from spreading.

Apoptosis plays an important part in tissue development both in plants and animals.
Balanced against the multiplication of cells due to mitosis, different tissues use different signals to induce apoptosis so affecting body form.
For example during the development of limbs, apoptosis causes the fingers and toes to separate from each other.
Scientists are working to understand how they can control which cells live and which undergo programmed cell death.
Anti-cancer drugs and radiation, for example, work by triggering apoptosis in diseased cells.
As we have seen many diseases and disorders are linked with the life and death of cells, so learning how to modulate apoptosis could be the first step towards treating these conditions.



TEM photomicrograph of prostate cells undergoing apoptosis



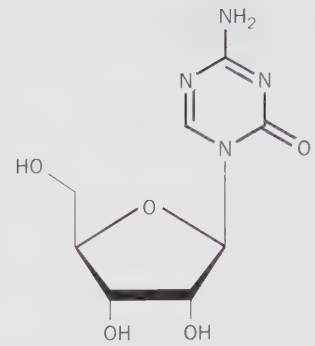
Stages in apoptosis

Biology at work: Epigenetics and disease

There is now a growing body of evidence that DNA methylation and histone acetylation have a key role to play in many diseases, including autism, dementia, diabetes and cancer.

Tumour suppressor genes usually encode proteins that are responsible for stopping tumours developing. Many of these tumour suppressor genes are more heavily methylated in cancerous cells than healthy cells, so as we saw on page 393, this will prevent them from being expressed and so they cannot do their job to prevent cancer occurring.

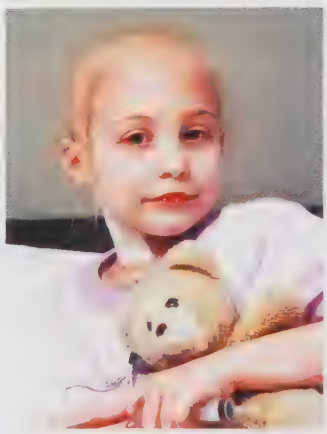
Understanding the epigenetic changes that occur in diseases is important for understanding the prognosis of the disease and developing new therapies. In particular, the ability to turn on or off genes that have been disrupted by epigenetic changes with chromatin remodelling drugs is a promising area of research. Extensive trials are needed though, as the drugs must target diseased cells in particular, and not cause extensive epigenetic changes in healthy cells. This could result in more dysregulated cells and further disease. However, drugs such as azacitidine which prevents DNA from being methylated are already being used to treat some cancers.



Molecular structure of 5-azacytidine, sold under the trade name Vidaza, which is an epigenetic drug used to treat several cancers

There have been many exciting new developments in the treatments of various cancers with epigenetic drugs recently. For example, Dr Jeanne Pierre Issa has led the first trial in humans to test a new methylation-blocking drug called SGI-110 in the treatment of two types of **leukaemia**.

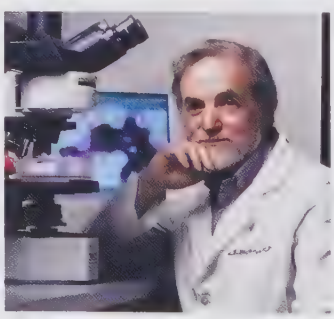
Leukaemia describes a group of cancers which originate in the bone marrow, and there is lots of evidence that leukaemic cells have disrupted patterns of DNA methylation. **Myelodysplastic syndrome** (**MDS**) is a type of leukaemia in which the bone marrow does not make enough healthy blood cells and there are abnormal (**blast**) cells in the blood and/or bone marrow. **Acute myelogenous leukaemia** (**AML**) is characterised by the rapid growth of abnormal white blood cells.



Over 8000 people were diagnosed with leukaemia in the UK in 2011. Finding new therapies to treat them is an exciting area of epigenetic research

Sixty-six patients with MDS or AML were enrolled in Dr Issa's trial, which tested a series of doses in two treatment schedules of SGI-110. Two patients with AML, whose disease had returned after previous treatments, have complete remission; and one had partial remission. The patients who had complete remission had the greatest decrease in methylation and the highest levels of the drug in their circulatory system. Treatment was generally well tolerated, with only moderate side-effects. The lack of side-effects makes sense, Dr Issa explained, because "cancer cells are much more reliant on DNA methylation for survival than normal cells."

Work by other scientists has shown the effectiveness of combining methylation-blocking drugs such as decitabine with azacitidine. Dr Stephen Baylin's team at Johns Hopkins University School of Medicine found that low doses of the drugs together had anti-tumour effects in cell lines and in mouse models of different cancer types – including leukaemia and breast and colon cancers. Post-treatment analyses of treated cells showed decreased DNA methylation and the reactivation of genes that can affect tumour growth and cell death.



"Our research has contributed heavily to the concept that epigenetically-mediated loss of gene function is a major player in the progression of human cancer. It is also contributing to the translational goal of targeting reversal of abnormal gene silencing as a cancer prevention and/or therapy strategy" Dr Baylin

Biology at work: Genetic screening

Genetic screening (often called genetic testing) is used to find out whether a person is carrying an altered gene (mutation) that causes a particular medical condition.

The United Kingdom Genetic Testing Network (administered by the NHS) holds a directory of genetic disorders for which there are tests available. In 2012 this directory contained a list of 579 disorders and 729 associated genes.

Genetic screening is useful to:

- confirm a diagnosis if someone has symptoms,
- show whether a person is a carrier for a genetic disease,
- help expectant parents know if an unborn child will have a genetic condition.

A geneticist analysing human chromosomes by fluorescent microscopy

Types of genetic screening

Genetic screening looks at DNA taken from blood, body fluids or tissues and there are three main types of test.

- **Molecular genetic tests** (or gene tests) look at single genes to identify mutations. The disease CF is tested for in this way.
- **Chromosomal genetic tests** look at the structure, number and arrangement of a person's chromosomes. A **karyotype** produces a picture of a complete set of chromosomes and allows for the identification of conditions like **Down's Syndrome**.
- **Fluorescent in-situ hybridisation** (**FISH**) analysis can detect very small pieces of chromosomes that might be missing or extra (for example in **Duchenne muscular dystrophy**).
- **Biochemical tests** look at the key proteins coded for by DNA. Abnormal amounts or levels of activity in these proteins can signal gene defects.
  These tests are often used in screening of children just after birth. One test can be used to detect the metabolic condition **phenylketonuria**.

Scientist preparing a DNA probe

How are tests carried out?

Chromosomal tests often involve relatively simple staining and microscopy techniques.
However, gene tests are more complex and often involve **DNA probes**.

A DNA probe is a short length of DNA which has a base sequence complementary to (and, therefore, able to bind with) the sequence on the altered gene. These probes usually have fluorescent chemical markers attached to them and during a test the probe is combined with DNA extracted from the patient. If the altered gene is present, the probe will bind to it and the marker is used to identify its presence.

Another type of gene test uses a technique called sequencing.
In this test the base sequence of DNA or RNA from a patient is compared with that of a normal version of the DNA or RNA.

Genetic counselling

Genetic counselling is available to anyone undergoing or thinking of undergoing genetic screening. It involves talking to an expert to allow patients to consider the psychological and other consequences should they test positive for a genetic condition.

It is important that genetic screening is followed by expert counselling

Biology at work: Genetic mapping

In simple terms **genetic mapping** is the process of establishing the location and relative position of genes on specific chromosomes.
The work of the **Human Genome Project** has led to the availability of easy to use, high resolution genetic maps. These maps have enabled scientists to identify which chromosome contains the gene associated with a particular disease, and precisely where it lies on that chromosome.
Genetic maps are an important step in helping to diagnose, identify risk factors for, and eventually develop treatments for genetic diseases.

How are maps created?

To create a genetic map scientists extract DNA from blood or tissue samples from members of a family where a particular disease is prevalent.
They then look for the unique sequences of bases seen only in those family members who actually have the disease.
These sequences are known as **polymorphisms** (or more commonly markers).
With the aid of genetic maps scientists use DNA markers to locate the approximate position of the gene associated with the disease.

Types of genetic map

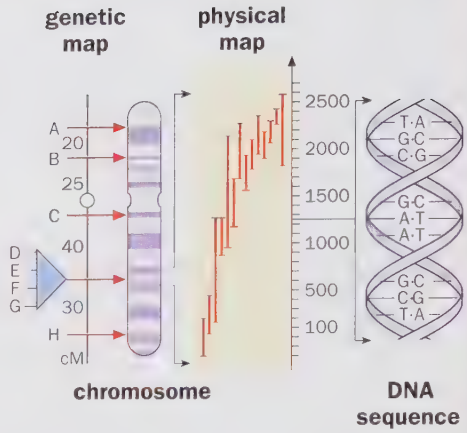
Linkage maps show the arrangement of genes and markers along a chromosome.
These maps make use of the features of inheritance known as linkage and recombination that you will have read about on page 381.
If a disease-causing gene is close to a DNA marker then they are more likely to stay together during recombination and be passed on together from parent to child.
If each family member with the disease also inherits the DNA marker it is likely that the gene causing the disease is positioned near to the marker.

The result is that inherited diseases can be located on the map by following the inheritance of the DNA marker.

Physical genetic maps can be divided into a number of types including:

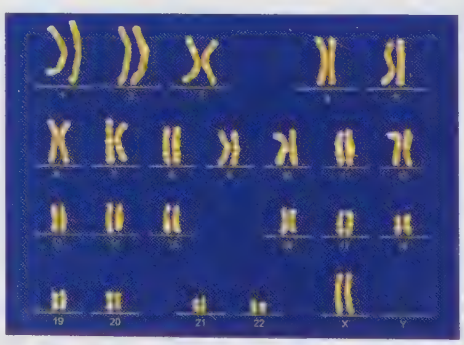
- **Cytogenetic maps** which are the most familiar but the lowest resolution. They show the distinctive banding patterns observed by light microscopy of stained chromosomes.
- **Sequence maps** are the highest resolution physical map and are created by producing fragments of DNA that can be assembled to produce a highly detailed map of the entire nucleotide sequence. The actual distance between markers can be worked out using gel electrophoresis (see page 23 for a reminder about this technique).

Genetic maps have been used to find several important disease genes; and in 2014 scientists announced a breakthrough in the study of schizophrenia. Schizophrenia is a debilitating mental disorder that makes it hard to tell the difference between what is real and not real. Scientists carried out a study of 150 000 people, of whom 37 000 had been diagnosed with schizophrenia. This study found 108 genetic markers for people at risk of developing the condition, of these 83 had not been previously reported.
This work provided the first real genetic evidence linking the immune system to schizophrenia.



This model of a genetic map shows the location of 8 markers (A–H) along the chromosome. Physical maps are not representations but overlapping collections of DNA fragments.

A scientist carrying out genetic mapping



G-Banded human female chromosomes

Summary

- A gene is a section of DNA that codes for a particular character. It is found at a particular position (the locus) on a pair of homologous chromosomes. There may be different forms of the same gene called alleles.
- If both alleles are the same, the organism is homozygous. If the alleles are different, the organism is heterozygous.
- During meiosis, only one of a pair of alleles can be passed into a gamete (this is Mendel's first law).
- A dominant allele expresses itself in the heterozygous state, masking the recessive allele.
- The phenotype is what is presented to the environment. It may be the physical appearance but can include biochemical characteristics.
- The genotype is the genetic make-up of an individual.
- A monohybrid cross is the study of one gene; a dihybrid cross involves the inheritance of two separate genes.
- In dihybrid inheritance, either of a pair of alleles may combine randomly with either of another pair (this is Mendel's second law).
- A test cross is carried out to see whether an individual is homozygous or heterozygous.
- Genes present on the same chromosome are said to be linked and are inherited together. Crossing over in prophase I of meiosis can separate linked genes.
- Codominance is where the heterozygous individual has a phenotype intermediate between the two homozygous parents. Both alleles are expressed and contribute to the phenotype.
- Genes carried on the same sex chromosome are said to be sex-linked.
- In epistasis, two genes at different loci interact to control a single characteristic.
- The Hardy-Weinberg principle allows scientists to determine whether evolution has occurred.
- Gene expression involves the regulation of transcription and translation.
- Epigenetics involves heritable changes in gene function without changes to the base sequence of DNA.
- Apoptosis can act as a mechanism to change body plans.

Questions

1 A red-flowered plant with the genotype **TtRr** is tall but self-pollination gives rise to a seed which produces tall, red-flowered; tall, white-flowered; short, red-flowered; and short, white-flowered plants in the ratio of 9:3:3:1 respectively. The short, white-flowered plants have the genotype **ttrr**. The following cross was carried out: **TtRr** × **ttRr** and can be represented in this diagram.

	TR	Tr	tR	tr
tR	TtRR	TtRr		ttRr
tr	TtRr		ttRr	

a) Copy and complete the diagram of the genetic cross.

b) Give the phenotypes of all the genotypes from the cross, in their correct ratio.

2 Mendel's first law states that the characteristics of an organism are determined by internal factors which occur in pairs, and that only one of a pair of such factors can be represented in a single gamete.

a) Give the modern name for
   i) an internal factor controlling part of an organism's characteristics,
   ii) different forms of the internal factor.

b) Explain why these factors occur in pairs rather than singly.

c) Explain why only one of a pair of such factors can be represented in a single gamete.

3 In guinea pigs, the allele for black coat **B** is dominant to the allele for albino **b**, and the allele for rough coat **R** is dominant to the allele for smooth coat **r**.
A heterozygous black, smooth-coated guinea pig is mated with a heterozygous rough-coated, albino guinea pig.
Show the cross diagrammatically and give the ratio of the phenotypes of the offspring.

4 In maize, the allele for coloured grain **C** is dominant to the allele for colourless **c**, and the allele for rounded grain **R** is dominant to the allele for wrinkled grain **r**.

a) Explain what we mean by
   i) a gene,
   ii) an allele.

b) Maize plants heterozygous for both characteristics were crossed and the following phenotypes were obtained:

   83 coloured, smooth
   38 coloured, wrinkled
   29 colourless, smooth
   8 colourless, wrinkled

   Why do you think that the results were not an exact 9:3:3:1 ratio?

c) Some of the colourless, rounded grain would have the genotype **ccRr** and some would be **ccRR**. Explain how you would use a test cross to distinguish between the two genotypes.

5 Two genes in a mouse interact to control ***three*** possible coat colours: grey, black and chocolate.
The two genes are located on different chromosomes (autosomes).
Each gene has two alleles: **A** is dominant to **a**, and **B** is dominant to **b**.
Examples of four genotypes and their phenotypes are shown in the table:

Genotype	Phenotype
AABb	grey
Aabb	grey
aaBb	black
aabb	chocolate

a) What colour coat would you expect each of the following genotypes to give:
   i) **AABB**,
   ii) **AaBb**,
   iii) **AAbb**,
   iv) **aaBB**?

b) An **AABB** male was crossed with an **aabb** female.
   i) Give the genotype of the F_1.
   ii) Give the phenotypes of the F_2 and their ratio.

c) A female from the F_1 in b) i) was crossed with a chocolate male. What would be the ratio of the phenotypes for this cross?

6 In a breed of domestic fowl, pea comb is dominant to single comb, but feather colour shows codominance. Black feathers and white feathers are homozygous, that is **BB** and **WW** respectively, whereas the heterozygous condition **BW** gives the intermediate 'blue' feathers.

a) Show the cross between the birds that are heterozygous for both alleles.

b) What proportions of the offspring would be
   i) pea-combed,
   ii) black-feathered,
   iii) blue-feathered,
   iv) pea-combed and blue-feathered,
   v) single-combed and white-feathered?

7 The inheritance of coat colour in cats is influenced by a gene present on the X chromosome but not on the Y. The allele for black coat colour can be represented by the symbol **B**, and that for ginger coat colour by **G**. These alleles are codominant and the hairs of heterozygous cats show bands of both black and ginger, called tortoiseshell.

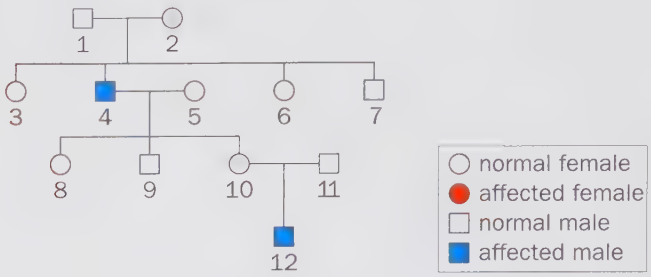
a) Define the term codominant.

b) Use a diagram to show a cross between a ginger male and a tortoiseshell female.

8 a) State two reasons why female haemophiliacs are rarely found in a population.

b) Use suitable symbols to draw a genetic diagram to show the cross between a carrier female and a haemophiliac male.

9 Pituitary dwarfism is an inherited condition in humans, in which affected individuals have very short limbs. The allele for pituitary dwarfism **d** is recessive to the allele for normal limbs **D**, and its locus is situated on the X chromosome. The pedigree diagram shows part of one affected family.



a) Identify and explain one piece of evidence from the diagram to show that the allele for pituitary dwarfism is recessive to the allele for normal limb length.

b) Explain why the genotype of
   i) individual 10 must be $X^D X^d$,
   ii) individual 11 must be $X^D Y$.

10 The shells of a species of snail may be banded or unbanded. The absence or presence of bands is controlled by a single gene with two alleles. The allele for unbanded, B, is dominant to the allele for banded, b. In a population of 175 individuals the frequency of the B allele is 0.6, and the frequency of the b allele is 0.4.

a) If the snails mated randomly, what frequencies of the B and b alleles would be expected in the next generation?

b) Use the Hardy-Weinberg equation to calculate the number of individuals with each phenotype. Show your working.

11 a) What are transcription factors?

b) Explain fully the role of the following in epigenetic regulation:
   i) methylation of DNA,
   ii) acetylation of histone proteins.

c) Explain what is meant by homeobox genes.

22 Gene technology

Gene technology enables scientists to manipulate DNA in many ways.
Individual genes can now be located in the DNA of an organism.
An individual gene can be isolated, removed and cloned.
The DNA of one organism can be combined with the DNA of another.
Genes can also be made from the RNA of an organism.
There are already many commercial applications of genetic engineering, and future developments may well provide the means to alleviate suffering and cure disease.
However, there are many reservations about the long-term effects of a genetically-modified world.

Technician at microscope injecting cells with DNA

The basic principles of genetic engineering

Restriction endonucleases are enzymes that cut DNA into small fragments.
This allows individual genes to be isolated.

A gene from one organism can be inserted into the DNA of another.
The gene that has been isolated for insertion is called **donor DNA**.
The donor DNA is inserted into the host DNA of another organism.
DNA that contains genetic material from ***two*** different organisms is called **recombinant DNA**.

Restriction endonucleases are used to cut the donor DNA and the host DNA into smaller pieces.
The enzymes make staggered cuts, called 'sticky ends', which allow the donor DNA to be spliced into the host DNA.

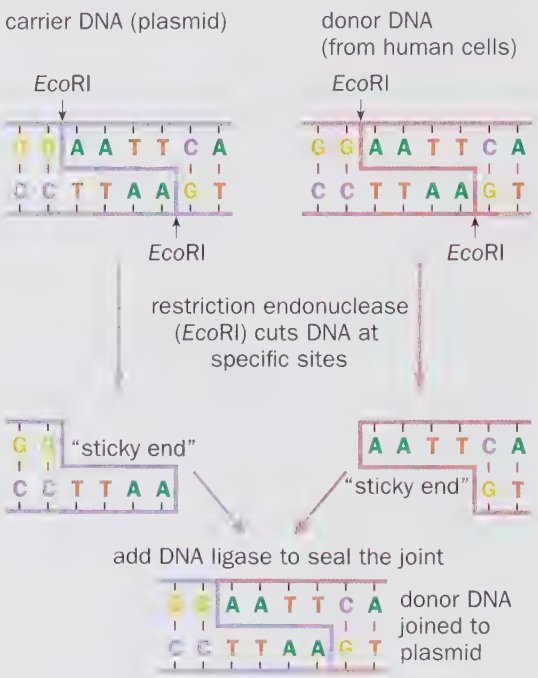
Another group of enzymes, called **DNA ligases**, are used to join the sticky ends of the donor DNA and the host DNA together.
The sticky ends are complementary.
For instance, an ATG end will 'stick' to a TAC end.

As you will see, host cells with recombinant DNA can be used to clone genes or to produce important substances such as antibiotics, hormones and enzymes.

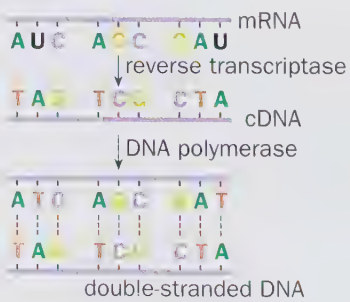
An enzyme called **reverse transcriptase** can be used to make a gene from RNA.
First, the RNA is extracted, which is a mirror image copy of the desired gene.
Then reverse transcriptase is used to make a single strand of **copy DNA** (**cDNA**) from the isolated RNA.

The single strand of cDNA can be used as a template to make the second strand, so forming the double-stranded DNA helix of the gene.

The cDNA coding for rennin has been spliced into a plasmid in *Escherichia coli* cells.
Non-animal rennin can then be produced by replication of the bacterial cells in large industrial fermenters.



How 'sticky ends' are made



The use of reverse transcription and DNA polymerase to make double-stranded DNA

Production of insulin

Some human diseases are caused by the inability of a person to produce certain chemicals.
For instance, diabetics are unable to make their own insulin and haemophiliacs are unable to produce factor VIII.
Many of these chemicals are proteins coded for by DNA.
In the past, some of these diseases have been treated using chemicals extracted from animals.
Sometimes this resulted in side-effects produced by the patient's immune system.

Genetic engineering has now made it possible to use bacteria to mass-produce human insulin.

First, the gene that codes for insulin is identified and isolated from a healthy human cell.
Then the donor DNA is spliced into the host bacterial DNA.
The bacterial cells are grown in a nutrient medium in a large industrial fermenter, which provides ideal conditions for growth.

The bacterial cells multiply rapidly and soon there are millions of cells all carrying the insulin-producing gene.
The gene is transcribed and translated and the insulin produced.
This type of insulin does not have the side-effects associated with animal insulin.

A man gives himself an insulin injection

Industrial fermentation

Fermentation involves the aerobic and anaerobic respiration of microbes.
Industrial fermentation is now widely used to culture cells such as bacteria and yeasts.
Genetically-altered cells can be produced on a large scale, producing useful chemicals such as antibiotics and biogas.
Strictly speaking, fermentation refers to anaerobic respiration but it is generally accepted to include aerobic respiration as well.

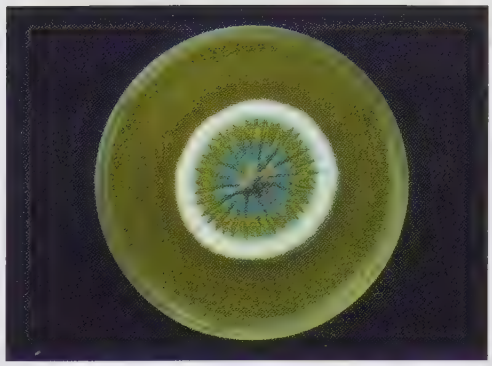
There are many advantages in using microorganisms, such as bacteria and yeasts, in industrial fermentation.

- They have a very rapid rate of growth.
- They can be grown continuously on a large scale.
- Their cells have a high protein content.
- They can utilise waste products as substrates, for example agricultural waste.
- They usually produce products that are non-toxic.
- Because they are living organisms, their chemical reactions are controlled by enzymes.
  This means that fermentation can take place at lower temperatures and therefore the production is cheaper.

A large industrial fermenter

Batch and continuous cultivation

One important use of large-scale industrial fermentation is the production of antibiotics such as penicillin.
The fermenter is inoculated with a culture of a suitable fungus, in this case *Penicillium chrysogenum*, which then proceeds to grow under the ideal conditions maintained inside the fermenter.



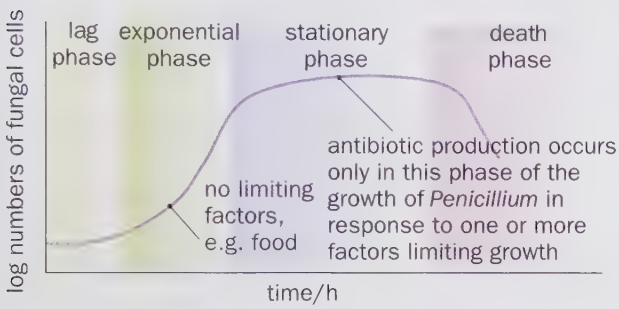
Penicillium growing on agar

These include:

- ***Adding nutrients*** such as sugar or starch as a source of carbon for respiration.
  Ammonia or urea is added as a source of nitrogen to make proteins.
  Vitamin B complex is added for respiration.
- ***Maintaining a constant temperature*** of about 30 °C.
- ***Maintaining a constant pH*** of about 6.5.

Batch cultivation

It takes about 30 hours for penicillin production to start.
Penicillin is secreted into the surrounding liquid by the fungus.

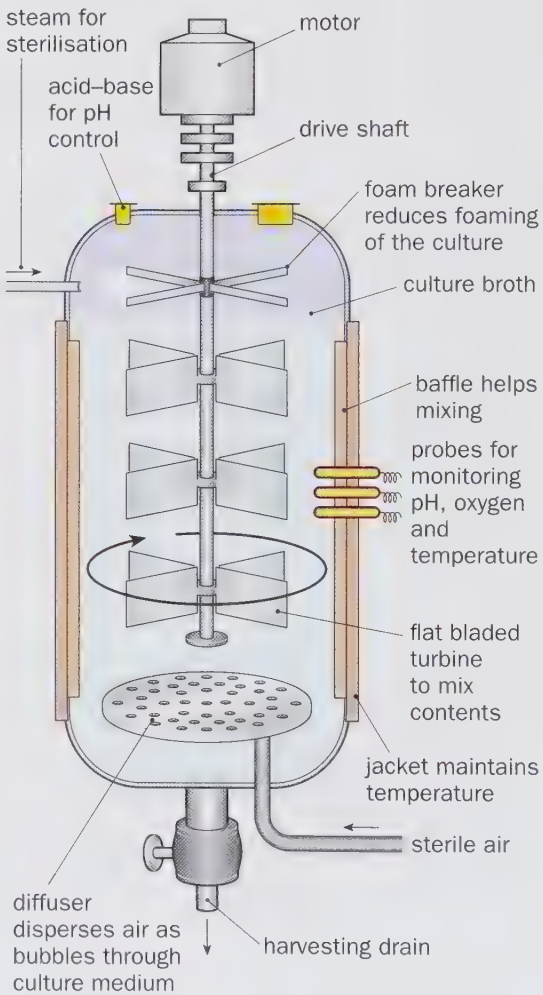


Look at the graph. You can see there is a delay.
This is because penicillin is a **secondary metabolite**, a substance which is not necessary for the growth of the fungus.
Secondary metabolites are not produced until ***after*** the exponential phase of growth is completed.
After about 6 days, the mixture in the fermenter is filtered, the penicillin is extracted using a solvent and purified into a crystalline salt.
This type of fermentation is known as **batch cultivation**.
After the 6-day period, the fermenter is emptied, cleaned and sterilised ready for the next batch.

Continuous cultivation

This type of cultivation allows production to continue for several weeks, since raw materials are added throughout the process and the products are continuously being removed.
This method is only suitable for products that are known as **primary metabolites**.
These are substances that are essential for the life of the microorganism and, as a result, they are produced throughout the growth of the organism.

Industrial fermenters are huge tanks in which conditions are carefully controlled.
They may hold 500 000 dm^3 or more and allow the economic production of vast quantities of important products.



Continuous cultivation in an industrial fermenter

Gene cloning using vectors

Sometimes large quantities of a particular gene are required.
As you will see on page 407, a possible treatment for **cystic fibrosis** (**CF**) requires many copies of the appropriate healthy human gene.
Techniques in gene cloning allow this to be achieved.

The DNA fragment containing the wanted gene is inserted into the DNA of a host cell.
The host cell is known as a **vector**.
The recombinant DNA acts as a carrier molecule for the gene that is to be copied.
As the host cell reproduces and replicates its DNA, clones of the required gene are made at the same time.

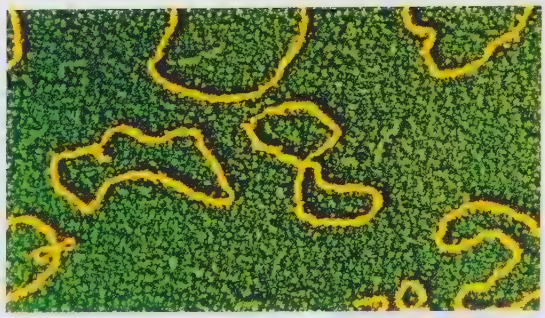
Bacteria, viruses and even some eukaryotic cells have all been used as vectors.
Bacteriophages can be used as vectors since they are able to inject the recombinant DNA into bacterial cells such as *E. coli*.
As you saw in Chapter 3, the host cell then replicates the virus many times, making multiple copies of the recombinant DNA.

The most commonly-used vector is a bacterial **plasmid**.
In addition to their single loop of DNA, bacterial cells also contain small circles of DNA called plasmids.
Plasmids are easy to work with since they can replicate very quickly, producing many copies of the original gene.

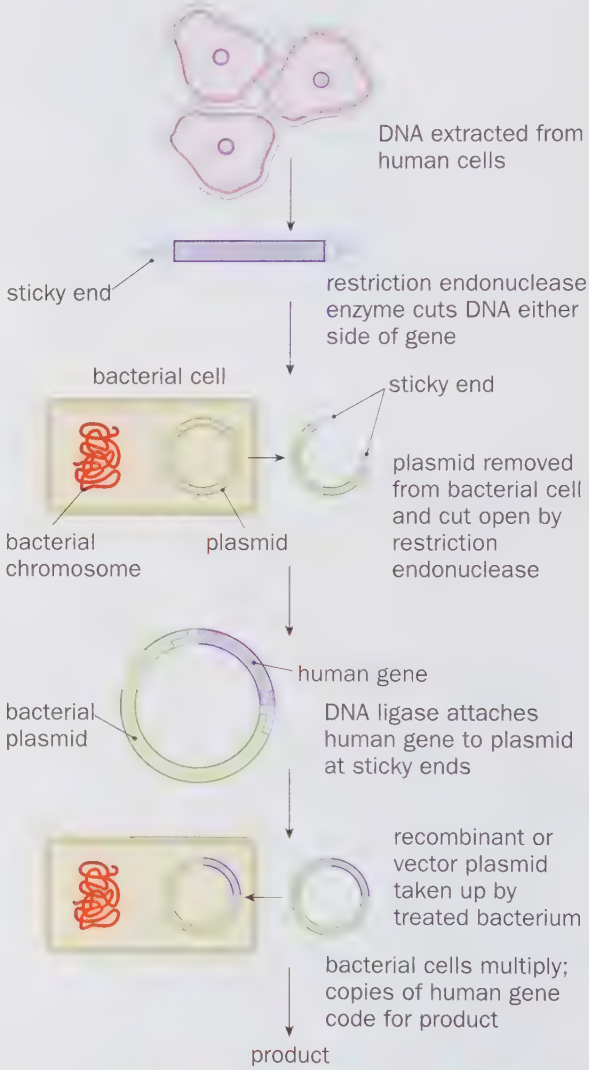
Plasmids can be isolated and cut open by restriction endonucleases.
A human gene that has been cut out of a human chromosome by restriction endonuclease can be spliced into the plasmid using DNA ligase.
The recombinant plasmid is then inserted into the host bacterium.
The bacterial cells are grown in nutrient medium in industrial fermenters.
In these conditions, the bacteria multiply rapidly, making many copies of the human gene.
The human DNA is transcribed and translated by the bacterial cells, so producing the human protein.
The protein is then separated and purified.

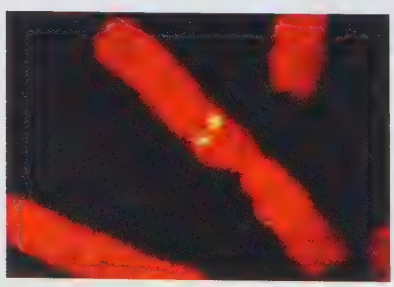
Eukaryotic cells have also been used as vectors to clone recombinant DNA.
Yeast cells often have naturally-occurring plasmids in them.
They can be used to make **yeast artificial chromosomes** (**YACs**).
These are used for cloning larger DNA fragments.

Sometimes **marker genes** are used to indicate that new genes have been incorporated into host cells.
Marker genes are linked to the desired gene, so they give clear evidence that the desired gene has been carried to the host cell.
Some of them may be radioactive so that the position of the labelled gene can be easily located.
Bacterial cells containing genetically engineered plasmids can also be identified by the use of marker genes that confer antibiotic resistance.
So the cells into which the gene has been inserted can be identified by the fact that the host cell is now resistant to a certain antibiotic.



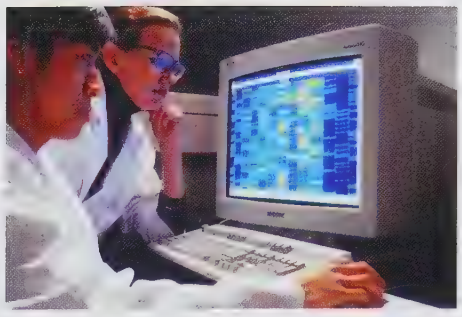
Scanning electron micrograph of bacterial plasmids





Radioactive marker genes on a chromosome

Polymerase chain reaction



The **polymerase chain reaction** (**PCR**) allows gene cloning to take place in a test-tube.
The reaction enables many identical copies of double-stranded DNA to be made without the use of bacteria.
Each strand is copied, producing two new strands, then each of these is copied and so on, doubling the amount of DNA at each cycle.
This is semi-conservative replication of DNA in a test-tube.

The raw materials

The original sample of DNA is dissolved in a buffer solution and mixed with the following:

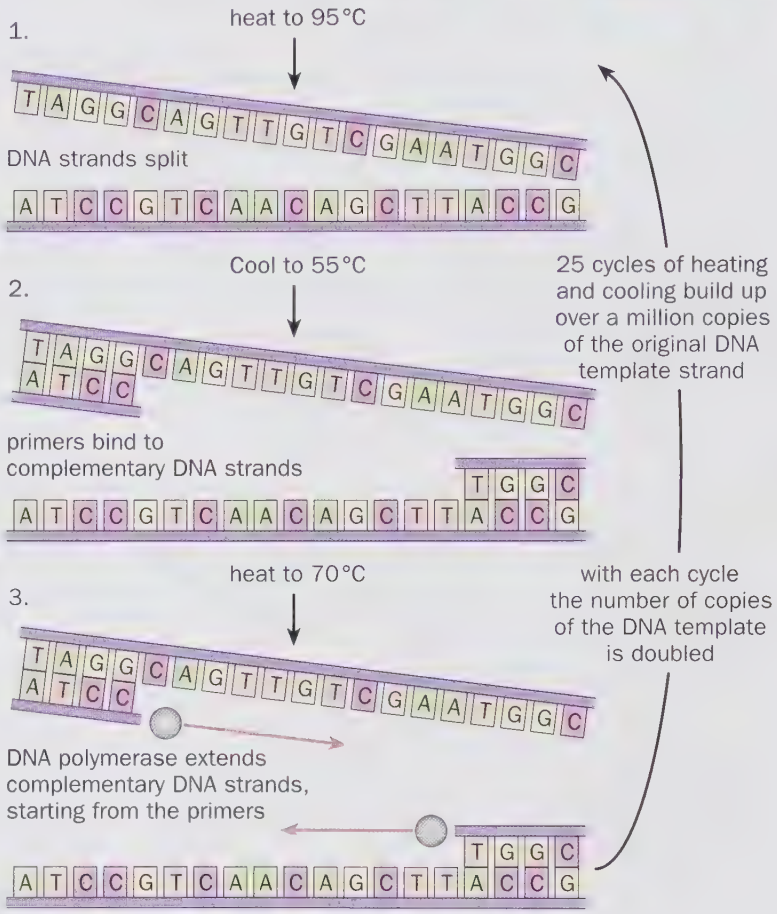
- DNA polymerase (this is a heat-stable form of the enzyme extracted from the thermophilic bacterium *Thermus aquaticus*),
- the four different types of nucleotide containing the bases adenine, guanine, cytosine and thymine,
- short pieces of DNA called **primers**, which act as signals to the DNA polymerase enzyme to start copying.

Stages of the PCR cycle

1 **Strand separation:** the original DNA (called target DNA) is heated to 95 °C for 5 minutes and denatured.
It separates out into two single strand lengths of DNA.

2 **Primer binding:** the solution is rapidly cooled to 55 °C to enable the primers to bind to the complementary base sequences on each of the single strands of DNA.
This provides a starting point for the DNA replication.

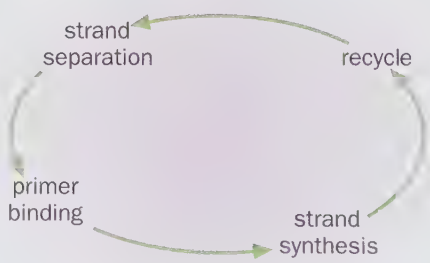
3 **Strand synthesis:** the solution is heated to 70 °C. The thermostable DNA polymerase enzyme catalyses the synthesis of a complementary strand for each of the single strands of DNA using the supply of nucleotides. The DNA polymerase produces two identical double strands of DNA.



Stages of the PCR cycle

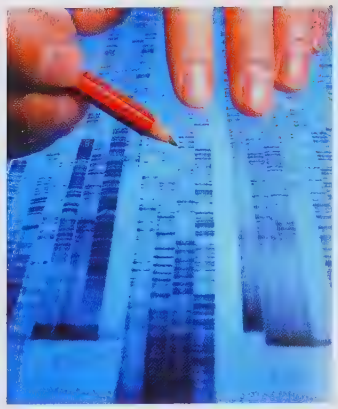
The process is then repeated by changing the temperature of the solution to 95 °C, then 55 °C and then 70 °C, so doubling the amount of DNA produced each time.

Many processes in DNA technology require large amounts of DNA, for example DNA sequencing and DNA fingerprinting. Often only small samples are available, so PCR can be used to create vast quantities.
It can be used to increase the tiny amount of DNA sample in a speck of blood. This gives forensic scientists enough material to use for genetic fingerprinting, which may lead to the identification of a criminal. Apart from its use as a forensic tool, genetic fingerprinting has also been used to detect inherited diseases, to monitor bone marrow transplants, and to confirm animal pedigrees.



The PCR cycle

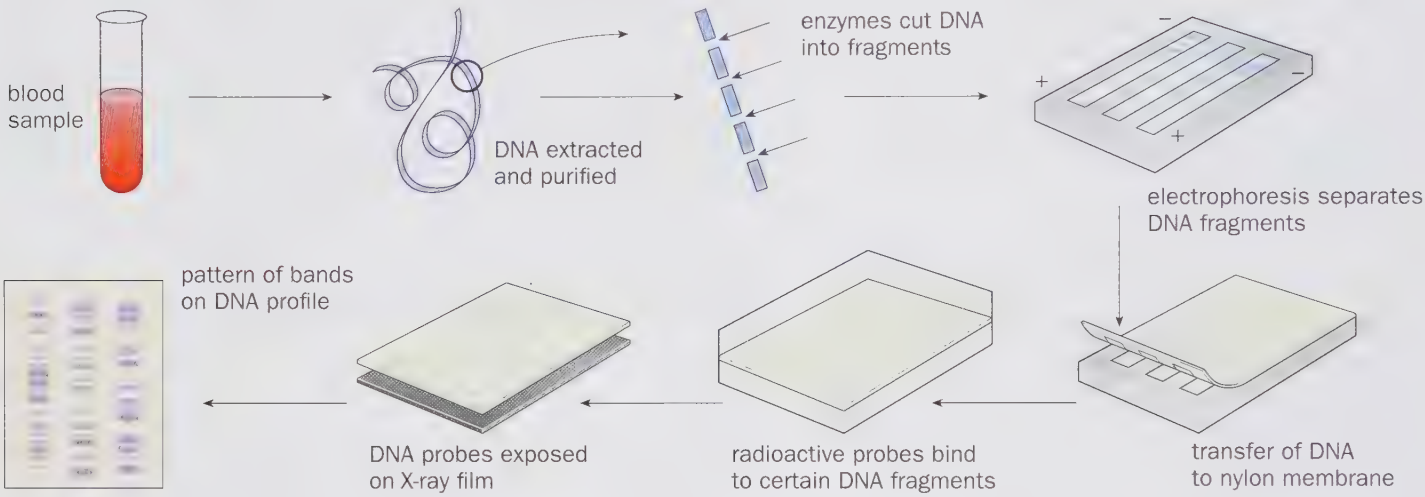
Genetic fingerprinting



Genetic fingerprints viewed on an autoradiogram

The DNA in your cells is as unique as your fingerprints.
Unless you have an identical twin, your '**genetic fingerprint**' or **DNA profile** is different from everyone else's.
There are certain regions of DNA along chromosomes that code for the production of proteins. These are called **exons**. Between exons are regions of non-coding DNA called **introns**. These regions of non-coding DNA contain a block of repeated nucleotides called **variable number tandem repeats (VNTRs)**.
It is the number of times that these blocks of VNTRs occur that produces the variation in individuals.

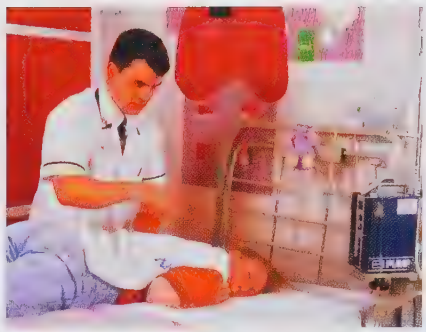
The technique of genetic fingerprinting can be used to provide forensic evidence and thus solve crimes.
Since body cells contain the same DNA, virtually any tissue can be used.
Only a small amount of, say, blood, hair root, skin cells or semen is needed.



- The DNA is extracted from the sample and cut into millions of small fragments using restriction endonucleases, aimed at specific VNTRs. These enzymes are specific, making cuts in specific places for each particular individual.
- The DNA fragments are separated using **electrophoresis**. This involves exposing the fragments of DNA to an electric current in a trough of gel. The DNA fragments are negatively charged and so they move towards the anode (positive terminal) when an electric field is applied. The smaller the fragment, the faster it moves, so the DNA becomes separated into bands according to the size of the fragments.
- The DNA fragments are transferred to a nylon membrane by a process called **Southern blotting**. This does not alter the pattern of the fragments resulting from the electrophoresis.
- Radioactive DNA probes are used to attach to specific parts of the fragments.
- Any probe sequences that have not bound to the DNA fragments are washed off and the membrane is dried.
- The nylon sheet with DNA fragments attached is placed under X-ray film.
- The radioactive probes on the DNA fragments expose the film.
- This produces a visible pattern of light and dark bands (where a radioactive probe is present) rather like a barcode. Everyone's barcode is different.

Scientist working on DNA profiles

Gene therapy



Cystic fibrosis sufferer

Cystic fibrosis (CF) is an inherited disease that affects 1 in 2000 people in the UK.
Sufferers of the disease produce thick, sticky mucus from the cells that line particular passages in the body.
This can block the bronchioles and alveoli of the lungs, causing congestion and difficulty in breathing.
The pancreatic duct can also become blocked with mucus so that the reduced release of pancreatic juice results in inadequate digestion.

Patients are treated by vigorous chest physiotherapy.
The mucus is difficult to remove and is a breeding ground for germs.
Sufferers often get infections and have to be treated with strong antibiotics.
Each infection leaves the lungs further damaged and the patient's health deteriorates progressively.
The physiotherapy, of course, does nothing to relieve the digestive disorder.

CF is caused by a defective allele, resulting from the deletion of a base triplet (see page 206 on gene mutations).
The deletion of the base triplet means that an amino acid is omitted from the coded protein.
The allele is recessive, so to inherit the disease ***both*** alleles have to code for CF.

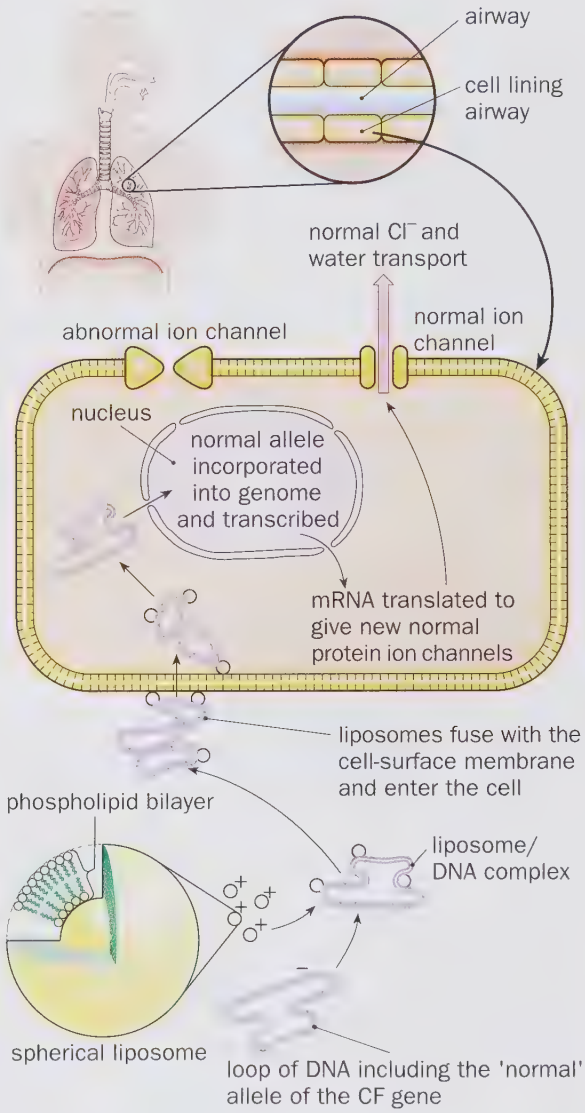
The normal allele codes for the production of a protein found in the cell membrane called **cystic fibrosis transmembrane regulator** (CFTR).
CFTR transports chloride ions (Cl^-) out of cells into mucus.
Sodium ions (Na^+) follow and water is drawn out of the cells by osmosis.
As a result, the mucus that lines the respiratory passages is a normal watery consistency.

However, if a person has CF, their CFTR protein lacks the amino acid phenylalanine in just one place in the chain.
As a result, chloride ions are not transported out of the cell.
So instead of being watered down, the mucus remains thick and sticky. This results in the air passages becoming blocked, making it difficult to breathe properly.

Scientists are researching a genetic treatment for CF.
They hope that gene therapy will work like this:

- A normal allele of the gene is isolated and cut out by restriction enzymes.
- The gene is then cloned many times by PCR.
- The genes are encapsulated in tiny spheres of lipids called **liposomes**.
- An aerosol inhaler is used to add the non-defective gene to the epithelial cells of the lungs of a CF sufferer.
- The liposomes fuse with the phospholipid bilayer of the cell-surface membrane and the DNA enters the affected cells, which then start to express the inserted gene by making the correct protein.

In the past, trials have been carried out using a type of virus called an adenovirus as a vector to insert the CFTR gene into affected cells.
But these tended to produce reactions in damaged lung tissue.

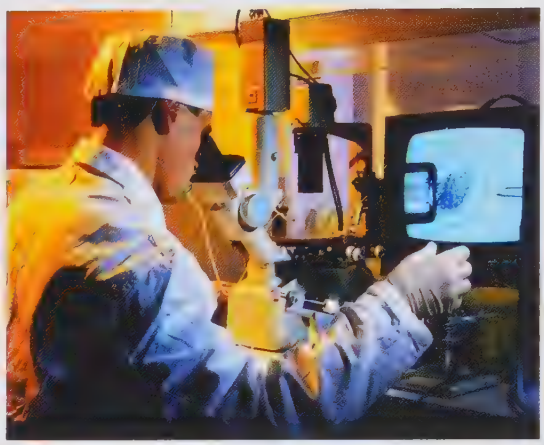


Transgenic organisms

A **transgenic** organism has had the DNA from another organism of another species transferred into it.
Its genotype has been altered, effectively producing a new strain of organism.
They are also called **genetically modified organisms**.

Tracey is a transgenic sheep. She is healthy and normal except in one respect: she has had a human gene inserted into her DNA.
The gene codes for the production of a protein called alpha-1-antitrypsin (AAT).
The protein is secreted in Tracey's milk and extracted from it.
The protein is valuable since it may be part of a potential treatment for human lung diseases such as emphysema and CF.

How do you think the human gene got into Tracey's cells?
The human gene for AAT was isolated and cloned.
It was then injected into a fertilised egg and 'adopted' by one of the chromosomes.
The fertilised egg divided by mitosis, producing identical cells each containing the AAT gene.
The early embryo was then transplanted into a surrogate mother and Tracey was eventually born.



Genetic manipulation of a bovine embryo

This technique has been used to make other valuable proteins that cannot be synthesised by laboratory methods.
The blood-clotting protein factor VIII has been extracted from the milk of transgenic sheep into which the human gene was inserted.
This has been used as a treatment for haemophilia.

Bovine somatotrophin

Bovine somatotrophin (**BST**) is a natural hormone secreted by the pituitary in a cow.
It has three physiological effects:

- it stimulates growth in young animals,
- it affects carbohydrate and fat metabolism,
- it increases milk production in cows.

A modern milking parlour

Scientists inserted the gene that codes for BST into bacteria.
The bacteria was replicated in large-scale industrial fermenters.
As a result, large amounts of BST hormone became available.
The hormone was injected into cows and was found to increase milk production by as much as 20%.

However, treated cows sometimes become infertile, either through a lack of response to artificial insemination or by aborting developing embryos.
BST-treated cattle are also prone to **mastitis** (an inflammation of the udders), and there is evidence that it increases the cow's susceptibility to disease by depleting the immune system.
Health fears have arisen over drinking milk produced by cattle injected with BST, because small quantities of BST appear in the milk and in the meat.
BST should not be a risk to most consumers, because it is a protein that will be digested into its constituent amino acids in the human gut.

However, there is concern about its effect on pregnant and lactating mothers.
Critics argue that not enough is known about the long-term effects of drinking BST milk.
Others see its use as an unnatural and cruel way of extracting milk from cows.

Transgenic plants

Foreign genes can be introduced into plant cells quite naturally.
The soil bacterium *Agrobacterium tumefaciens* is the cause of 'crown gall' infection in some plants.
The bacterium attacks wounds and causes the plant cells to multiply and form a tumour.

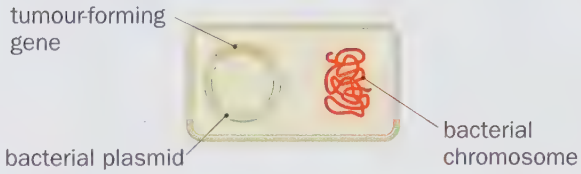
It does this by inserting genes from its own plasmids into one of the plant's chromosomes.
The plasmid gene links up with the plant's DNA and stimulates the tumour growth.
Plant geneticists have been successful in replacing the tumour-forming genes in the bacterial plasmids with useful genes.
These can be inserted into the plant's DNA by a vector.

For instance, this technique has been used to introduce into potato plants genes that promote resistance to diseases such as potato roll leaf virus, which can have a severe economic effect on crop yield.

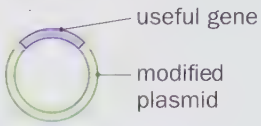
Legume plants, such as peas, beans and clover, have nitrogen-fixing bacteria in their roots.
The bacteria provide the plant with a source of nitrogen as nitrates and obtain nutrients made by the plant in return.
Soon it may be possible to transfer the nitrogen-fixing genes directly into other plants such as wheat and rice.
Such plants would no longer need artificial fertilisers, as they would be able to fix their own nitrogen.

This may limit the use of fertilisers, which can have an adverse effect on the environment.
So genetically modified plants may be able to reduce the impact of modern crop production techniques on the environment because fewer agrochemicals will be needed.
Such a view has to be balanced by the threat to environmental stability that could come from the release of genetically modified organisms into biological communities.

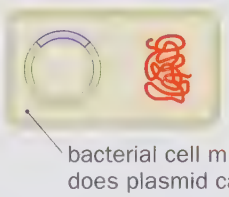
1. plasmid extracted from the bacterium *A. tumefaciens*



2. the useful gene replaces the tumour-forming gene in the plasmid



3. the modified plasmid is inserted back into the bacterium



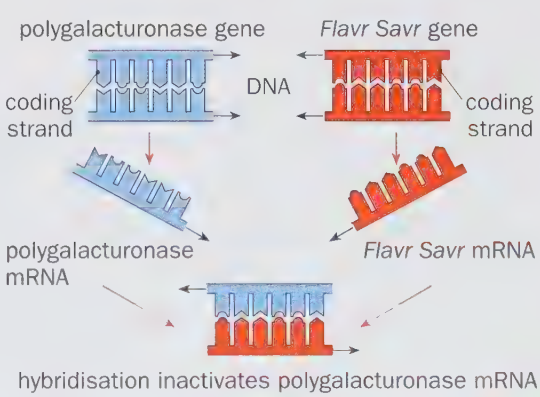
4. when the bacterium infects the plant the useful gene is introduced to one of the plant's chromosomes instead of the tumour-forming gene

Flavr Savr tomatoes

Most tomatoes are picked when they are green so that they are still firm on arrival at the shops.
The green tomatoes are treated with **ethene** gas to turn them red (this does not affect their natural flavour).
Normally tomatoes produce an enzyme called **polygalacturonase**, which breaks down the pectin in cell walls, making them mushy and thus reducing their shelf life.

The treated tomatoes have not gone mouldy

To stop the tomatoes from going soft, scientists have found a way of blocking the production of polygalacturonase.
Polygalacturonase is coded for by a single gene, which produces a messenger RNA (mRNA) molecule.
In *Flavr Savr* tomatoes this mRNA is blocked by a complementary **antisense mRNA** produced by a reverse *Flavr Savr* gene.
The antisense mRNA binds to the polygalacturonase mRNA, and so prevents the translation of the polygalacturonase gene into the sequence of amino acids that make up the enzyme.
The result is that *Flavr Savr* tomatoes are tastier and have a longer shelf life.

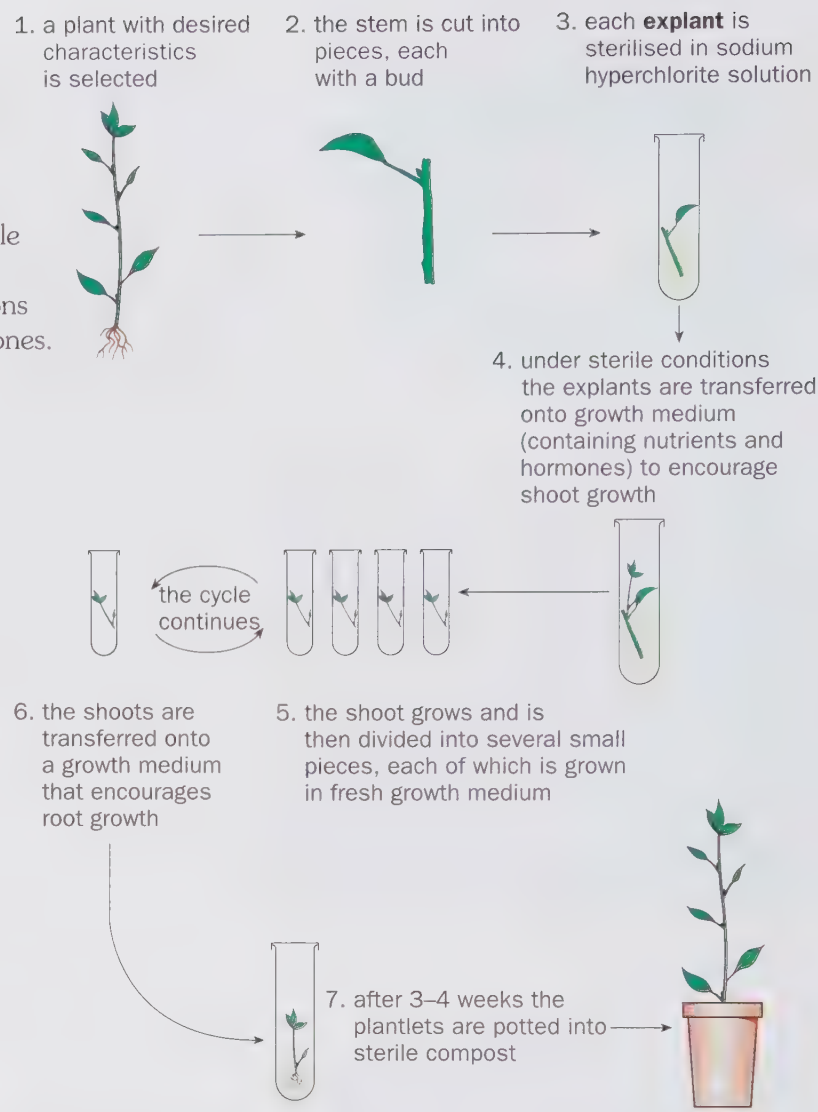


Plant tissue culture

Plant tissue culture, or **micropropagation**, involves cloning plants.
Cloning results in the production of large numbers of genetically identical plants.
Clones are taken from stock plants that have desirable characteristics and that are commercially important.
They are grown in tissue culture under sterile conditions with controlled concentrations of nutrients and hormones.

Plant tissue culture is a form of vegetative propagation and has many advantages over the more traditional methods of propagation such as grafting or taking cuttings.

- It is very time-effective, since it dispenses with the need for pollination and seed production.
- It ensures that the good qualities of the stock plants are retained, for example resistance to disease and insects, or high fruit yield.
- It enables large numbers of new plants to be produced from a single parent.
- It is extremely cost effective.
- It is easy to transport or store large numbers of plants under sterile conditions.
- It has conservation use in the recovery programmes of endangered plants.
- It helps to eliminate plant diseases since only healthy stock are selected and sterile conditions are used.
- It eliminates the seasonal restrictions on germination.



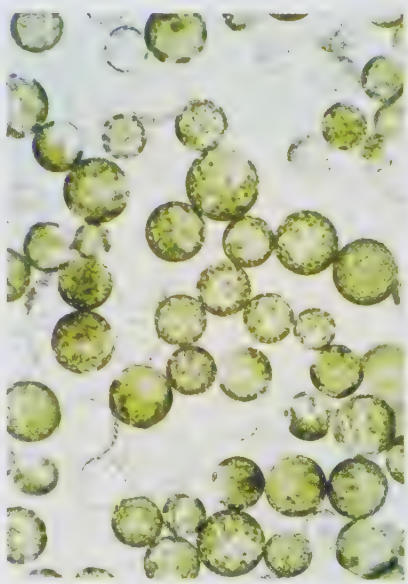
Stages in micropropagation

Cloning from protoplasts

Techniques have been developed by which complete plants can be grown from single cells.
A small piece of stock plant tissue is taken and treated with cellulase enzymes.
The enzymes remove the cellulose cell walls and split the cells up.
The naked cells are called **protoplasts**.

The protoplasts are then cultured under sterile conditions and grow to form an undifferentiated mass of cells called **callus**.
The callus tissue is then grown, using sterile culture techniques, to produce complete plants.
This technique is an effective way of producing large numbers of plants from one commercially valuable plant.
It has been used to produce high value ornamental plants such as orchids.

The protoplasts of closely related species can be fused.
This results in hybrid plants and induces variation.
By fusing protoplasts of different strains, it is possible to transfer genes, for example the gene for blight-resistance in potato plants.



Tobacco leaf protoplasts

Cloning farm animals

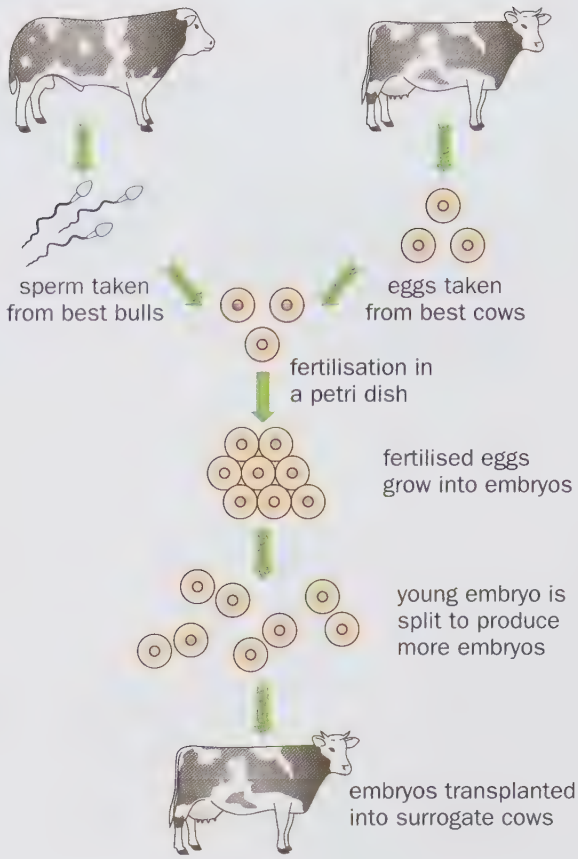
Embryo cloning

Embryo cloning has been used to produce many genetically identical individuals.

On average, a ewe or cow will produce one offspring per pregnancy.
Embryo cloning has made it possible for farmers to rapidly increase their livestock numbers.

First, eggs taken from the best cows are fertilised in a petri dish by sperm from the best bulls.
This is known as **in-vitro fertilisation**.
The fertilised egg divides to form a ball of cells.
At this stage the young embryos are surgically split up to produce a number of separate embryos.
Each separate embryo is a genetically identical clone (though it does have half its genes from the father and half from the mother).

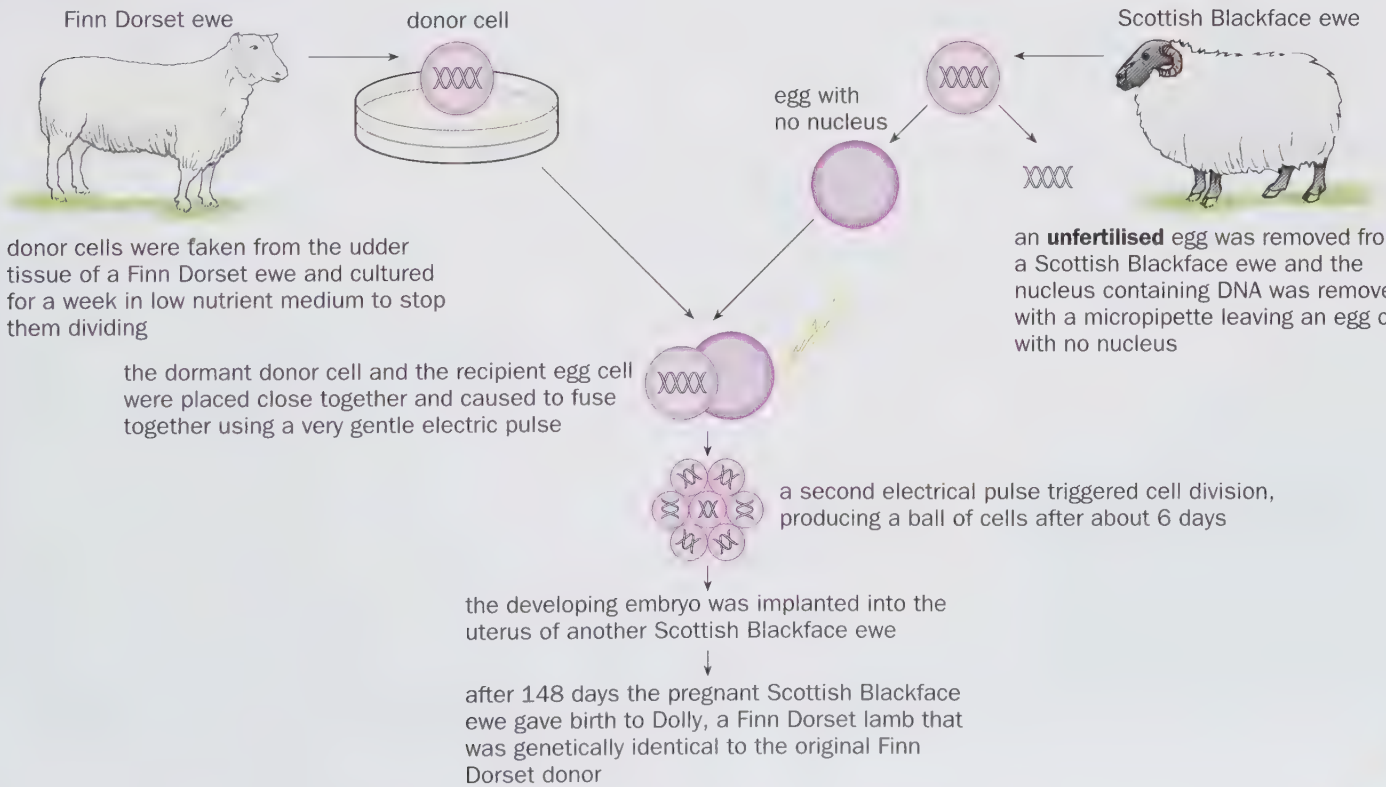
The embryos are then transplanted into other cows called **surrogates**.
They grow and are eventually born, producing several individuals with the same characteristics as the parents.



Stages in embryo cloning

Cloning by nuclear transfer

This technique enables clones to be produced from ***one*** individual.
It has allowed the cloning of superior livestock such as cows with a high milk yield.
If the cow were to be mated with a bull, the resulting genotype might not give the same unique characteristics.
Cloning produces identical copies and preserves desirable features for future generations.



The GM food debate

Genetically modified (GM) food, in the form of tomato puree, vegetarian cheese or products containing genetically modified soya, has probably been eaten by most consumers, given its increased use in a variety of foods over the past decade.
There are few topics in biology that have inspired such a polarising debate, as the following two quotes evidence:

> *"I happen to believe that this kind of genetic modification takes mankind into realms that belong to God and to God alone."*
>
> Charles, Prince of Wales

> *"In all honesty, if scientists don't play God, who will?"*
>
> James Watson, co-discoverer of DNA with Francis Crick

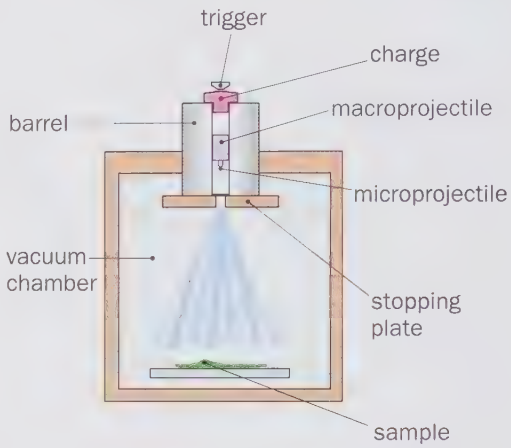
Prince Charles is not a supporter of genetic modification

GM foods – the benefits

The scientific community and biotechnology companies stress the safety of GM foods and their benefits:

- ***Solving global hunger*** – genetic modification could feed the world through the development of crops that will tolerate drought, saline soils or frost, and thus increase food production in some areas.
- ***Environmentally friendly*** – genetic modification can confer resistance to insects, weeds and pathogens. Together with genes that improve nitrogen uptake, these improvements should lead to a decrease in the use of chemicals.
- ***Consumer benefits*** – genetically modified food plants have already been produced which can provide food with an improved flavour and better keeping qualities, which are easier to produce, and which require fewer additives.

The GM food debate has raised questions about the ethics and scientific assumptions of such developments.



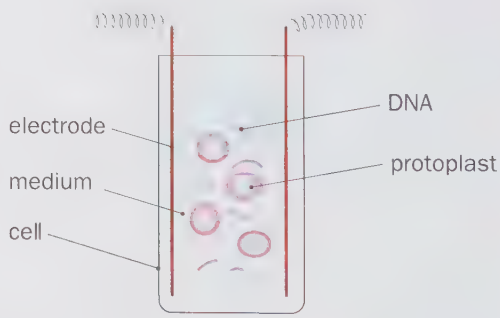
DNA is stuck onto minute gold or tungsten particles, which are then fired into the plant tissue. The plant DNA then takes up some of these particles. Ballistic impregnation is used with cereal crops

What is GM food?

Genetic engineering is the controlled modification of genetic material by artificial means and involves the isolation of specific regions of DNA using specialised enzymes, which cut the DNA at precise locations.
Selected DNA fragments can then be transferred into plant cells.
Food derived from such plants is referred to as genetically modified.
There are several ways in which **gene transfer** can occur:

- **plasmid transfer** (see page 409),
- **ballistic impregnation**,
- **electroporation**.

With all these techniques, only a small proportion of the novel DNA is incorporated into the plant's DNA.
To check how successful gene transfer has been, **marker genes** are linked to the DNA fragments before transfer.
These marker genes are usually genes that allow the plant to grow in the presence of a specific antibiotic or herbicide.
Other techniques, for example **antisense technology**, are used to neutralise the action of specific undesirable genes, such as those involved in the excessive ripening of fruit.



Microsecond pulses of a strong electrical field cause minute pores to appear, allowing DNA to enter from a surrounding solution. Electroporation – works with plant cells that have no cell walls such as pollen tubes

Examples of GM foods

GM crops enter the UK mainly as animal feed. There is no commercial growing, but there have been experimental trials of GM wheat and potatoes in recent years. Large quantities of GM soya and maize are imported into Europe and the UK.

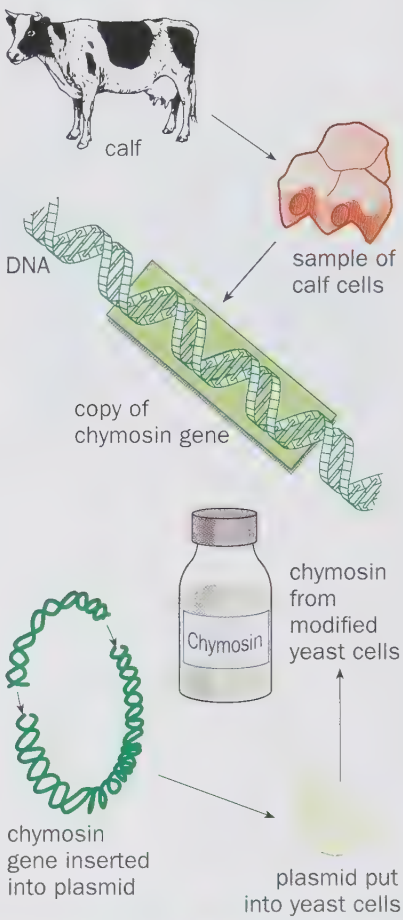
- ***Tomatoes*** – research was aimed at gaining a better understanding of the ripening process using antisense technology.
- ***GM yeast*** – microorganisms have been genetically modified to yield **chymosin**, which is identical to the enzyme obtained from animal rennets.
- ***GM maize*** – maize grown in the USA has been genetically modified to be resistant to an insect pest, the European corn borer.
- ***GM soya*** – the first herbicide-tolerant soya beans produced in the USA were approved for use in the European Union in 1996 as processed beans. More than 60% of all processed foods contain soya or soya products.
- ***Processing aids*** – current UK legislation requires that a food's ingredients should be listed. There are no such requirements to indicate those substances such as enzymes that are not found in the finished product. Meat and dairy products obtained from animals fed on GM feed are not labelled to show this.

GM foods – the concerns

Opposition to the increased use of GM foods revolves around five main areas of concern.

1. ***Environmental safety*** – there are worries that new GM food plants will become successful weeds, that they will transfer their new genes to wild relatives or similar crops nearby, and that those with insect-resistant genes will lead to the establishment of resistant populations of pests.
2. ***Food safety*** – concern focuses on the inclusion of marker genes for antibiotics and herbicides. In particular, the proteins these genes can produce, what levels should be acceptable and whether the genes are transferable to other organisms such as microbes in the intestine of the consumer. There is no recorded evidence currently of such transfer occurring between plants and microbes or humans.
3. ***Changes in farming structure*** – may occur which amplify the existing trends towards larger farms and more capital intensive systems. These tend to favour wealthy farmers in the northern hemisphere.
4. ***Biodiversity*** – fewer companies will increasingly control plant breeding, reducing the number of plant varieties available to farmers and leading to a reduction in the use of the old varieties and wild relatives. This could make plants more susceptible to attack by pests and diseases.
5. ***Animal health*** – developments in livestock production that affect animal welfare are increasingly unlikely to be accepted by regulatory authorities or the public. There are currently no products of animal biotechnology in food shops. In the future, such research could, however, benefit disease resistance in cattle in developing countries.

Whilst the biotechnology companies will continue to develop genetic modification techniques, public confidence and understanding is now seen as crucial to the acceptance of new GM food products.



GM maize production

Evaluation of genetic engineering

Like many beneficial scientific advances, for instance the discovery of antibiotics, the introduction of gene technology has brought with it the possibilities of misuse.

Body cell gene therapy brings the benefit of targeting a particular tissue.
In the case of CF, the therapy could solve the problem of congested lungs.
Body cell gene therapy could be used to target the blood cells to cure **thalassaemia**, a disease caused by a recessive mutant gene resulting in severe anaemia.
With body cell gene therapy, the sex cells are not involved, so the cure is not passed on to the offspring.

Far more controversial is **germ-line gene therapy**.
This involves repairing the original gene inside the fertilised egg.
The resulting individual grows and develops with healthy genes functioning in ***all body cells***.

This means that not only is the defective gene eliminated from the patient, but the risk of it being passed on to the offspring is also removed.
On the face of it, the ability to treat diseases such as CF and haemophilia in this way appears very attractive.

But ethical questions have to be raised as to whether we have the right to alter the genes of future generations.
We know very little about how genes function and control the development of human embryos.

Tampering with genes in the fertilised egg could result in unforeseen effects which are only discovered later in life or in subsequent generations.
Would we be only one step away from altering genes that code for skin colour, height or even intelligence?
This raises major ethical questions about the technique.
It is perhaps not surprising that research into germ-line gene therapyis currently banned in the UK.

The introduction of genetic engineering in the 1970s brought with it fears that new strains of microbes could escape from laboratories and cause outbreaks of disease.
In fact, scientists have worked with strains that are very poorly adapted to live inside the human body.
Work on potentially dangerous pathogens has been restricted to isolated laboratories with strict hygiene conditions, highly effective air filters and extensive monitoring of the atmosphere.

Worries that genetically modified organisms might escape into the environment and get into food chains have so far proved unfounded.
But the introduction of transgenic animals and plants is still viewed by many as interfering with nature. (See pages 412–13 on GM foods.)

On the one hand, gene technology may be able to provide the means to alleviate suffering and cure genetic diseases.
But on the other hand, there are justifiable reservations about the long-term effects that manipulation of the human genome and the production of genetically modified organisms may have.

Greenpeace activists removing GM maize in Norfolk, UK. Twenty-eight of the Greenpeace volunteers were aquitted of criminal damage at Norwich Crown Court on 20 September 2000

Biology at work: DNA profiling

In 1998, the Russian Government confirmed that nine sets of bones unearthed near Yekaterinburg in the late 1970s were those of the last tsar and his family. The Bolsheviks executed Tsar Nicholas II and his family after the Russian revolution in 1917.
The positive identification of the remains 80 years after their burial was only possible with the use of forensic techniques, including DNA profiling.

The Russian Imperial Family in 1913

For years, forensic scientists have used **genetic markers** in the form of blood groups to eliminate or include suspects in criminal investigations. However, a sufficient quantity of blood in good condition was necessary, and old and degraded samples were difficult to analyse accurately. Also, the use of blood groups could obviously not be used to identify actual individuals.
The first forensic use of DNA profiling in the UK occurred in the 1980s. This involved the successful conviction of a rape and murder suspect in Leicestershire, UK.

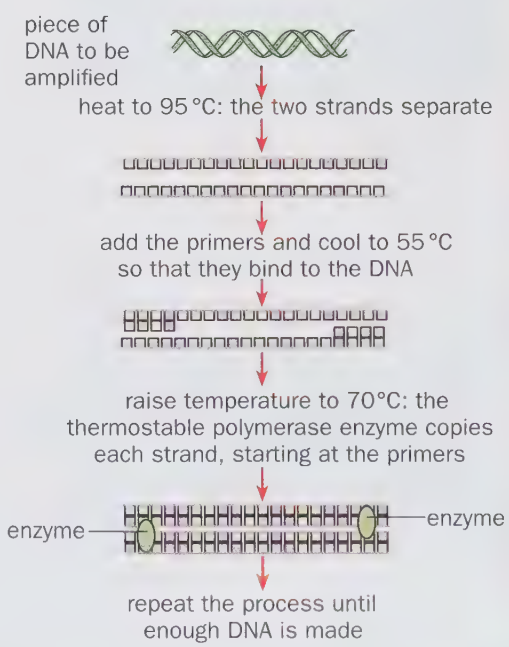
In 1985 Professor Alec Jeffreys of Leicester University demonstrated that chromosomes had regions of non-coding DNA.
He termed these **mini-satellites**, and they can be used to identify individuals. They are scattered throughout the chromosomes and consist of repeated blocks of nucleotides that do not code for any particular protein. It is the number of times these blocks are repeated that produces the variation in individuals.
If enough regions of variation, or **loci**, are examined, it is possible to obtain a profile which is exclusive to an individual.

Skeletal remains of the tsarist family are examined by a forensic scientist

Polymerase chain reaction (PCR)

The PCR technique is now a common method for creating copies of specific fragments of DNA (see page 405).
PCR rapidly amplifies a single DNA molecule into many billions of molecules.
It detects repeating, very short sequences of nucleotides.
These very short pieces of DNA are known as Variable Number Tandem Repeats (VNTRs).
Using VNTRs from the DNA in a single strand of hair at a crime scene, sufficient copies can be produced to carry out forensic tests.
This is a major advantage as it enables tests to be carried out accurately, quickly and regardless of the age of the sample.

It is for this reason that PCR was used in the identification of the bones of Tsar Nicholas II and his family.
The analysis confirmed that the bones came from a father, mother and three siblings.
Further analysis of the **mitochondrial DNA (mtDNA),** which is inherited maternally, showed that the mother shared the same maternal line as the present Duke of Edinburgh.
This was not conclusive evidence and it was only after the known remains of the Tsar's brother were exhumed that it was shown that they shared the same mtDNA sequence.



Biology at work: DNA sequencing

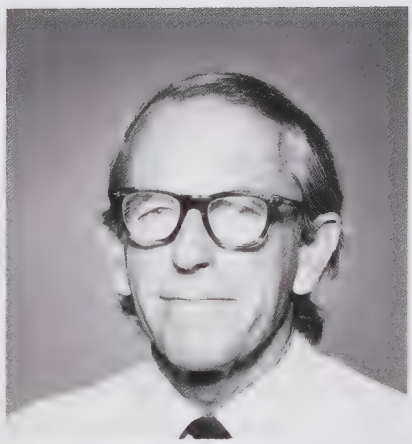
DNA sequencing is the process of determining the order of the bases A, T, C and G in a piece of DNA. The technique was at the heart of the **Human Genome Project**, which in 2003 published the entire sequence of the human genome. This was a major achievement not least because the human genome contains over 3 billion base pairs.

How is DNA sequenced?

DNA sequencing is a complex and rapidly developing area of biotechnology. Early techniques were very slow and labour intensive and only a few base pairs could be sequenced per year.

Considerable progress was made in the mid-1970s by Frederick Sanger whose improvements in speed and efficiency earned him the Nobel Prize for Chemistry in 1980. Sanger's technique was based on using DNA polymerase to synthesise DNA chains of varying lengths which could then be separated by electrophoresis. This technique was sensitive enough to distinguish DNA fragments that differed in size by only one nucleotide. Over the next 30 years the use of automated sequencing machines using Sanger's technique has meant that a sequence up to 1000 base pairs long can be read in a single reaction.

The very latest technique is called **pyrosequencing** and it can read up to 20 million bases in a single run.



Frederick Sanger, an early pioneer of DNA sequencing

DNA sequencing has been greatly helped by **bioinformatics** which is a hybrid science linking biological data with information storage and analysis techniques.

Why is DNA sequencing useful?

Knowing the sequence of a section of DNA has a number of uses, such as:

- finding genes (DNA is not composed entirely of genes),
- assisting in the identification of genes associated with particular diseases,
- determining the function of a gene,
- comparing DNA sequences from different organisms to study evolutionary relationships.

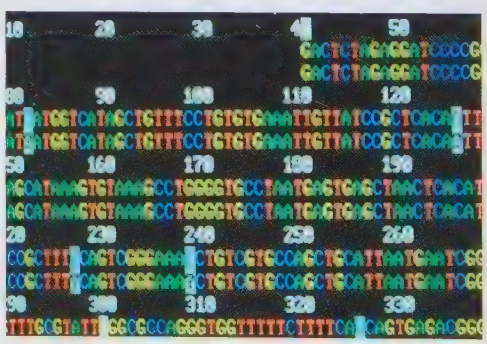
Technician preparing samples for DNA sequencing

Future developments

The latest techniques in DNA sequencing have meant that the cost of sequencing has been drastically reduced. In 2012, for example, an American company began offering individuals the opportunity to have their entire DNA sequence read for $1000 (£700).

In 2013, scientists in the UK began looking for 100 000 volunteers prepared to have their DNA sequenced and published online. The idea behind the **UK Personal Genome Project** is to provide data for scientists to further develop our human genetics, biology and health.

A similar project called **Genomics England** was also announced in 2013. The aim of this project is to sequence the genome of 100 000 NHS patients. The focus of this project is to research rare diseases, cancers and infectious diseases. These data, however, will not be published online and will remain private.



Computer analysis of part of the human genome

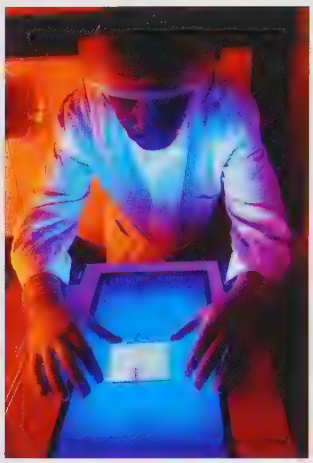
Biology at work: Genomics

An organism's complete set of DNA is called its **genome**, and in the case of humans the genome is made up of in excess of 3 billion pairs of bases.

Genomics is the branch of molecular genetics concerned with the study of genomes, specifically the identification and sequencing of their constituent genes.
It also covers the application of this knowledge in areas such as medicine, agriculture and evolution.
The main difference between **genomics** and **genetics** is that genetics analyses the functioning of single genes whereas genomics addresses all genes and their inter-relationships.

Genomics and medicine

Two of the key developments in genomics have been **DNA sequencing** (see page 416) and **gene mapping** (see page 398), and these techniques have significant implications for medical science. Scientists can now study how multiple genetic factors act together and with the environment in complex diseases like cancer and diabetes.
One of the most recent benefits of genome research is the field of **pharmacogenomics**. This uses information about the genetic make-up of a patient to tailor drug therapy to their individual needs.
Identification of genes linked to specific disease has also led to advances in **gene therapy** (see page 407).



A scientist sequencing the genome of the highly dangerous Ebola virus

Genomics and regulatory DNA

Sequencing and mapping DNA has led to the identification and study of **regulatory** or **non-coding DNA**. Only about 1.5% of DNA in the human genome carries the code for protein production, much of the remainder is thought to carry out various regulatory functions. You can read on page 406 about genetic fingerprinting. More accurately called **DNA profiling** this technique also makes use of sections of non-coding DNA (introns) identified by sequencing and mapping. DNA profiling has a number of important applications including crime scene analysis, paternity testing and identification of human remains from battlefields across the world.

DNA profiles of suspects in a crime investigation can be compared with DNA obtained from the crime scene

Comparative genomics

This area of study looks at the biological similarities and differences, as well as the evolutionary relationships between organisms. It works on the simple principle that the more closely related organisms are, the more DNA sequences they will have in common. A good example is the human and the chimpanzee who share about 98% of their genome. The remaining 2% will relate to various human traits such as language development.

Chapter 12 explains in more detail the importance of DNA and amino acid sequences in the area of classification based on biochemistry.

Genome study is a fast moving branch of science with new discoveries happening on a regular basis.
In 2014 scientists sequenced the genome of the Antarctic midge. This insect is adapted to very harsh conditions. Scientists hope that revealing its genome will help in understanding the mechanisms behind its survival.

The Antarctic midge has the smallest insect genome

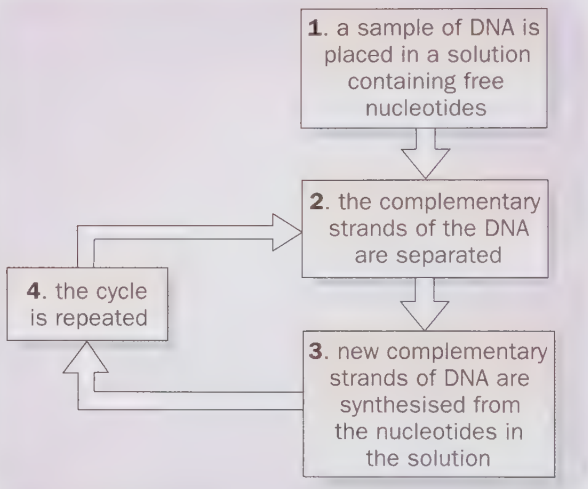
Summary

- Genetic engineering involves the extraction of a gene or genes from one organism and their transfer into a host organism.
- DNA derived from two different organisms is called recombinant DNA.
- The manipulation of DNA involves the use of enzymes such as restriction endonucleases, ligases and reverse transcriptases.
- The polymerase chain reaction can produce large amounts of identical DNA from a small sample.
- Genetic fingerprinting can produce a DNA barcode that is unique for each individual.
- A DNA fragment containing the wanted gene is inserted into a carrier DNA molecule known as a vector.
- Bacterial plasmids are the most commonly used vector and can reproduce the gene on a vast scale inside industrial fermenters.
- Bacterial plasmids can be labelled using marker genes. Some of these may be radioactive.
- It is hoped that gene therapy can be used to replicate non-defective genes for use in the treatment of diseases such as cystic fibrosis.
- Genetically modified organisms have the DNA of another species transferred into them.
- Cloning can be used to produce large numbers of genetically identical organisms in a relatively short period of time.

Questions

1 Copy and complete the following:
The isolation of specific genes during genetic engineering involves the formation of DNA fragments. These fragments are formed using ___ enzymes, which make staggered cuts in the DNA within specific base sequences. This leaves single stranded 'sticky ends' at the ends of each fragment. The same enzyme is used to open up a circular loop of bacterial DNA, which acts as a ___ for the DNA fragments. The complementary sticky ends of the bacterial DNA are joined to the DNA fragment using another enzyme called ___. DNA fragments can also be made from a ___ template. Reverse transcriptase is used to produce a single strand of DNA and the enzyme ___ catalyses the formation of a double helix. Finally, the new DNA is introduced into ___ cells. These can then be cloned on an industrial scale and large amounts of protein can be harvested. An example of a protein currently manufactured using this technique is ___.

2 The polymerase chain reaction (PCR) is a process that can be carried out in a laboratory to make large quantities of identical DNA from very small samples. The process is summarised in the flowchart.

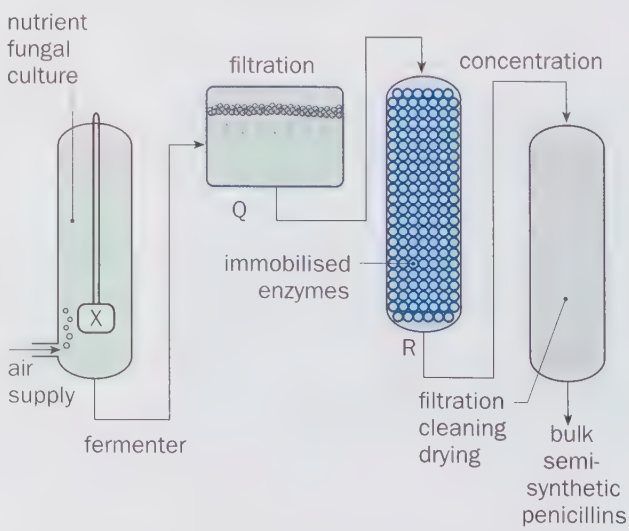


a) i) At the end of one cycle, two molecules of DNA have been produced from each original molecule. How many DNA molecules will have been produced from one molecule of DNA after five complete cycles?
ii) Suggest one practical use to which this technique might be put.

b) Give two ways in which the PCR differs from the process of transcription.

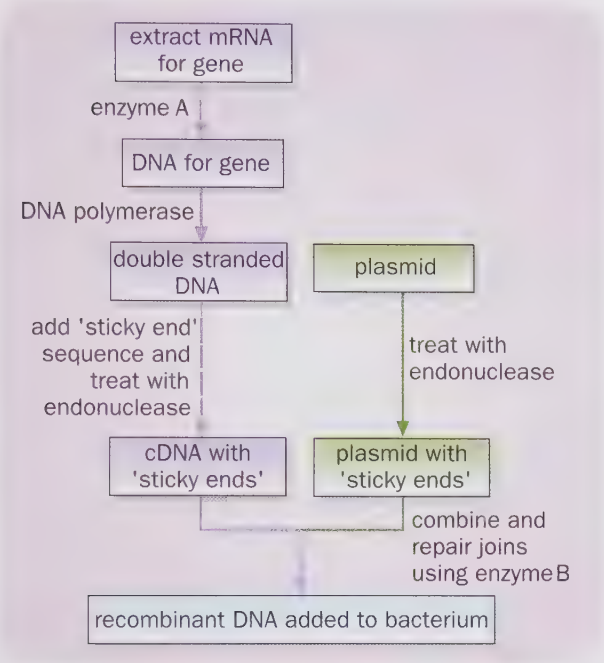
c) The PCR involves semi-conservative replication. Explain what is meant by semi-conservative replication.

3 Penicillin is an antibiotic derived from the fungus *Penicillium chrysogenum*.
The antibiotic can be produced on a commercial scale as shown in the diagram.



a) i) What is the purpose of structure X?
ii) Why is air supplied to the fermenter?
b) Explain the purpose of the filtration carried out at Q.
c) i) Suggest one reason for using enzymes at R.
ii) Suggest why ***immobilised*** enzymes are used.

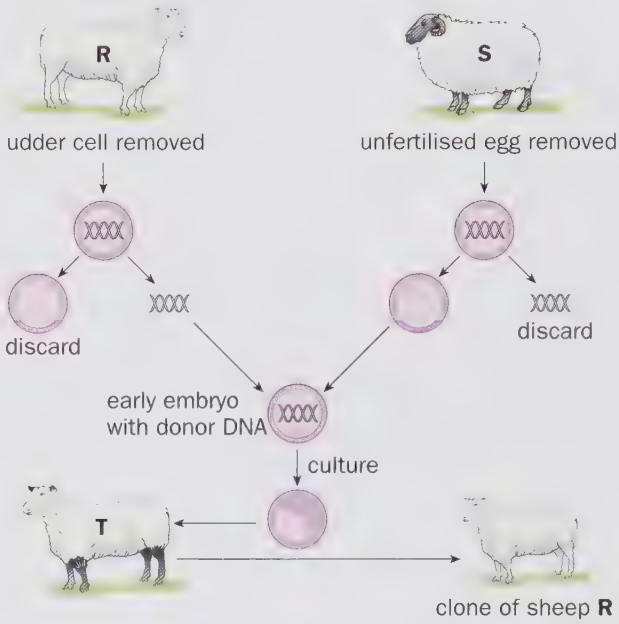
4 The diagram shows how a gene from a human may be inserted into a bacterium to produce a genetically modified organism.



a) Name the enzymes A and B.
b) Describe how treating plasmids with endonuclease produces sticky ends, and explain their importance.
c) Suggest one way in which genetically modified organisms may be used.

5 a) i) In the DNA of a herring, 28% of the nucleotides contain adenine. What percentage of the nucleotides would you expect to contain guanine?
ii) The percentage of nucleotides containing adenine is similar in both herring DNA and wheat DNA. Explain how organisms with the same proportion of adenine can be as different from each other as herring and wheat.
b) In processed food products, cheaper varieties of fish may be substituted for more expensive varieties. Food inspectors have developed a DNA test to identify the species of fish used.
i) In this test, a section of the DNA that codes for part of a protein known as cytochrome b was isolated. It was 351 base pairs in length. What is the maximum number of amino acids for which this section of DNA could code?
ii) The sequence of DNA was then cut into shorter pieces using a restriction endonuclease. The positions where the cuts were made depended on the species of fish from which the DNA came.
Explain why the restriction endonuclease cuts the DNA in different positions in different species of fish.

6 The diagram shows a method that has recently been used to clone sheep.



a) i) State the scientific term used to describe the developmental stage of the embryo transferred to the uterus of ewe T.
ii) State the scientific term used to describe ewe T.
iii) Name the first process that takes place in the uterus of ewe T after embryo transfer.
iv) Name the hormone that is essential for this process to take place.
b) Scientists used ewes from different homozygous varieties in order to check that the procedure was successful at each stage and that the lamb produced was a clone of R. Suggest what could have been deduced about the procedure if the lamb had been born:
i) with a black face,
ii) with black legs.
c) i) Suggest one reason why scientists did not think that it would be possible to clone sheep from udder cells.
ii) Some scientists did not regard sheep produced in this way as a pure clone.
Suggest one reason for this.
d) Suggest one reason why it would be undesirable to produce all farm animals in this way.

Genetics

1 Some cats have white patches on their coats. This effect is produced by action of the spotting gene, **S**. This gene has two codominant alleles, $\mathbf{S}^1$ and $\mathbf{S}^2$. The coats can have large white patches, small white patches or no white patches at all.

a) Define the term *codominance*. [1]

b) A cat with no white patches, homozygous for $\mathbf{S}^1$, was crossed with a cat which had small white patches. Some of the offspring had coats with small white patches and the rest had no white patches.
Draw a genetic diagram to show this cross and show the expected ratio of phenotypes on your diagram. [4]

Edexcel (formerly London) **[5]**

2 In mice, the dominant allele (**B**) of a gene for coat colour gives a black coat, the recessive allele (**b**) of this gene gives a brown coat. A second gene determines the density of the coat colour. The dominant allele (**D**) of this gene allows expression of coat colour, its recessive allele (**d**) dilutes the colour converting black to grey and brown to cream.

a) A breeder crossed a male black mouse with a female brown one. The offspring produced showed four different coat colours: black, grey, brown and cream.

i) State the genotypes for the black parent and the brown parent giving an explanation for your answer. [5]

ii) Copy and complete the Punnett square to show the genotypes of the gametes and the offspring. [2]

iii) State the expected phenotypic ratio. [1]

b) With the aid of a genetic diagram, explain how the breeder could determine which of the black offspring were homozygous for the full colour allele (**D**). [4]

Edexcel **[12]**

3 In the fruit fly, *Drosophila melanogaster*, the allele for grey body colour (**G**) is dominant to that for ebony body colour (**g**). The allele for normal wings (**N**) is dominant to that for curled wings (**n**).
A student crossed a grey-bodied, normal-winged fly with an ebony-bodied, curled-winged fly.
The offspring were as follows.

Phenotype	Numbers
grey body and normal wings	33
grey body and curled wings	23
ebony body and curled wings	28
ebony body and normal wings	16

a) Show how this cross should have produced offspring in the ratio 1 : 1 : 1 : 1. [2]

b) i) The Chi-squared test (χ^2) can be used to test whether the observed results fit the expectation. Complete the table below in which E represents the expected number of each type of fly in the above cross, and O represents the number actually observed. [2]

Phenotype	Number observed (O)	Number expected (E)	Difference (O − E)	Difference squared $(O - E)^2$
Grey body, normal wings				
Grey body, curled wings				
Ebony body, curled wings				
Ebony body, normal wings				

ii) Calculate the value of χ^2 using the following formula.

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$ [1]

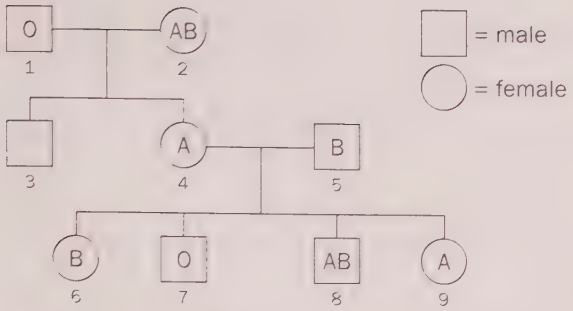
iii) Use the following extract from the χ^2 table to decide whether the observed numbers of offspring are significantly different from those expected. Explain how you reached your answer. [3]

Degrees of freedom	Probability (p)						
	0.90	0.50	0.20	0.10	0.05	0.02	0.01
1	0.02	0.46	1.64	2.71	3.84	5.41	6.64
2	0.21	1.39	3.22	4.61	5.99	7.82	9.21
3	0.58	2.37	4.64	6.25	7.82	9.84	11.34
4	1.06	3.36	5.99	7.78	9.49	11.67	13.28

AQA (formerly AEB) **[8]**

4 a) Distinguish between the terms *gene* and *allele*. [3]

b) The diagram at the top of the next page shows a family tree in which the blood group phenotypes are shown for some individuals.



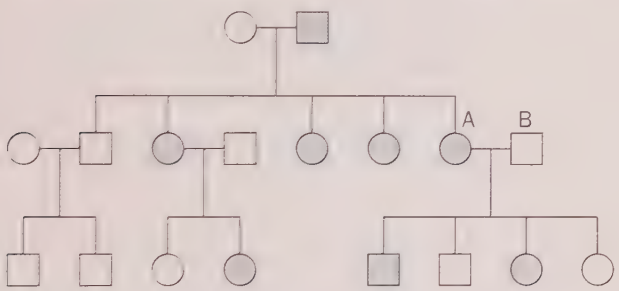
i) Using the symbols I^A, I^B and I^o to represent the alleles, indicate the genotypes of the following people: 1, 2, 4, 5, 6. [5]

ii) State the possible blood groups of person 3. Explain your answer. [3]

Edexcel **[11]**

5 a) Doctors in some clinics claim that they can separate individual sperm cells so that the sex of a human child can be pre-determined. Explain the genetic principles which enable the sex of a child to be pre-determined. [2]

b) The diagram below shows the inheritance of a rare hereditary form of rickets in a human family.



affected female
affected male
unaffected female
unaffected male

The condition is caused by a dominant allele (**R**). This allele may be present on the X chromosome ($\mathbf{X^R}$), but not on the Y chromosome.
Copy and complete the genetic diagram to explain how this type of rickets was inherited by the children of parents **A** and **B**.

	A	**B**
Parental phenotypes	*affected female*	*unaffected male*
Parental genotypes		
Genotypes of gametes		
Genotypes of children		
Phenotypes of children	affected male / unaffected male / affected female / unaffected female	

[3]

AQA (formerly NEAB) **[5]**

6 Huntington's disease is a human inherited condition resulting in gradual degeneration of nerve cells in the brain. It is caused by a dominant allele, but usually no symptoms are evident until the person is at least 30-years-old. It is very rare in most populations.

However, in one isolated area in Venezuela, 48% of the population possess a genotype which gives rise to Huntington's disease. Many of the inhabitants of this area can trace their origins back to a common ancestor 200 years ago.

a) Use the Hardy-Weinberg equation to estimate the percentage of this Venezuelan population which is heterozygous for Huntington's disease. Show your working. [3]

b) Suggest why

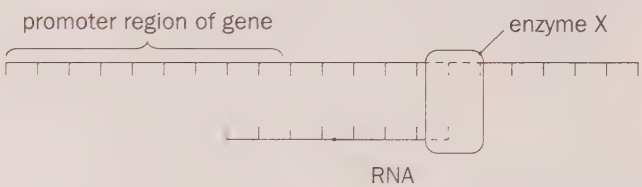
i) there is such a high incidence of Huntington's disease in this population.

ii) Huntington's disease has not been eliminated from this population by natural selection. [3]

AQA **[6]**

7 Figure 1 shows part of a gene that is being transcribed.

Figure 1



a) Name the enzyme X. [1]

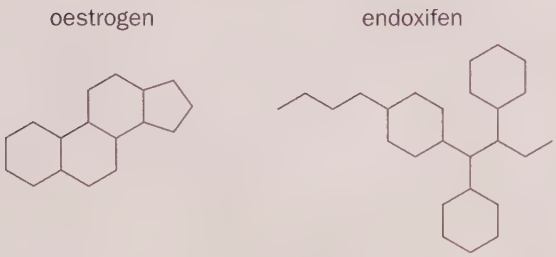
b) i) Oestrogen is a hormone that affects transcription. It forms a complex with a receptor in the cytoplasm of target cells. Explain how an activated oestrogen receptor affects that target cell. [3]

ii) Oestrogen only affects target cells. Explain why oestrogen does not affect other cells in the body. [1]

c) Some breast tumours are stimulated to grow by oestrogen. Tamoxifen is used to treat these breast tumours. In the liver, tamoxifen is converted into an active substance called endoxifen.

Figure 2 shows a molecule of oestrogen and a molecule of endoxifen

Figure 2

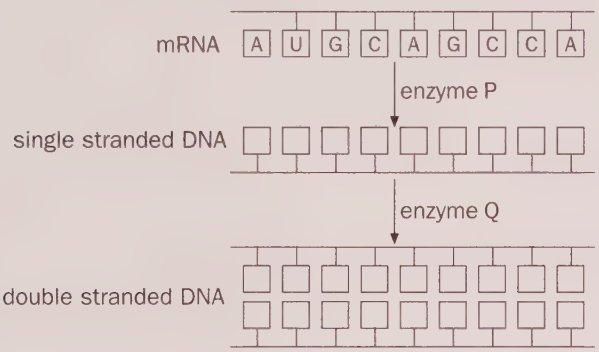


Use Figure 2 to suggest how endoxifen reduces the growth rate of these breast tumours. [2]

AQA **[7]**

Gene technology

8 One of the processes in gene technology involves the synthesis of DNA, using messenger RNA (mRNA) as a template. This is shown in the diagram below.



a) Name enzyme P. [1]

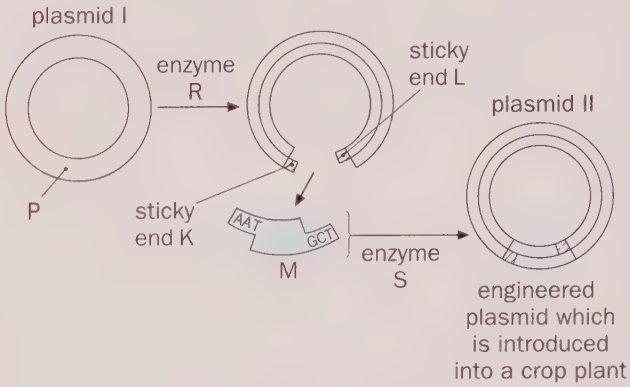
b) Copy the diagram above and write on the sequence of bases which would be present in the single stranded DNA. [2]

c) Name enzyme Q. [1]

d) The double stranded DNA can be inserted into a bacterial cell, which will then synthesise a protein which is coded for by this DNA. Name *one* protein which is produced in this way on an industrial scale. [1]

Edexcel **[5]**

9 Glyphosate is a broad-spectrum herbicide. The diagram below shows stages in the production of a crop plant that is resistant to glyphosate. M represents the gene for glyphosate resistance.



a) i) Name the molecule P which forms the plasmid. [1]

ii) Name enzyme R and enzyme S. [2]

iii) The diagram shows detail of the sticky ends on M. Write down the base sequence you would expect to find on sticky ends K and L of the plasmid. [2]

b) i) Suggest *one* advantage to farmers of growing crops which are resistant to glyphosate. [1]

ii) Suggest *one* disadvantage of using gene technology in this way to develop a crop plant with glyphosate resistance. [1]

WJEC **[7]**

10 The polymerase chain reaction (PCR) can be used to obtain many copies of a DNA sample.

a) The strands of DNA are separated during the PCR. Explain how. [2]

b) In the PCR, two different primers are added to the DNA.

i) Why are primers required? [1]

ii) Two different primers are required. Suggest why. [1]

iii) In addition to primers and the original DNA sample, other substances must be added to the tube in which the reaction occurs. Name *two* of these substances. [2]

c) Starting with a single molecule of DNA, a PCR was allowed to go through three complete cycles. Calculate the number of DNA molecules at the end of the three cycles. [1]

AQA **[7]**

23 Energy and ecosytems

Ecology is the study of how living organisms ***interact*** with each other and with their environment.
By ***interaction*** we mean the relationships that an organism has with its physical surroundings and with individuals of the same species and individuals of other species.
By ***environment*** we mean not only the physical (**abiotic**) conditions, such as light and temperature, but also the biological (**biotic**) conditions under which an organism lives.

Habitats and niches

The **habitat** is the place where an organism lives.
It is the physical (abiotic) environment, which provides the conditions that the organism needs to survive, such as the right amount of light, oxygen and water, and a suitable temperature.
The habitat of a trout is a fast-flowing stream, and the habitat of wild garlic is a woodland.
Most habitats are made up of a patchwork of ***microhabitats***.
For instance, in an oak tree, insects can live in or on the leaves, in cracks in the bark, inside an acorn or amongst the roots.
These are all examples of microhabitats.

How many habitats can you spot in this photograph?

The **niche** is the ***role*** that an organism plays in its environment.
It is quite simply the ***way of life*** of an organism in its natural surroundings.
For instance, the winter moth feeds on the buds of oak trees.
At the same time, it is the food of insectivorous birds and parasites.
This is the role or niche of the winter moth.
If two organisms occupy the same niche, they will **compete**.
In Britain, the North American grey squirrel and the native red squirrel seem to be competing for the same niche.
They both need the same food and nesting sites in the same kind of habitat.

Competing for the same niche

Populations, communities and ecosystems

Ecology can be studied at a number of different levels.
A **population** is a group of individuals of the ***same*** species, living in the same habitat. For example, a population of tawny owls in a woodland, or a population of banded snails in a hedgerow.
Since they are of the same species, the members of a population are able to interbreed.
A **community** consists of all the living things in a particular habitat.
For instance, all the inhabitants of a lake or a salt marsh.
A community is made up of the different populations of species that live in the habitat. An **ecosystem** is made up of the community (biotic component) and the habitat (abiotic component).
An ecosystem is a major ecological unit. For instance, a pond ecosystem consists of all the living organisms in the pond ***as well as*** the water, dissolved oxygen, suspended materials and pond bed.

Coral reef ecosystem

Feeding relationships

The individuals in a community can be classified by their method of feeding.

Living organisms can be classed as:

- **autotrophs** – those that can make their own food, or
- **heterotrophs** – those that cannot make their own food.

Autotrophs are able to make their own organic food from inorganic substances, using a source of energy.
Green plants are autotrophs. They use light energy to convert carbon dioxide and water into sugars by photosynthesis.
Algae and many types of bacteria can also photosynthesise using light energy.

Light penetrating the tree canopy

Other autotrophic bacteria use the energy from chemical reactions to synthesise organic food. This is called **chemosynthesis**.
Since autotrophs are the only living things that can produce organic food substances, they ultimately provide all the food for other members of the community.
For this reason we call autotrophs **producers**.

Heterotrophs cannot make their own organic food – they have to take it into their bodies ready-made.
In other words, they eat it or consume it.
All animals are **consumers** and ultimately they all depend upon producers for food.

- **Primary consumers** are herbivores. They eat producers.
- **Secondary consumers** are carnivores. They feed on primary consumers.
- **Tertiary consumers** are carnivores that eat other consumers. They are sometimes called top carnivores.

Secondary consumer and primary consumer

Each of these feeding categories is known as a **trophic level**.
'Trophic' comes from the Greek word meaning 'to feed'.
But how do you know which trophic level an animal belongs to?
You can get clues about what it eats from its teeth and by studying its feeding behaviour.

Decomposers and detritivores

These two groups of consumers are important since they feed on dead animals and plants. They release organic and inorganic nutrients, which may be used again.

Detritivores at work

- **Detritivores** are primary consumers that feed on fragments of dead organic material called **detritus**. They shred the detritus up into smaller particles.
  Detritivores include small animals like earthworms and woodlice in the soil, and freshwater shrimps and hog-lice in rivers and streams.
- **Decomposers** (**saprobionts**) include microbes such as fungi and bacteria. They also obtain their energy from dead and decaying organic material. They complete the process of decomposition started by detritivores. Decomposers fulfil an important role by releasing nutrients from dead organic matter, which can be recycled and used for new plant growth.

Chains and webs

Food chains show what eats what in a community.
They show the transfer of food energy from one trophic level to another.
Look at the food chain below:

oak leaves → caterpillar → blue tit → sparrow hawk

Notice that the arrows show the direction in which food energy is transferred.
Food chains always begin with a producer, usually a photosynthetic plant.
Here is a detritivore food chain:

dead leaves → woodlouse → carabid beetle → shrew → owl

We can use food chains to show feeding relationships in any community.
Here is a food chain for a lake community:

phytoplankton → water fleas → stickleback → perch → pike

If all the water fleas died:
What would happen to the number of sticklebacks?
What would happen to the number of phytoplankton?
A food chain seldom has more than five links. Why do you think this is?

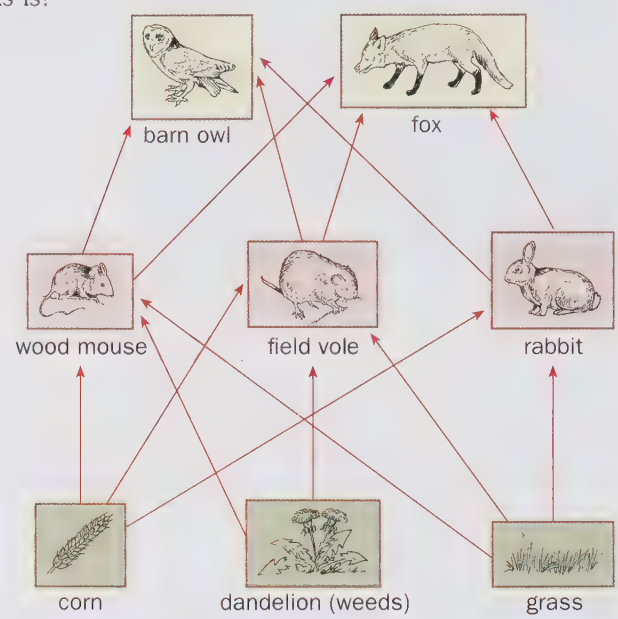
Food webs

Food chains give only a limited impression of the feeding relationships in a community.
They only ever show a consumer feeding on ***one*** type of animal or plant.
In reality, an animal will feed upon a variety of other organisms.

A **food web** gives a more complete picture of the feeding relationships in a community.
A food web consists of all the food chains in a community linked together.

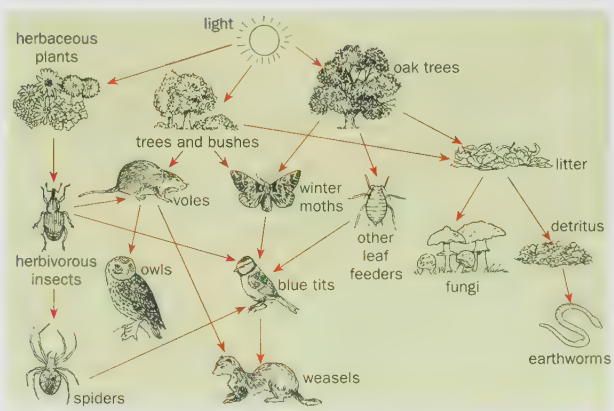
Look at this simple grassland food web.

Suppose all the field voles were killed by disease.
Why might the number of dandelions increase?
Why might the number of foxes decrease?
Explain why the numbers of wood mice might either increase or decrease.



Food web for a grassland community

Look at the food web for a woodland community and give one example of:
i) a decomposer,
ii) a tertiary consumer,
iii) an omnivore,
iv) a producer,
v) an invertebrate predator.
Try to draw a pyramid of number for the food chain
oak tree → winter moths → blue tits → weasels
How might a pyramid of biomass look different from the pyramid that you have drawn?



Food web for a woodland community

A major drawback of food webs is that they do not tell you ***how many*** living organisms are involved.
Many food webs are just too complex to complete.

Pyramids of number

Food webs describe the feeding relationships that exist within a community but they give no information about the numbers or ***quantities*** of organisms involved, or their mass, or the energy involved.

You can see from the diagram that it may take many plants to feed one herbivore and many herbivores to sustain just one carnivore.

Here are some numbers for this food chain:

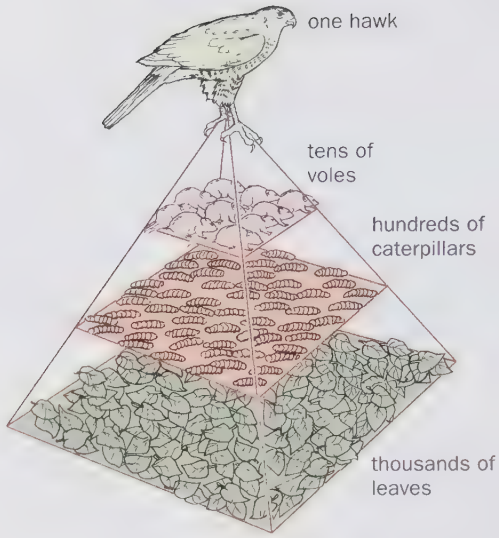
hawk	1
voles	10
caterpillars	100
groundsel plants	600

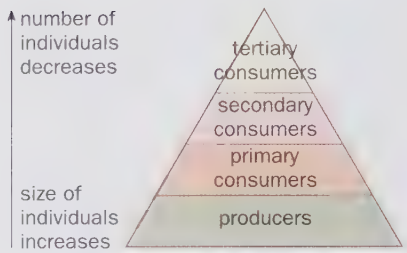
We can display this information in a **pyramid of number**. This is essentially a bar chart that is plotted horizontally. The area of each bar is proportional to the number of individuals at that trophic level.
The producers are placed at the base of the pyramid and each successive consumer level is placed above them.

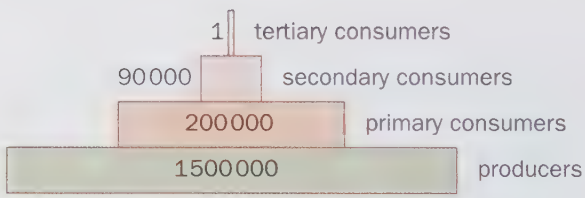
- What happens to the ***numbers*** of individuals as you go up this pyramid?
- What happens to the ***size*** of each individual organism as you go up the pyramid?

To construct a pyramid of number for a particular community, you must first randomly sample the organisms.
This may involve quadrat analysis, the use of nets or humane trapping.

The sample is divided up into each trophic level and the numbers of individuals counted.
The units for pyramids of number are usually expressed as individuals per square metre.







Pyramid of number for a grassland community in 0.1 hectare

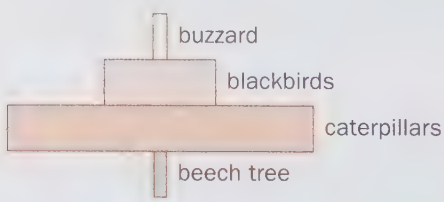
Problems with pyramids of number

Pyramids of number are an improvement upon food chains and food webs since they do give ***quantitative*** information. However, their major drawback is that they do not take into account the ***relative mass*** of organisms at each trophic level.

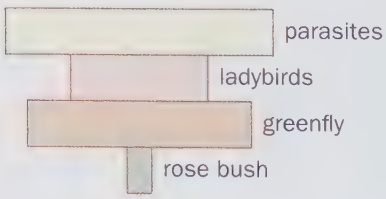
For instance, both an oak tree and a grass plant each count as just ***one*** organism.
But clearly one oak tree can support many more herbivores than one grass plant can.
This limitation in the technique can result in some unusual shaped pyramids, as you can see here.

In this pyramid of number, the tertiary consumers are parasites. Many of them are able to feed on just one ladybird.

This top-heavy pyramid is said to be inverted.



Beech tree pyramid



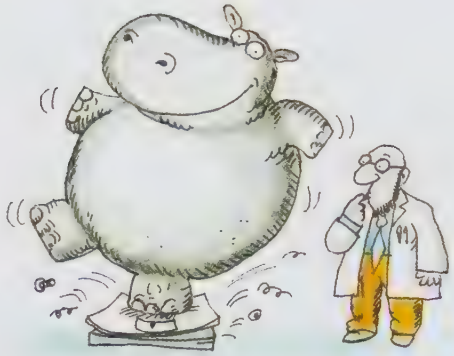
Inverted pyramid

Pyramids of biomass

One way to overcome this problem of the size of the organism is to measure the **biomass** at each trophic level instead of the number of organisms present.

Biomass is the mass of living material present at a given time. So a biomass pyramid will show the mass of living organisms, at a particular trophic level, per unit area or volume, at a particular time (often expressed in $kg\,m^{-2}$).
This is also referred to as the **standing crop**.

To construct a biomass pyramid, you first need to collect some data. You need to take random samples, usually harvesting all the organisms within a quadrat.
Then divide the organisms up into their respective trophic levels and weigh them. This is known as **wet mass** or **fresh mass**.
Find the average mass for each trophic level.
Then multiply the average mass by the estimated number of organisms to give the biomass at each trophic level.
Some scientists prefer to use **dry mass**, since the water content of organisms (especially plants) can vary a great deal.
However, obtaining dry mass data involves heating the sample in an oven at 110 °C to remove all the water.
This is neither practicable nor desirable since it inevitably kills the plants and animals that have been sampled.



Biomass is the weight of living material

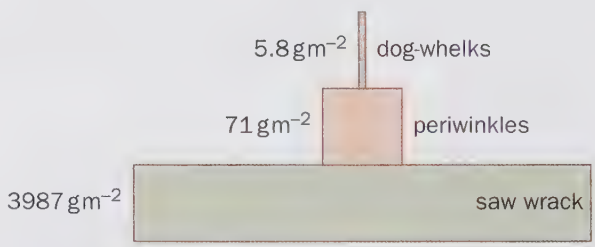
Biomass sampling on a rocky shore

Drawbacks with biomass pyramids

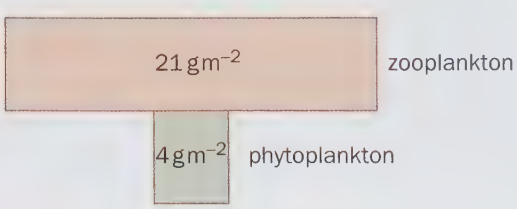
- The biomass recorded at any one instance is just a 'snap shot' in time. Biomass pyramids give no indication of the ***rate*** at which organisms grow. **Productivity** is the rate at which organic materials are produced per unit area or volume per unit time. Biomass data cannot take into account how fast organic materials are produced. For example, the grass in a field grows fast, it has ***high productivity***. However, because it is grazed by herbivores such as sheep or cattle, its biomass at any one point in time will be low.
- Phytoplankton are tiny producers that grow very quickly in the sea. They grow and divide every 3 or 4 days.
  We say that they have high productivity or that they have a high **turnover rate**. But they are constantly harvested by the primary consumers present in the habitat.
  So their total biomass at any particular time is relatively small.
  But over a period of, say, a year, their biomass will be huge.
  So this particular biomass pyramid looks inverted because it reflects only a few days of phytoplankton growth.
- Biomass can also vary with the seasons.
  The biomass of a beech tree will be far greater in summer than it is in winter. Why do you think this is?

  In the winter the tree will have shed the leaves, flowers and fruits that develop in the summer.

  Pyramids of biomass overcome the problem of size of individuals but they do not take productivity into account.



Biomass pyramid for a rocky shore community

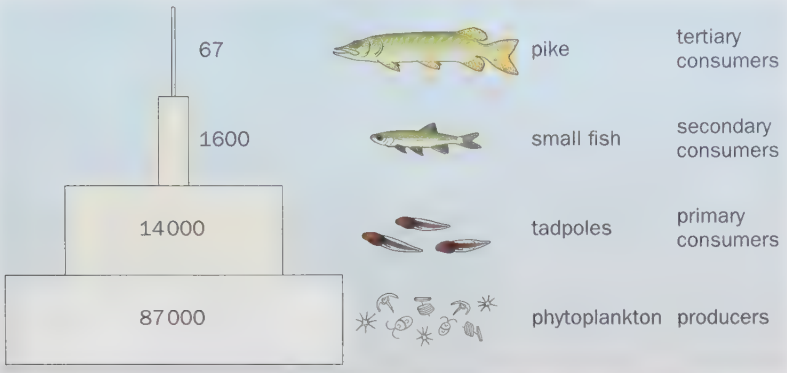


Biomass pyramid for a plankton community

Pyramids of energy

The most accurate way to represent the feeding relationships in a community is to use an **energy pyramid**.
An energy pyramid shows the amount of energy transferred from one trophic level to the next, per unit area or volume, per unit time.

This energy pyramid shows that 87 000 kJ m^{-2} $year^{-1}$ is transferred to the tadpoles (primary consumers) from the water plants (producers).
The tadpoles then pass 14 000 kJ m^{-2} $year^{-1}$ to the small fish (secondary consumers) and so on.



A pyramid of energy for a lake community (figures are in kJ m^{-2} $year^{-1}$)

However, if the tadpoles gained 87 000 kJ m^{-2} $year^{-1}$ from the water plants but only passed on 14 000 kJ m^{-2} $year^{-1}$ to the small fish, then where did the other 73 000 kJ m^{-2} $year^{-1}$ go to?

What have the tadpoles done with all that energy?

They will have used up a lot in respiration, using energy to swim around.
They will also have passed out some energy in waste materials.
The only energy that the tadpoles do pass on is that which they have used to make new body cells as they grow.

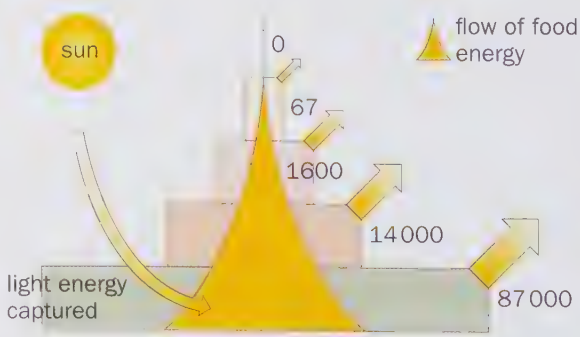
A large proportion of energy is always 'lost' between trophic levels in these ways.
Of the 87 000 kJ m^{-2} $year^{-1}$ of energy at the start, only 67 kJ m^{-2} $year^{-1}$ will end up in biomass as part of the pike's body.

Since only some of the energy is passed on, energy pyramids are never inverted.
The pyramid's shape is not affected by the size of the organisms nor their numbers, it simply reflects the amount of energy that is passed on.

Unlike pyramids of number or biomass, energy pyramids make it easy to compare the efficiency of energy transfer from one trophic level to the next in different communities.
They reflect the productivity at each trophic level.

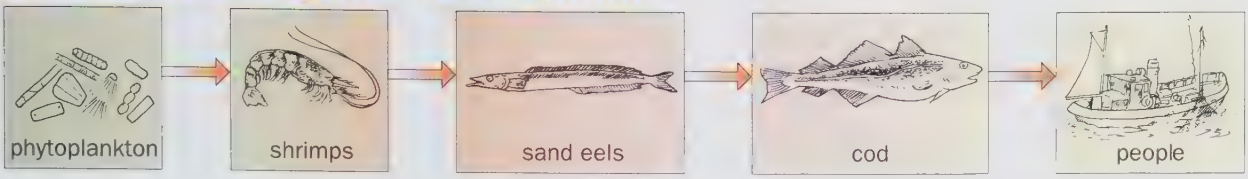
Although energy pyramids give a better representation of the transfer of food energy between trophic levels than pyramids of number or biomass, the data is difficult to obtain.
It requires incineration of the sample in a calorimeter to estimate energy content.
This destructive sampling is seldom practicable nor desirable.

The larva of the great diving beetle also predates upon tadpoles

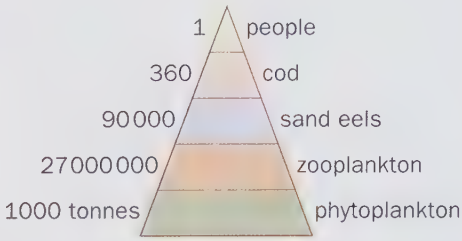


A pyramid of energy for a lake ecosystem in kJ m^{-2} $year^{-1}$

Shortening the food chain

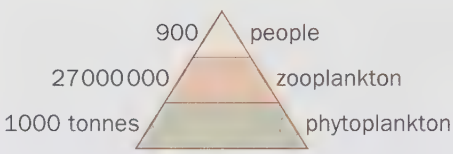


Look at the pyramid of number on the right.
It shows the estimated number of individuals that could be supported by 1000 tonnes of phytoplankton per year.
Humans are at the top of this pyramid.
How many cod would one human eat in a year?
It works out at about 1 cod a day.

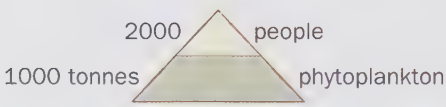


What if the food chain is shortened and people ate sand eels instead of cod?
Thirty people could be supported in this way, that's assuming that each person could get by on about 10 sand eels a day.

What if the food chain is shortened again?
People now feed on zooplankton such as shrimps.
How would you fancy 100 shrimps a day?
If so, the food chain could support 900 people a year.



What if we were to remove the last animal link in the food chain and become vegetarian?
Feeding upon 2kg of phytoplankton a day may not appeal to you.
But this could sustain 2000 people per year!



What is the message for us from this simple exercise?
Quite simply, a vegetarian diet can support far more people.
By eliminating links in the food chain, more people at the end of the food chain can be fed.
This is because we are reducing the 90% 'wastage' of energy that occurs between one trophic level and the next.
The energy that is uneaten, undigested or used in respiration at each trophic level.
Quite simply, the longer the food chain, the more energy will be lost.

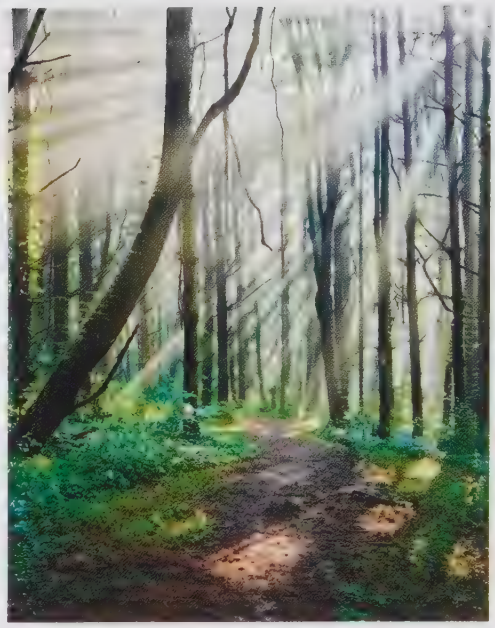
Why do you think that people living in underdeveloped countries tend to have vegetarian diets?
In developed countries, people have a varied diet including poultry, fish, lamb and beef.
What does this tell you about the economies of these countries?

The human population is increasing at an alarming rate.
How do you think this will affect the future price of meat?

The rising price of food will inevitably push us down the food pyramid towards a vegetarian diet.

Food distribution in Somalia

Energy flow through producers



The energy in all ecosystems comes originally from sunlight.
This energy can be transferred from one form to another
but cannot be created or destroyed.
Solar energy enters the food chain at producer level during photosynthesis.
Some of this energy is then passed on to consumers, but eventually
all of it will leave the system as heat.

All living organisms depend directly or indirectly upon **primary production**.
Primary production is the production of organic materials by producers.
Green plants (and algae and some bacteria) are able to transfer solar
energy into the chemical energy in sugar during photosynthesis.

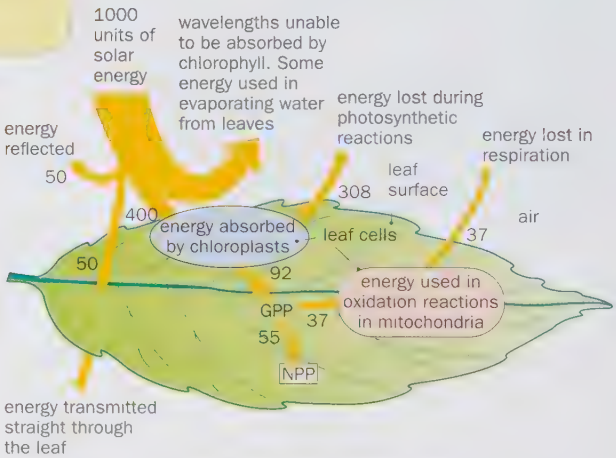
Photosynthesis is far less efficient than we may imagine.
Most of the sunlight that falls on a plant is not even absorbed.
Only light within the wavelengths 380 nm (blue) and 720 nm (red)
can be absorbed by chlorophyll and other plant pigments.
This light is called **photosynthetically active radiation (PAR)**.
This is the only light that can be used in photosynthesis.
But not all PAR will be absorbed by chlorophyll.
Some will be reflected and some will pass straight through the leaf
(in which case, we say that it is transmitted).

Photosynthetic efficiency is a measure of how well
a plant is able to capture light energy.

$$\text{photosynthetic efficiency} = \frac{\text{amount of energy incorporated into carbohydrate}}{\text{amount of energy falling on the plant}}$$

Even in ideal conditions the overall efficiency of energy
conversion in photosynthesis is less than 8%.
In reality, ideal conditions seldom prevail, due to daily and
seasonal fluctuations in light and temperature, lack of nutrients
and water stress.

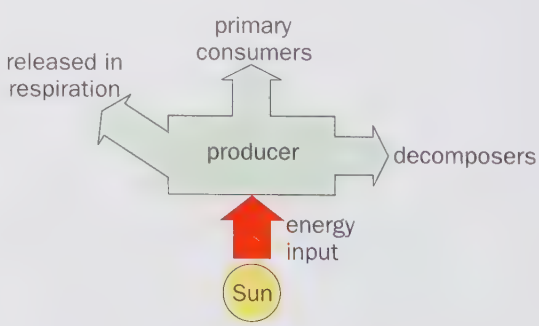
The light energy captured by chlorophyll is used to make ATP
(adenosine triphosphate). The plant uses most of this ATP to
carry out its own metabolic processes.
The sugars made in photosynthesis accumulate as **gross
primary production (GPP)**.
A great deal of this is used up in respiration, providing energy to
drive the plant's life processes.
What is left over is called **net primary production (NPP)**.



The amount of solar energy intercepted by green plants depends a great deal on geographical location. In Britain this is estimated as approximately $1 \times 106\,kJm^{-2}\,year^{-1}$, but at least 95% of this is unavailable to plants for photosynthesis

$$\text{net primary production} = \text{gross primary production} - \text{respiration}$$

NPP represents the potential food available to primary consumers.
By eating plants, herbivores are, in effect, receiving light energy in
the form of organic molecules such as carbohydrates, fats and proteins.
Some of this potential food may be transferred to decomposers.
This can happen when leaves are shed, fruits and seeds are dispersed
and when the plant itself dies.
Decomposers benefit by obtaining energy from the dead plant tissues.



Energy flow through a green plant

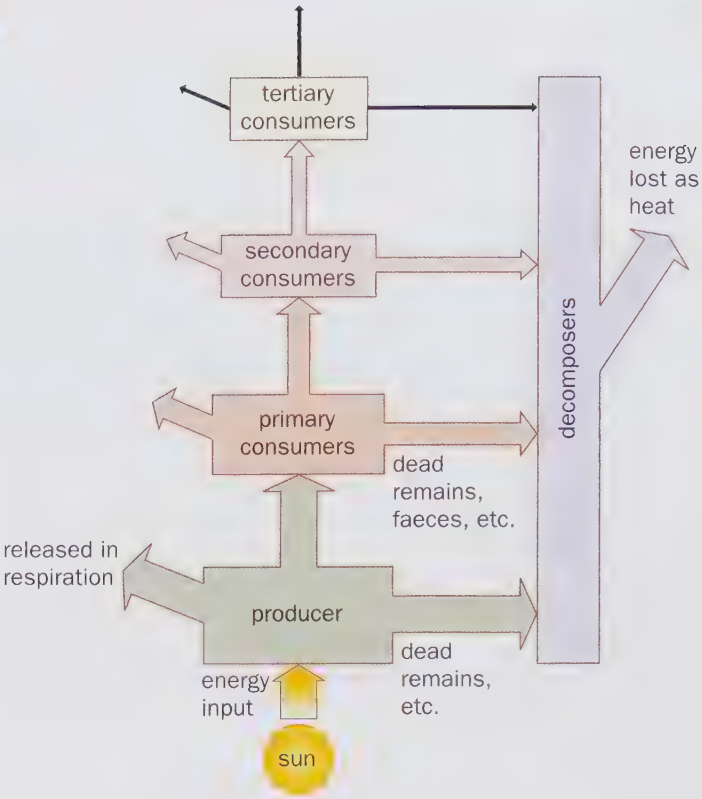
Energy flow through consumers

Transfer of energy from producers to primary consumers, or from plants to herbivores, also involves energy 'wastage'. It is estimated that for every 100 g of plant material available, only about 10 g ends up as new herbivore biomass. This represents a conversion efficiency of 10%.

So what are the reasons for the 90% energy wastage between trophic levels?

- Some plant material may not be consumed in the first place, for example the bark of a tree or a plant's roots. The energy will eventually go to decomposers in dead remains.
- Some plant material is not digested and passes out of the herbivore's body in the faeces.
- Much of the energy in the food will be used by the herbivore for respiration.

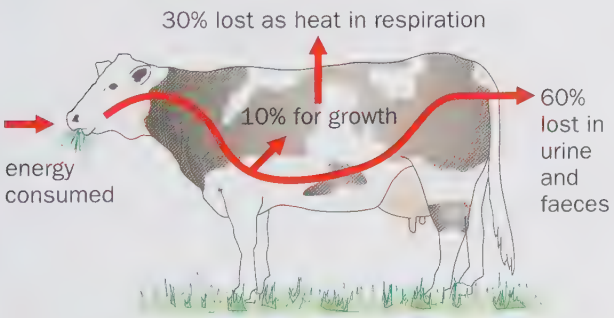
Similar losses of energy occur between subsequent trophic levels. Carnivores are more efficient at energy conversion. Some are able to achieve as much as 20%. That is, 20% of the herbivore biomass eaten ends up as carnivore biomass. This is because they are able to digest high protein diets more efficiently. It is this loss of energy at each trophic level that gives ecological pyramids their characteristic shape.



Energy flow through a community

Energy budgets

If the amounts of energy entering, being used up and leaving an animal can be measured, then we can work out an **energy budget**.



Energy budget of a cow

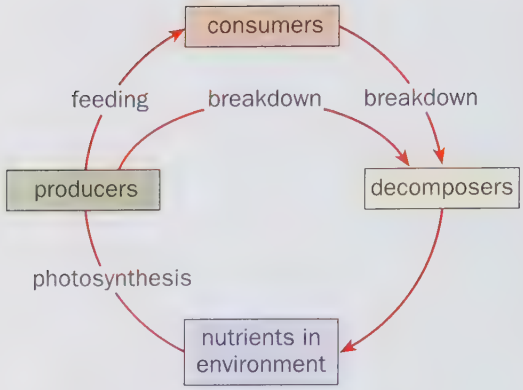
Look at the energy budget of the cow. You can see that, of the energy in the grass that the cow consumes, over half of it is passed out of the body in the faeces. A lot of energy is used up in respiration to fuel the chemical reactions in the body and for movement. A proportion goes to produce new biomass in growth. The remainder is excreted as metabolic waste in urine. The energy budget of a cow can be summarised as:

energy intake = **energy transfer in respiration** + **energy transfer into biomass** + **energy in faeces** + **energy in urine**

Nutrient cycling

As you know, all living organisms need energy.
Energy enters ecosystems as sunlight trapped by producers.
It is then transferred up various trophic levels, eventually being released as heat.

But living organisms also need organic and inorganic nutrients.
Plants obtain inorganic nutrients from the soil and water.
As with energy, these nutrients are transferred to consumers along the food chain.
But unlike energy, these nutrients are later released back into the environment so that they can be reused.
There is a fixed amount of nutrients on Earth and they constantly move from air, water and soil into living organisms and back again.
We say that nutrients are **cycled** between the biotic and abiotic environments.

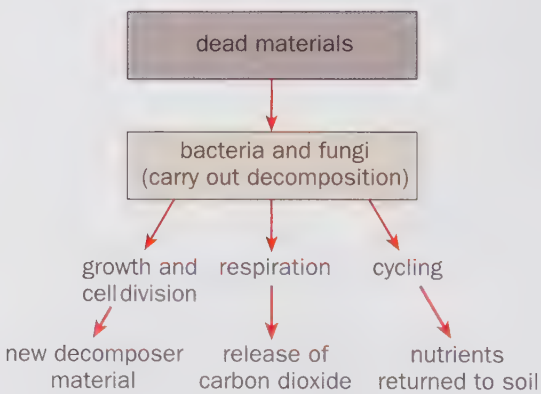


Cycling of nutrients in an ecosystem

Most living matter (95%) is composed of just six elements: carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur.
A good way to remember this is the mnemonic 'CHNOPS'.
These are **macronutrients** needed in large amounts by green plants (as we saw in Chapter 18).
Other nutrients, called **trace elements**, are only needed in minute amounts, for example iron, manganese, copper, zinc and boron.
However, if these are lacking, plants will develop deficiency symptoms.

Decomposition

Fragments of dead and decaying matter are called detritus.
Can you remember what we call the small animals that feed on detritus?
Detritivores include animals like worms, woodlice and maggots.
They shred up detritus into minute particles that decomposers are able to act upon.
Without detritivores, the process of decomposition would take much longer.



The roles of bacteria and fungi in decomposition

Decomposers or saprobionts (bacteria and fungi) release enzymes which break down their food by **extracellular digestion**.
They then absorb the digested products in much the same way as we absorb digested food from our gut.
The decomposers use this food for growth and energy.
The decomposers may be eaten by other organisms, passing on nutrients in this way, or the nutrients may be released into the soil or water.

Decomposer food chain:

dead leaves → fungus → beetle → frog

Detritivore food chain:

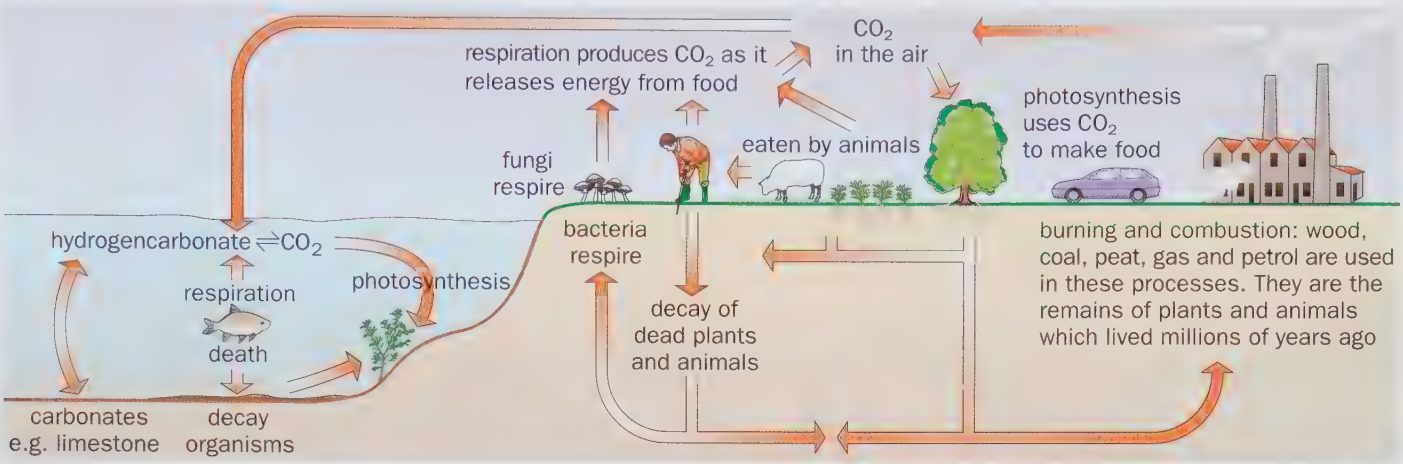
dead animal → blowfly maggots → blackbird → sparrowhawk

As you will see in the carbon cycle and the nitrogen cycle, decomposers play a key role in the cycling of nutrients.

The carbon cycle

All living organisms need carbon.
Organic molecules such as carbohydrates, proteins, fats, nucleic acids and other important compounds all contain carbon.

Two processes dominate the carbon cycle: photosynthesis and respiration.



Carbon dioxide (CO_2) is readily available in the air and, because it is highly soluble in water, it is freely available to freshwater and marine plants.
As you can see from the diagram, carbon dioxide from the air and water is fixed into organic compounds during photosynthesis.

$$\underset{\text{carbon dioxide}}{6CO_2} + \underset{\text{water}}{6H_2O} \xrightarrow[\text{chlorophyll}]{\text{sunlight}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{oxygen}}{6O_2}$$

Photosynthesis provides the input for carbon into food chains.
Primary consumers eat the producers and may, in turn, be eaten by secondary consumers, and so on.
All animals rely upon plants, directly or indirectly, for their source of carbon.

How is carbon released back into the environment?

- Plants and animals use a lot of their organic food in respiration. The carbon is released back into the environment as carbon dioxide. This carbon dioxide can be used for photosynthesis again.

$$\underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{oxygen}}{6O_2} \longrightarrow \underset{\text{carbon dioxide}}{6CO_2} + \underset{\text{water}}{6H_2O} + \text{energy}$$

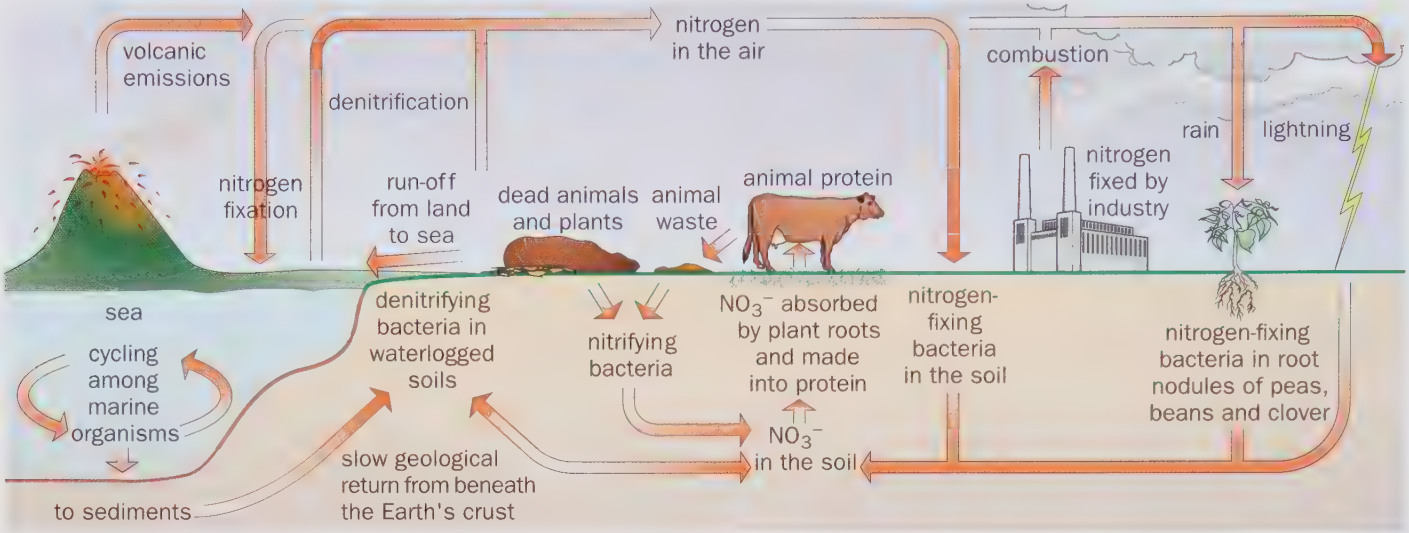
- Decomposers use dead plants and animals for food. They use some of their food in respiration, releasing carbon dioxide.
- Fossil fuels such as oil, coal, peat and gas contain carbon. When they are burned, carbon dioxide is released back into air and water.

These processes put back carbon dioxide into the air and water at about the same rate as plants remove it for photosynthesis.
So the amount of carbon dioxide in the atmosphere and water should stay the same.
As you will know, the level of carbon dioxide in the air is increasing.
The possible consequences of this in terms of the 'greenhouse effect' and global warming are dealt with in Chapter 25.

The burning of fossil fuels releases carbon dioxide back into the environment

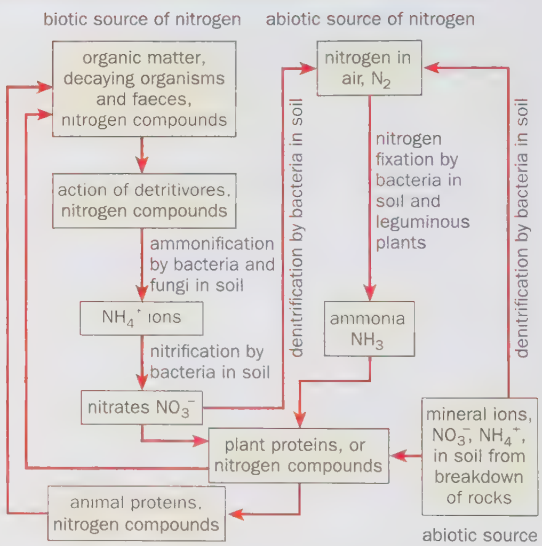
The nitrogen cycle

Despite making up nearly 80% of the air, nitrogen (N_2) is an unreactive gas.
Living organisms need nitrogen for amino acids, proteins, nucleic acids and other important organic molecules.
But plants and animals are unable to use nitrogen gas.
It has to be converted to **nitrates** (NO_3^-) before it can be absorbed and used by plants.
It can then be transferred to consumers along the food chains.
So how does nitrogen gas get changed into nitrates?



If you look carefully at the diagram you will see that the key organisms in the nitrogen cycle are ***bacteria***.

- **Decomposing bacteria** (saprobionts), along with fungi, decompose dead plants and animals, faeces and urine into simpler molecules. The complex molecules such as proteins, amino acids and urea are broken down and nitrogen is released to the environment in the form of ammonium ions (NH_4^+). This is called **ammonification**.
- **Nitrifying bacteria** are able to convert the NH_4^+ ions into nitrates, under aerobic conditions. *Nitrosomonas* oxidises NH_4^+ ions to nitrite. The nitrite is then oxidised to nitrate by *Nitrobacter*. This process is called **nitrification**. The nitrates formed can be absorbed by plants and so make proteins and nucleic acids and enter the food chains.
- **Nitrogen-fixing bacteria** take nitrogen gas out of the air and convert it into ammonia. Plants convert ammonia into organic nitrogen-containing compounds. Free-living microorganisms such as *Azotobacter* in the soil and *Nostoc*, a cyanobacterium found in freshwater, account for 90% of nitrogen fixation.
  The bacterium *Rhizobium* is found in the roots of legume plants such as peas, beans, clover and gorse. The roots swell to form **root nodules**. The bacterium forms a relationship with the legume plant which is beneficial to both partners.The bacterium fixes nitrogen and gives the plant a source of nitrogen; in return, the bacterium obtains carbohydrate from the host plant. This type of relationship where both partners win is called **mutualism**.
- **Denitrifying bacteria** get their energy source by converting nitrates and ammonium ions back into nitrogen gas.
  Denitrification occurs in the absence of oxygen, for example in water-logged soils. *Pseudomonas* and *Thiobacillus* gain their energy from denitrification.



The role of bacteria in the nitrogen cycle

Root nodules of a legume plant

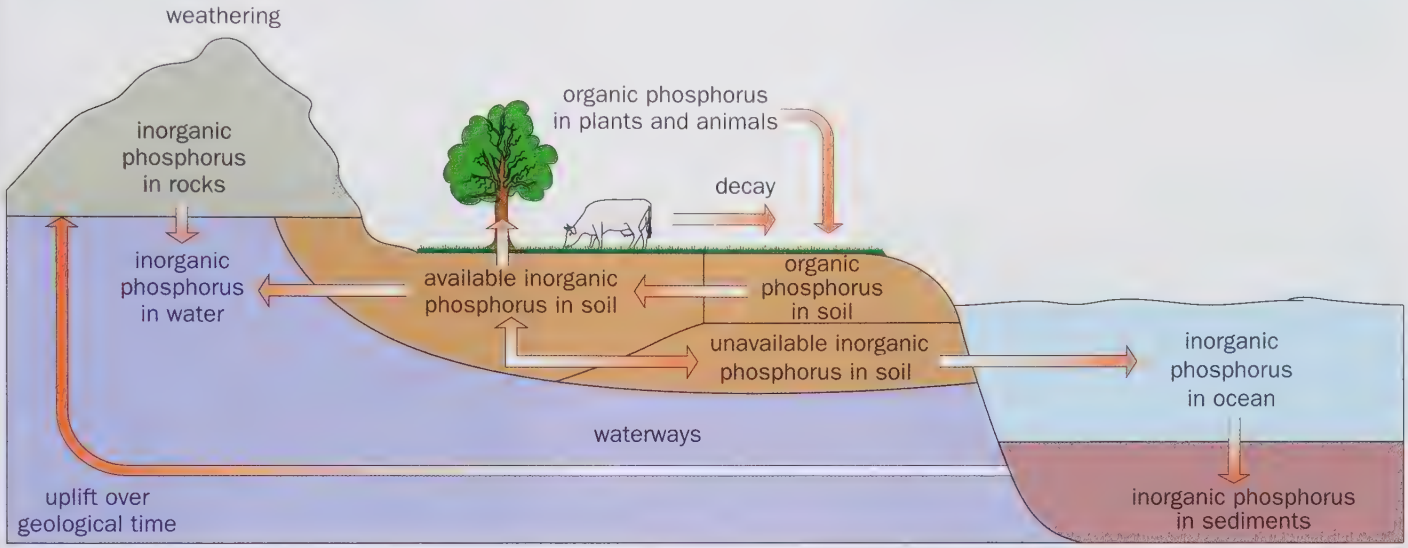
The phosphorus cycle

Phosphorus is an essential nutrient for plants and animals. It forms part of important biological molecules such as DNA and RNA, ATP, and as phospholipid in cell membranes. Phosphorus, along with calcium, is important to vertebrates; in the human body, 80% of phosphorus is found in bones and teeth.

Phosphorus can be found in numerous compounds, such as **phosphate ions** ($\mathbf{PO_4^{3}}$) which are located in water, soil and sediments. Unlike the compounds of the carbon and nitrogen cycle, phosphorus cannot be found in the air in its gaseous state. Also the amount of phosphorus in the soil is relatively small and this can limit plant growth.

Sources of phosphorus

The largest reservoir of phosphorus is in sedimentary rock, and it is in these rocks that the phosphorus cycle begins. When it rains, PO_4^{3-} ions are removed from the rocks by weathering and distributed in soils and water.



The phosphorus cycle

Plants take up PO_4^{3-} ions from the soil. In uncultivated soil, **mycorrhizae** can play a key role in obtaining these ions, as well as other inorganic ions and water. The fungus forms an association with the plant's roots, greatly extending its area in the soil and, therefore, its ability to obtain essential nutrients. The phosphate then moves from plants to animals along the food chain. Inside the plant or animal, phosphate is incorporated into key biological molecules such as DNA. The phosphate absorbed by animal tissue through consumption eventually returns to the soil through excretion in urine and faeces. Decomposition of dead plants and animals returns organic phosphate to the soil from organic compounds.

Within the soil, bacteria break down organic compounds releasing inorganic forms of phosphorus which are then available to plants.

Most of the phosphorus in the soil will end up in waterways and eventually oceans. Here it can be incorporated into sediments over time. Eventually, phosphorus is released again through weathering and the cycle starts over.

Phosphorus and plant growth

As mentioned, most phosphorus is locked up in sediments and rocks and little becomes available to plants in the soil. Some bacteria can convert phosphate into phosphorus-containing organic molecules that cannot be absorbed by plant roots. Also inorganic phosphate binds tightly to molecules in the soil and becomes adsorbed onto soil particles making it unavailable to plants. Soils with a pH lower than 4, or greater than 8, tend to combine with other soil compounds so less is available for plant growth. You can read about the problems with phosphate-rich fertilisers on page 466.

Biology at work: Sustainable timber production

The First World War alerted the UK government to the dangers of running out of timber, so it set up the Forestry Commission in 1919. Its brief was to establish a national forestry reserve.
Since then, vast areas of non-native conifers have been planted, particularly in Scotland.
Currently, woodland covers 10% of the UK, with 9% made up of these plantations and only 1% by native woodlands.
Conifers have been planted rather than native hardwoods because they produce more timber in a shorter time and can grow on infertile soils.

Coniferous plantation in Perthshire, Scotland

The two main aims of the government's forestry policy are

- the sustained management of existing woods and forests, and
- a steady expansion of tree cover to increase the many diverse benefits that forests provide.

Can a landscape rich in wildlife also produce a ***sustainable*** supply of timber? The following actions will help:

- better management of existing woodlands,
- planting more native trees,
- using natural regeneration and more sensitive planting methods,
- siting well-managed commercial forests on surplus agricultural land.

Cutting down coniferous timber in USA

The UK is a major importer and user of timber.
Most of this comes from the temperate forests of Scandinavia and North America, often involving clear-felling of old growth.
We could reduce the need to import so much timber by reducing consumption of wood products, recycling paper, and only using wood which has come from sustainably managed forests.

Native woodlands

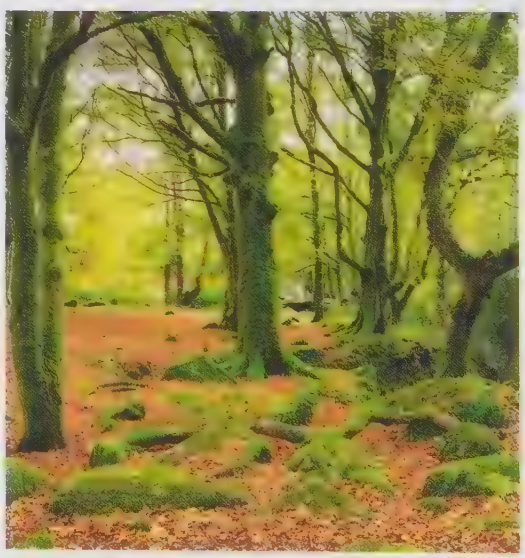
The native woodlands that are left should be preserved.
They should be allowed to grow and expand to form a network of woodland reserves across the country.

There should be a clear commitment towards sustainable forestry in order to

- maintain and enhance biodiversity by providing woodland habitats for the great variety of species that live there,
- produce timber at a rate the forest can sustain,
- use non-renewable resources sparingly to avoid needless consumption and pollution.

To achieve this there should be

- major research and survey work carried out to classify woodlands according to their environmental quality,
- restoration of woodlands by allowing them to grow naturally or, where this is difficult, by planting native species,
- sensitive management of woodlands to mimic natural ecological processes.



Native woodland in Cornwall

Biology at work: Intensive farming

Intensive farming involves attempts to maximise food production through the careful control of growing conditions.

It can be seen in a number of examples, such as the use of chemicals and glasshouses in crop production, careful monitoring of food and environmental conditions in poultry farming, and also in fish farming.

In the EU 80% of farmed fish production is comprised of just four species: rainbow trout, Atlantic salmon, gilthead seabream and European seabass.
In the UK salmon and trout are the main farmed fish, with trout reaching a marketable size in around 11 months.

A sea loch salmon farm using cages

Fish farms are usually based on a series of ponds, or cages suspended in freshwater or the sea.
Where ponds are used it is obviously easier to control growing conditions.
The depth, for example, is kept to about 1.5m to avoid the development of a **thermocline**.
This is where a cooler layer of water (unsuitable for growth) develops below the warmer surface layers.

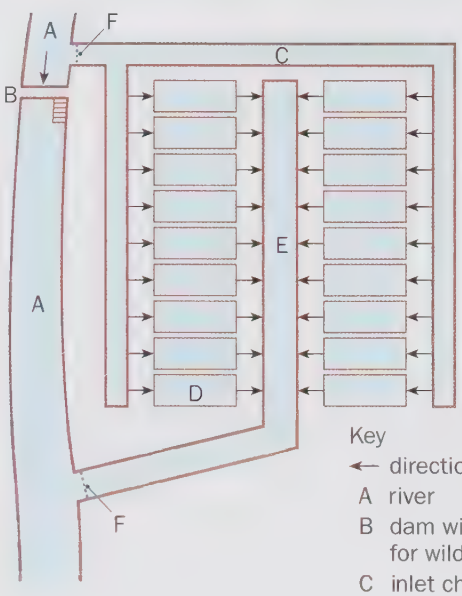
A trout hatchery

In intensive farming, fish are kept at a very high stock density meaning that they cannot obtain sufficient food and oxygen from their environment without human input.

The quality and reliability of the water is crucial to successful growth.
It should be clear, with a neutral to slightly alkaline pH.
The flow rate must provide both adequate oxygenation and also adequate dispersal of metabolic wastes such as ammonia and carbon dioxide.
A high flow rate will cause the fish to expend energy simply to maintain their position. This energy is therefore not available for growth.

A high protein diet is given, usually in the form of fish meal together with a mix of minerals and vitamins.
The subject of feeding farmed fish is controversial because fish meal is usually derived from wild caught fish.
It is estimated that it takes between 2 and 3kg of wild fish to produce 1kg of farmed fish.
The spread of disease in overcrowded conditions is another area of concern, not least because of the danger of infection spreading to local wild populations.
The use of drugs in fish farms is also controversial because the use of antibiotics in food production is thought to increase the prevalence of antibiotic resistance in humans.

At a time of increasing depletion of wild stocks, fish farming, despite its controversies, is seen by many as a solution to the increased market demand for fish and fish protein.



The layout of ponds in a trout farm

Key
← direction of water flow
A river
B dam with fish ladder for wild fish
C inlet channels
D ponds
E outlet channel
F fish screen

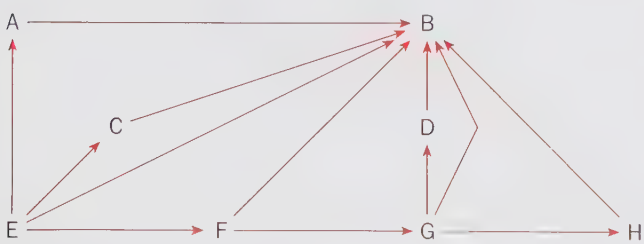
Summary

- Ecology is the study of how living organisms interact with each other and with their environment.
- A habitat is the place where an organism lives. A niche is the role that an organism plays in its environment.
- A population is a group of individuals of the same species. A community consists of all the species in a particular habitat. An ecosystem is made up of the community and the habitat.
- Feeding relationships can involve producers, consumers, detritivores and decomposers.
- Food chains and food webs chart the feeding relationships in a community.
- Pyramids of number give information about the quantities of organisms involved.
- Biomass pyramids reflect the mass of organisms involved but do not show their productivity.
- Energy pyramids are the most accurate method of representing feeding relationships.
- The overall efficiency of energy conversion in photosynthesis is low.
- Net primary production = gross primary production − respiration.
- Transfer of energy from producers to consumers involves energy 'wastage'.
- The longer the food chain, the more energy will be lost.
- Decomposers are the key organisms involved in the cycling of nutrients in ecosystems.
- Bacteria and fungi play a number of vital roles in the cycling of nitrogen and phosphorus.

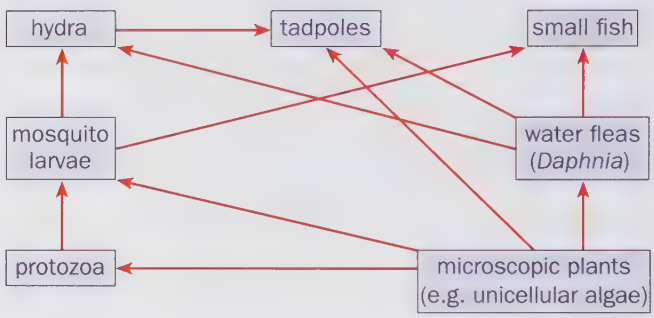
Questions

1 What is meant by each of the following ecological terms:
a) a community,
b) a population,
c) a niche?

2 Look at the diagram showing the food web.
Each letter in the diagram represents a different species of organism.
a) Suggest one letter that represents
i) a decomposer,
ii) a herbivore,
iii) a secondary consumer,
iv) trophic level 1.
b) Identify, by letter, one organism in the food web that is found in the trophic level which contains most energy.



3 The diagram shows part of a food web in a freshwater pond.
a) Name examples of the following from this food web.
i) an omnivore,
ii) a producer,
iii) a secondary consumer,
iv) a tertiary consumer.



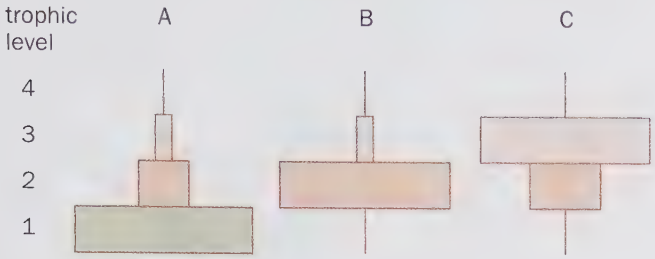
b) Can you find a food chain in this web that consists of five trophic levels?
Draw a pyramid of energy to represent this chain.
Label your diagram to show the organisms at each energy level.

4 The table shows the numbers of organisms obtained at each trophic level in a sampling study of an oak tree.

Trophic level	Number of organisms
producer	1
primary consumer	260 000
secondary consumer	40
tertiary consumer	3

a) Draw a pyramid of number to represent this food chain.
b) Suggest two reasons why there is such a large difference between the numbers of primary and secondary consumers.

5 Here are three pyramids of number from different food chains.



a) Name the four trophic levels in pyramid A and give a typical example of an organism found at each trophic level. (The organisms you choose should all come from the same food chain.)

b) Account for the shapes of pyramids B and C.

6 The table shows the net primary productivities in some aquatic communities.

Community type	Mean net primary productivity
swamp and marsh	2000
continental shelf	360
lake and stream	250
open ocean	125

a) i) What is meant by net primary productivity?

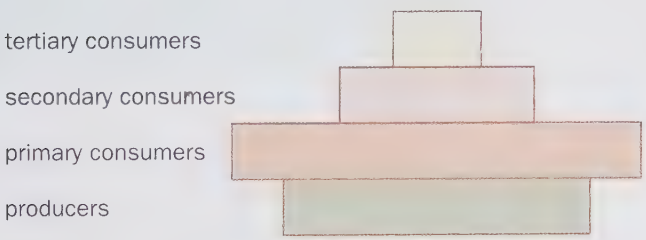
ii) What units could net primary productivity be measured in?

b) Why do you think that the net primary productivity is much higher for the continental shelf community than for the open ocean community?

c) The diagram shows a biomass pyramid for a lake in June.

i) Why is the biomass of the producers smaller than that of the primary consumers?

ii) Draw an energy pyramid for this community.



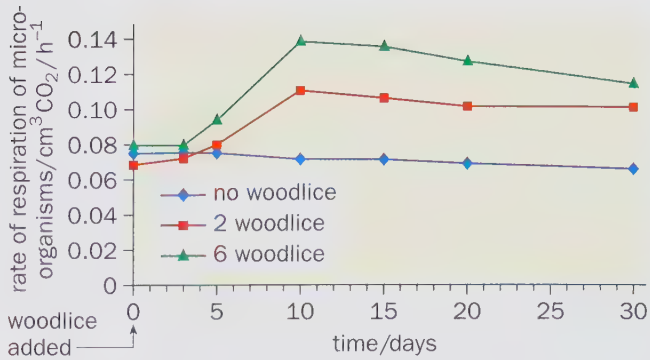
7 Detritivores and decomposers are both important in the processes of decomposition. The graph shows the activities of microorganisms involved in the breakdown of leaf litter and the effects of the number of woodlice (detritivores).

a) Why is rate of respiration a good way of measuring the activity of microorganisms?

b) What is meant by a detritivore?

c) i) Describe the effects of introducing woodlice on the activity of the microorganisms.

ii) Try to suggest a reason for these effects.

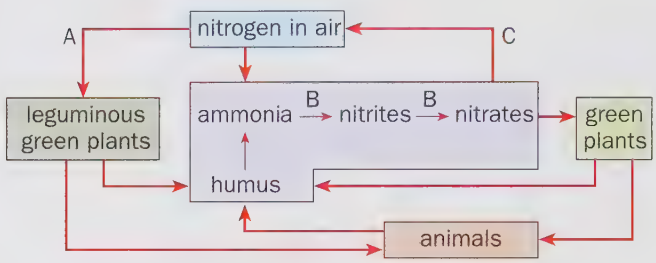


8 a) Name one compound produced by plants which needs a source of nitrogen.

b) The diagram shows some stages of the nitrogen cycle.

i) Why are animals not essential to the nitrogen cycle?

ii) Name the processes carried out by bacteria at points A, B and C in this cycle.



9 An experiment was carried out to investigate the disappearance of leaf litter. Discs cut out of oak leaves were put into nylon mesh bags and buried in the soil. The table shows the disappearance of the oak discs from bags of 7 mm and 0.5 mm mesh over a period of months.

a) Plot the results of the experiment as line graphs.

b) i) Describe the effect of mesh size on the rate of disappearance of leaf litter between the months of June and October.

ii) Explain the variation in the rate of disappearance of leaf litter from the 0.5 mm mesh bags during the experiment.

iii) The nitrogen released as a result of this decomposition acts as a fertiliser, but only some of it is available to plants. Suggest one way in which nitrogen is lost from the soil.

Month	Percentage oak leaf area remaining in bags of mesh size	
	7 mm	0.5 mm
June	100	100
August	81	94
October	30	91
December	13	66
February	9	62
April	6	60

24 Interactions between organisms

Isle Royale is an island on Lake Superior in North America.
It is thought to be the only site where the moose, a large herbivore, and its predator, the wolf, co-exist unhunted by humans.

The moose reached the island in about 1908, probably by swimming from the mainland. Their numbers increased as they browsed on the leaves of trees and aquatic plants.
Visitors to the island in 1934 found many dead and emaciated moose carcasses. The population had crashed due to overgrazing.
In 1948, wolves reached the island across the ice.
They established a pack and started to kill the moose for food.
At present there are about 1000 moose on Isle Royale, producing about 440 calves a year and supporting about 24 wolves.
The populations appear to be in balance with each other and with the vegetation on which the moose browses.

Populations

A population is a group of individuals of the same species living in the same place at the same time and interbreeding.
These are all populations:

- aphids on a sycamore tree,
- a shoal of herring in the sea,
- bluebells in a wood.

Why do you think that individuals live in populations?

- Individuals may be more successful in breeding and rearing their young.
- Many animals gain protection from predators in a group.
- Some animals can locate new food resources as a group.

But as a population grows, organisms **compete** for scarce resources such as food and space.
Some organisms will be better adapted to compete than others.
They are more likely to survive and pass on their genes to their offspring.

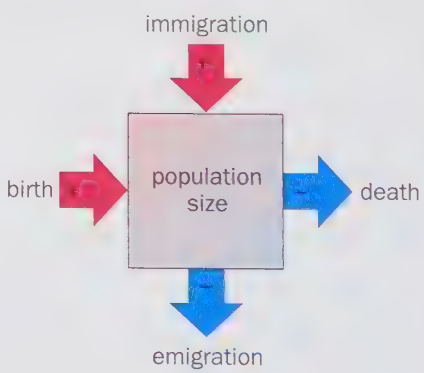
A population of sardine in Oman

What determines population growth?

The growth of a particular population at a particular time is determined by
birth rate – the reproductive capacity of the population,
mortality – the death rate of organisms in the population.

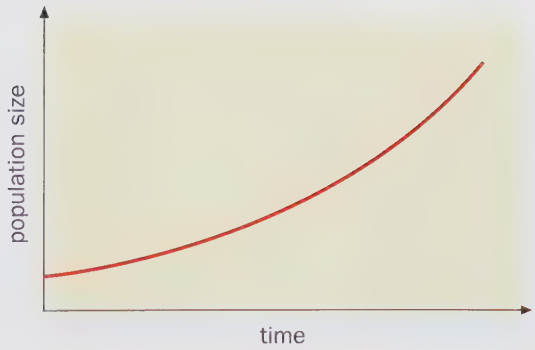
Organisms can also enter or leave a population.
It is easy to imagine animals being able to do this, but plants too can use seed dispersal to enter or leave a population.

Immigration is the movement of organisms into a population.
Emigration is the movement of organisms out of a population.



How populations grow

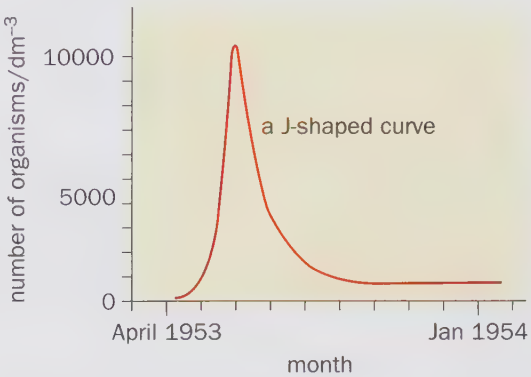
What happens when a species colonises a new area?
If conditions are favourable, the birth rate will exceed the death rate and lead to **exponential growth**.
With this type of growth the population ***doubles*** per unit time.
Fifteen organisms become 30, then over the same period of time, 30 organisms double to 60, 60 to 120, and so on.
As you can see, exponential growth can lead to a massive explosion in numbers.



Exponential population growth

Some species of algae can achieve this sort of growth if conditions of light, temperature and nutrients are favourable. This can result in algal blooms, but if nutrients are used up and become a ***limiting factor***, then the population can fall as quickly as it rose.

The characteristic **J-shaped** growth curve is often called a 'boom and bust' curve. Insect pests that produce many generations in a single year often have this sort of growth curve.



Changes in the population size of a brown alga

Population growth involves three main factors:

- the **biotic potential** of the population,
- **environmental resistance** of the habitat,
- the **carrying capacity** (**k**) of the environment.

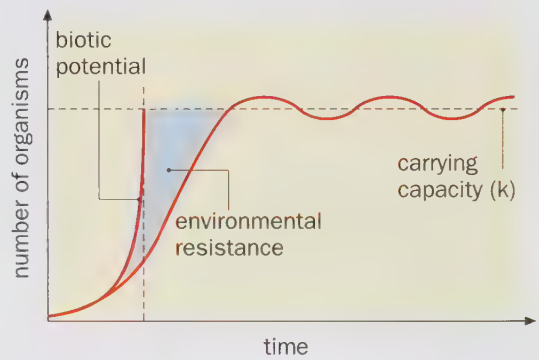
The **biotic potential** of a particular population is the maximum rate at which it can reproduce, given all the resources it needs. Environmental resistance means that populations seldom ever achieve their biotic potential.

Environmental resistance includes all the factors that may limit the growth of a population, such as accumulation of waste products, scarcity of resources such as food and space, or adverse climatic conditions.

Environmental resistance also takes in biotic factors such as the effects of predators, parasites and competitors.

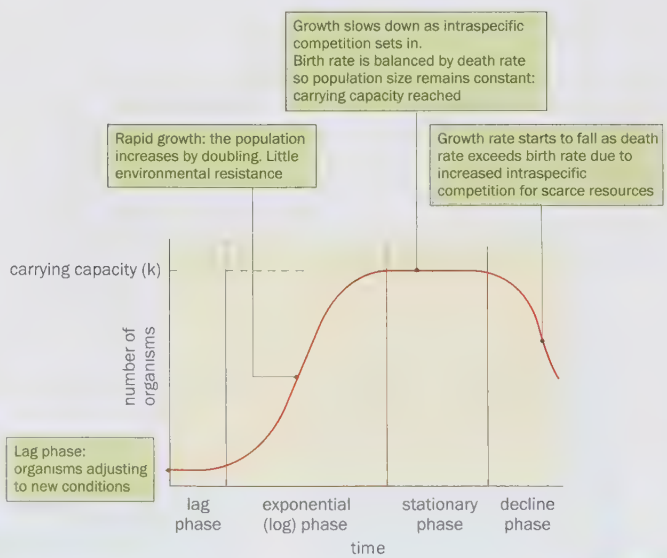
When the rate of increase stops and the birth rate balances the death rate, the population has reached the **carrying capacity** of the environment.

This is the maximum population size that can be supported by a particular environment.



The S-shaped curve

As a population encounters environmental resistance, such as food shortage, its rate of growth slows and it approaches the carrying capacity.
The **S-shaped** curve is typical of species colonising new habitats. There is a period of slow growth as the species adapts to the habitat, followed by a period of rapid growth with little environmental resistance.
The graph then levels off as the population reaches its carrying capacity. If a particular factor becomes scarce, it can limit the growth of the population, which can then go into decline.
Look at the different phases demonstrated in an S-shaped curve.



Checks on population increase

Population growth slows down due to environmental resistance. This may be due to abiotic or biotic factors.

Migrating wildebeest

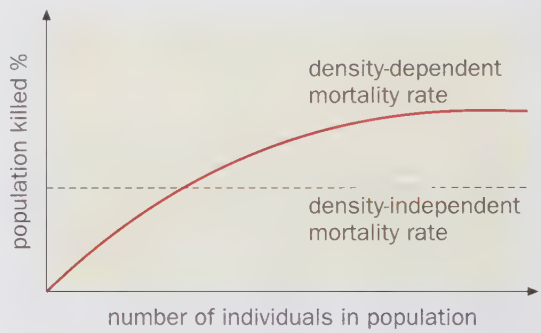
Abiotic factors

Abiotic factors include climatic factors, such as extremes of temperature, drought, floods or storms.
Lack of shelter exposes individuals to a harsh climate.
Plants need light of the correct intensity, wavelength and duration if they are to achieve optimum growth.
Shortage of oxygen can limit the numbers of aquatic species.
Water quality is affected by pollution and intolerant species may die.
Pollution from the local accumulation of toxic materials can affect populations on land, sea and air.

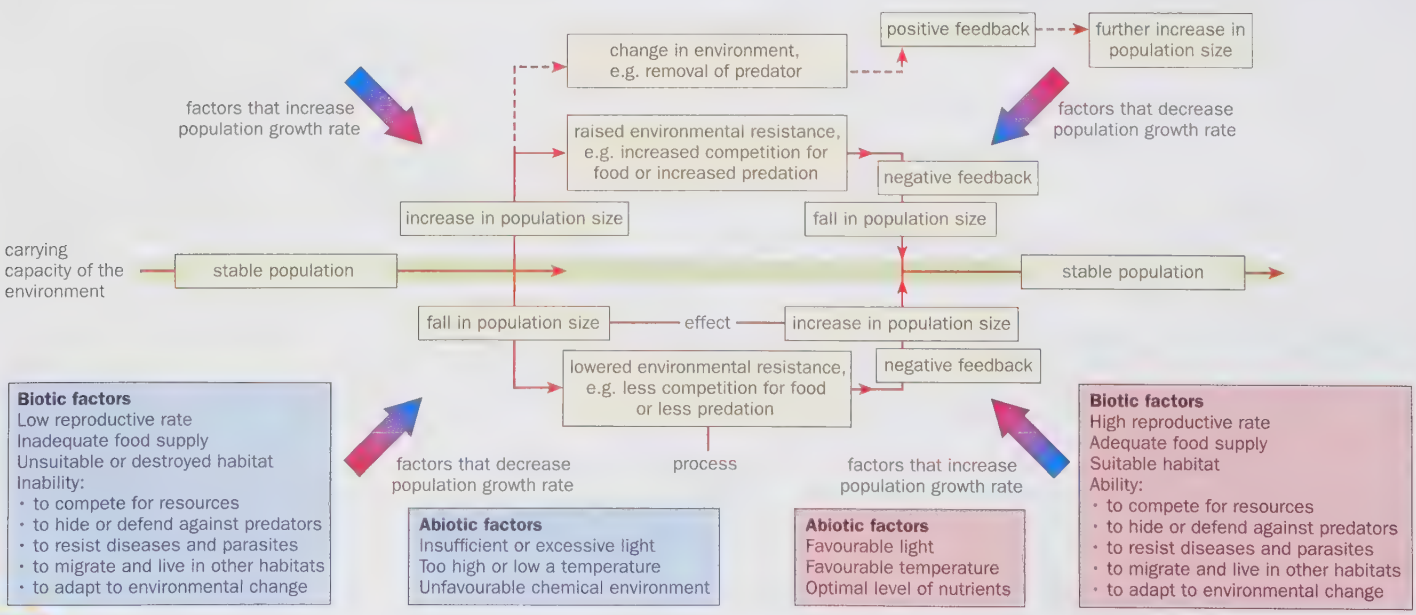
Biotic factors

Individual plants and animals may have to compete for scarce resources such as food, light, water and space.
Predators can reduce the numbers of a prey species.
The higher the density of a population, the more rapidly a disease could spread through it. Similarly, parasites reduce the ability of the host species to survive and reproduce.

Density-independent factors affect ***all*** the plants or animals in a population irrespective of the population size.
For example, a severe winter will affect ***all*** the birds in a population of robins. Density-independent factors may be abiotic, for example chemical pollution affecting water fleas in a pond, or biotic, for example the effects of disease on a vole population.



Density-dependent factors vary in the effect that they have on a population, depending on the size of the population.
So the size (or density) of a population affects its growth rate.
For a fixed food supply, the larger the population gets, the less food there will be for each individual and the slower the growth rate.
Other density-dependent factors include space, competition, predation and parasites.
Density-dependent factors are ***always*** biotic.



Competition

The word 'competition' suggests a race in which all the competitors try their hardest to win.
In nature, plants and animals struggle for survival.
They compete with each other for scarce resources.
As you have seen, plants compete for light, space, water and nutrients.
Animals compete for things like food, space and mates.
Many animals have to establish a **territory** if they are to attract a mate and breed.
There are two types of competition: **intraspecific** and **interspecific competition**.

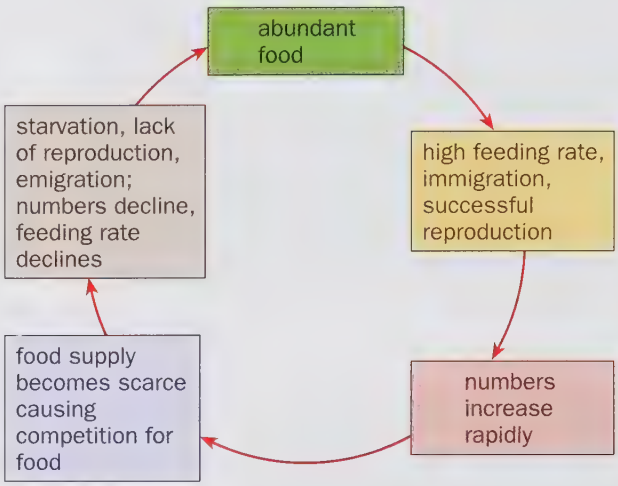
Intraspecific competition between lesser black-backed gulls

Intraspecific competition

Intraspecific competition is competition between organisms of the ***same*** species. Seedlings of the same species of plant compete for light, water, space and nutrients.
Gulls of the same species compete for nesting sites.

Intraspecific competition is ***density-dependent***.
As the population density increases, a greater proportion of the population fails to survive.
Growth rate, reproduction and length of life are also affected.
For instance, in crop plants, intraspecific competition can result in smaller plants with a decrease in biomass, or in fewer seeds being produced per plant.

Living organisms tend to produce far more offspring than the habitat can support.
It is this overproduction that results in intraspecific competition.
Those organisms that are best adapted to take advantage of scarce resources have a better chance of survival.
This ensures that their alleles are passed on to their offspring.
It also ensures that environmental resources are not over-exploited, as in the case of too many herbivores over-grazing vegetation.

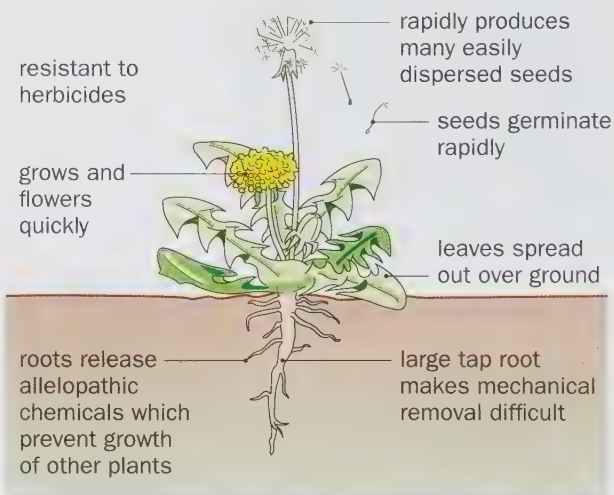


Changing population numbers caused by intraspecific competition

Interspecific competition

This is competition between organisms of ***different*** species.
In winter, different species of garden bird visit a bird table.
They all compete for the food that is put out.

Weeds are excellent competitors.
A weed is a plant growing where it is not wanted, for instance the field poppy (*Papaver rhoeas*) growing in a barley crop.
Poppies compete with barley for light, water, nutrients and space.
They grow and complete their life cycle before the crop plant has reached its full height and shaded them out.
But how are they able to do this?
The diagram will give you some clues.



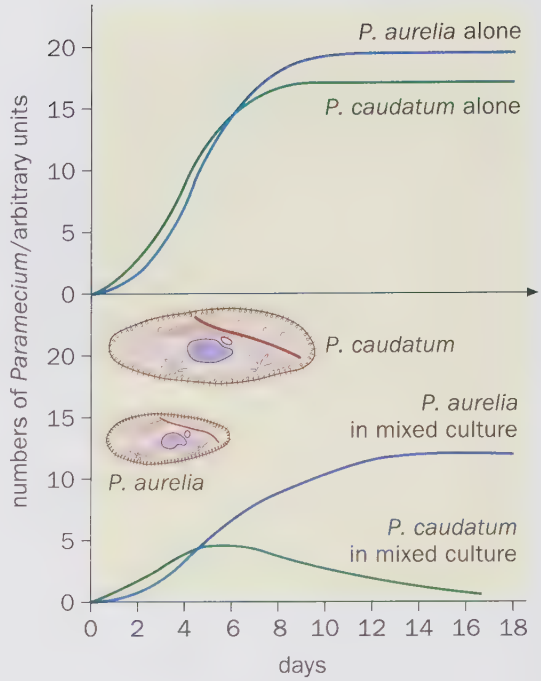
Weeds are successful interspecific competitors

- Weeds are able to reproduce quickly and produce huge numbers of seeds.
- Their seeds germinate quickly in poor soil.
- Weeds grow very quickly, and flower and set seed before other plants can.
- They can grow in poor soil, often become resistant to herbicides, and have adaptations such as spiny leaves and poisonous chemicals which make them unpalatable to grazers such as cattle.

Competitive exclusion

Interspecific competition is most intense when two different species attempt to occupy the same niche.

A Russian biologist, G. F. Gause, cultured two different species of the unicellular organism *Paramecium* in laboratory conditions.
When grown separately with controlled amounts of food, *P. caudatum* and *P. aurelia* both showed typical S-shaped growth curves.
When they were grown together, the two species were able to co-exist for a limited period of time.
But eventually, *P. aurelia*, the smaller, faster-growing species, tended to out-compete the larger, slower-growing *P. caudatum*.
P. caudatum eventually died since it could not occupy the same niche as *P. aurelia*.
This work demonstrates the **competitive exclusion principle** under controlled laboratory conditions.

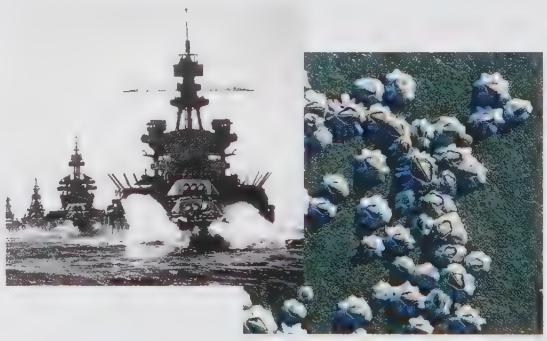


Competition between two species of Paramecium

There are two native barnacles that are common on UK shores, *Semibalanus balanoides* and *Chthamalus montagui*.
Chthamalus is able to live higher up on the shore than *Semibalanus*, because it is more resistant to desiccation (water loss).
Lower down the shore, *Semibalanus* is able to feed for longer periods and grows more quickly.
So on the lower shore *Semibalanus* out-competes *Chthamalus* for space.

During the Second World War, an Australian barnacle, *Elminius modestus*, invaded British shores.
It arrived in this country on the hulls of ships and quickly spread around the south coast.
In many situations, *Elminius* has been able to out-compete both *Semibalanus* and *Chthamalus* because

- *Elminius* can withstand lower temperatures than *Chthamalus*,
- *Elminius* can withstand higher temperatures than *Semibalanus*,
- *Elminius* can tolerate lower salinities so can colonise estuaries,
- *Elminius* has a faster feeding rate and rate of growth.



Elminius modestus

The ability of *Elminius* to occupy a niche at the expense of the native barnacles demonstrates competitive exclusion.

The native red squirrel was found in both deciduous and coniferous woodland throughout the UK. In the 1870s, the grey squirrel was introduced into the UK from North America.
By the beginning of the 20th century, the grey squirrel had spread at the expense of the native red squirrel.
Today, red squirrels are only found in coniferous woods and isolated areas which grey squirrels have not colonised.
Grey squirrels do not physically drive out red squirrels.
So the question is ***how*** does the grey out-compete the red?
The answer may well be in their food source. Grey squirrels can digest acorns and hazelnuts more efficiently than red squirrels can.
Grey squirrels also seem to be broad-leaved woodland specialists, whereas the smaller, nimbler reds do better in conifers where they feed on pine cones high in the canopy.
It seems that competitive exclusion is at work and that the niches of the two squirrels are so similar that only one can survive in a particular habitat.

Native UK red squirrel

Grey squirrel

Predation

Predators kill other animals (their **prey**) for food.
Predators are usually larger and fewer in number than their prey.
If they are to survive, then they must be well-adapted for killing.

What do you think makes a good predator?

- It needs the weapons to kill: claws or talons to grasp the prey, and sharp teeth or beak to kill and tear it up.
- It needs speed to pursue the prey, and camouflage to escape detection when stalking.

There are other less obvious strategies that also help predators.

- Group hunting enables predators to surround the prey and also means that together they can kill larger prey.
- Catching prey that is young, old, sick or injured means that the prey is easier to overpower and kill.
  This also 'weeds out' some of the weaker individuals in the prey population, leaving better-adapted individuals to pass on their genes.
- Catching large prey provides the predator with more food per kill.
- Having a variety of prey species reduces the chances of starvation in the event that any one prey species declines in number.
- Migrating to areas where the prey is more plentiful.

Brown bear catching salmon

Spotted hyenas with prey

A prey's guide to survival

However well-adapted the predator, some of the prey escape.
Prey species are adapted to avoid capture, although they are never completely successful.

- Many try to out-run, out-swim or out-fly their predators.
- Staying in large groups, like herds of antelope and shoals of fish, distracts a predator from aiming at one particular individual.
- Some potential prey, for example bees and wasps, can sting a predator. Others simply taste horrible, like some types of ladybird.
  Either way, predators learn to take them off the menu.
  These species often have **warning colouration** to act as a reminder to the predator.
- Some prey species 'mimic' these warning colourations.
  A hoverfly has yellow and black stripes like those of a wasp, although it has no sting.
- Camouflage is often used by prey species to avoid capture.
  Many animals blend in with their surroundings, whilst others look like something else, twigs or leaves for instance.
- **Startle mechanisms** involve a sudden and conspicuous change in appearance of the prey, designed to confuse or alarm the predator.
  Can you see how the eyed hawkmoth might startle and scare off a hungry bird?

A six-spot Burnet moth showing warning colours

Katydid insect

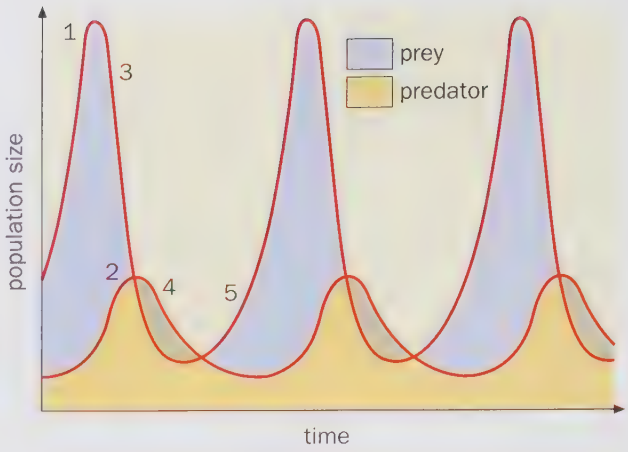
Eyed hawkmoth

Predator–prey cycles

It's pretty obvious that predators affect the size of prey populations. But have you ever considered the effect that the prey can have upon the predator population?

What would happen to the predator population if its prey were affected by disease?
Such drastic events are not common place, but if its prey becomes scarce then the predator suffers too.

The diagram shows a model for a predator–prey cycle that has been worked out mathematically.



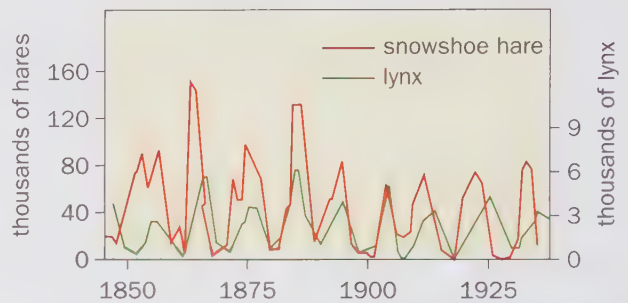
1 The prey have plenty of food.
They survive to breed and their numbers increase.
2 The increase in prey numbers means that there is more food for the predator. So more predators survive to breed and their numbers increase.
3 As there are now more predators, the rate of predation rises and the number of prey goes down.
4 There is now less prey for the predator to feed upon.
Fewer predators survive to breed and their numbers decline.
5 With fewer predators, more prey will survive to breed.
Prey numbers will increase and so the cycle continues.

Predator–prey cycles are naturally self-regulating.
Notice that there are fewer predators than prey and also that predators tend to reproduce more slowly than their prey.

- So the fluctuations in predator numbers are smaller than those in numbers of prey, and
- the fluctuations in predator numbers lag behind the fluctuations in numbers of prey.

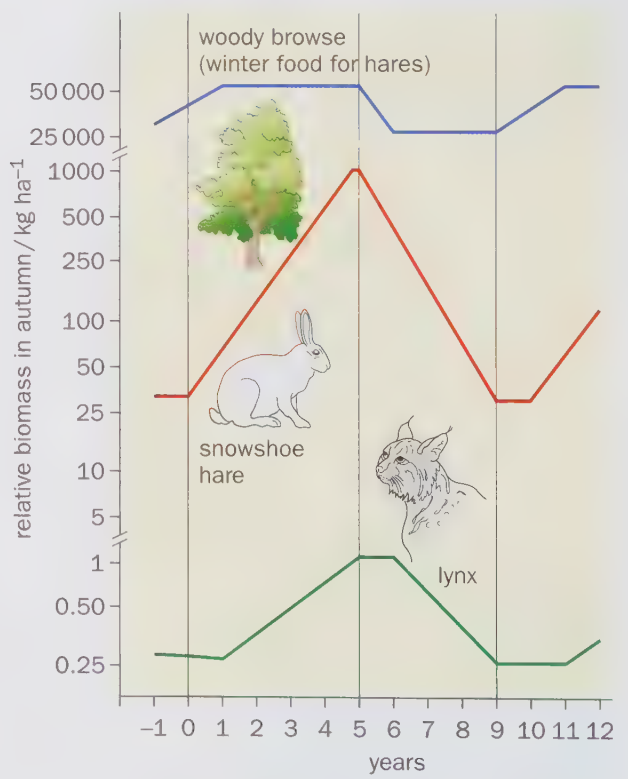
The lynx and the snowshoe hare

One of the most widely documented examples of a predator–prey cycle is that of the snowshoe hare and its main predator, the lynx.
Records of their numbers were kept by the Hudson Bay Fur Trading Company in Canada, between 1845 and 1935.
The numbers of hare and lynx pelts brought in by trappers were accurately recorded over this period.
The relationship between predator and prey can be clearly seen in the graphs.



The relationship between the numbers of lynx (predator) and snowshoe hare (prey)

Predator–prey relationships are also affected by interactions at other levels, for instance plant–herbivore relations.
The snowshoe hare feeds on conifer buds and twigs of aspen, alder and willow, so-called browsing vegetation.
If the amount of browsing vegetation falls below that needed to support the hare population over winter, malnutrition occurs.
Weakened hares are much more vulnerable to predation.
Intense predation lowers the number of hares and means that inevitably the lynx will suffer its own food shortage.
Meanwhile, since the vegetation is not being browsed by so many hares, growth increases.
With the decrease in predation and the growing abundance of winter food, the hare population rises, starting another cycle.

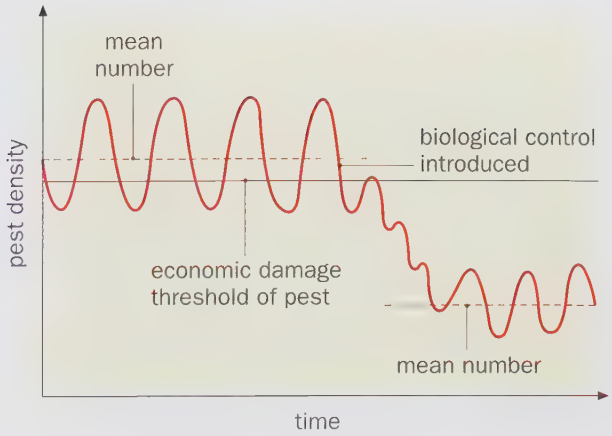


Three-way interaction between woody vegetation, snowshoe hare and lynx

Biological control

A pest is any organism that competes with or adversely affects a population of plants or animals that are of economic importance to humans. As with many living things, the population of a pest is normally regulated by its natural predators and parasites. Many alien species, introduced into a country by accident, can become pests since they are no longer controlled by a natural predator. Biological control is the use of predators, parasites and pathogens to keep the numbers of pests below the **economic damage threshold**.

The economic damage threshold is reached when the pest numbers are causing so much damage (say to a crop) that it is worth spending money on controlling the pest. The predator, parasite or pathogen used is called the **biological control agent** or **biocontrol agent**.



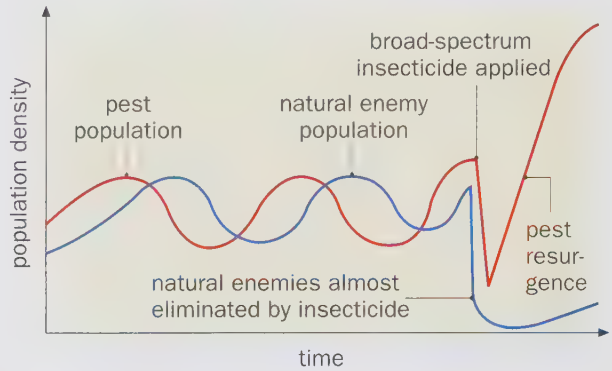
Graph showing the principle of biological control

Biocontrol agents

- Biocontrol agents must be ***specific*** to the pest. They must target the pest and no other species.
- If the biocontrol agent becomes established and is successful in controlling the pest for long periods of time, then the high initial expense is justified, and over the long term this form of control is relatively inexpensive.
- Biological control has none of the detrimental effects upon the environment associated with persistent use of chemical pesticides.

There are, however, drawbacks associated with biological control.

- If a farmer has a sudden invasion by a pest, rapid control is required in order to minimise damage to the crop. Biocontrol agents are fairly slow to react to a surge in pest numbers.
- Most crops suffer from several pests during their growth. This would require several biocontrol agents for one crop.
- Crops may have only occasional pest attacks. In the years that the pest is absent, the biocontrol agent will die out and so will have to be continually reintroduced.



Graph showing resurgence of a pest after the application of a broad-spectrum insecticide

Comparison	Control method	
	Biological	Chemical
time to take effect	slow	fast
pollution, danger to humans and domestic animals	none	considerable
ecological dangers	few	considerable
permanence of control	permanent	usually temporary, repeated treatment needed
development of pest resistance	very rare if ever	common

A classic case

In 1868, the cottony-cushion scale insect (*Icerya purchasi*) was accidentally introduced to California from Australia.
Away from its natural enemies, the scale insect decimated the citrus groves, threatening the future of the industry.
American scientists went to Australia to search for a predator of the scale insect.
They found a ladybird predator (*Rodolia cardinalis*).
The ladybird was imported into California in 1888 and quickly succeeded in controlling the scale insect threat.
Rodolia has proved to be a successful biocontrol agent in 35 countries.

Try drawing a graph to show the relationship between the numbers of a pest and the numbers of its biocontrol agent over time.
Will the graph look similar to a predator–prey cycle?

Cottony-cushion scale insects on citrus trees

Succession

An area of bare ground left to nature does not remain devoid of vegetation for long.
Weeds are usually the first plants to invade the site, followed by grasses and tall herbs.
After a long period of time, the area will turn to woodland.
This one-directional process of community change with time is called **succession**.

Succession is the change in structure and species composition of a community over time.

The different stages in a succession when particular communities dominate are known as **seres**.
Succession continues until the community reaches an equilibrium with its environment and no further change occurs.
This is then known as the **climax community**.

Primary succession

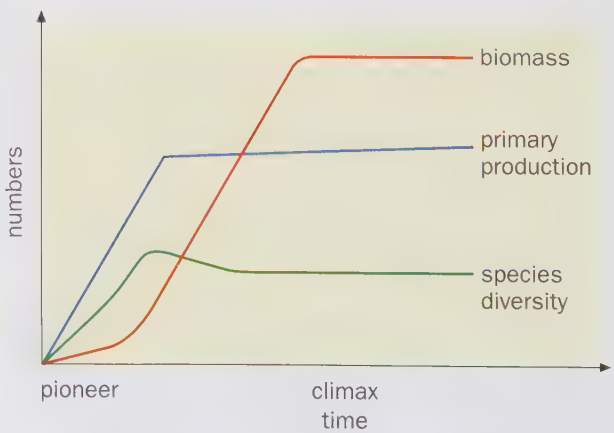
Primary succession occurs on newly-formed habitats that have not previously supported a community. Examples can be found on rocks exposed by landslides, volcanic larva flows and sand dunes. The first organisms to colonise the bare rock are lichens and algae, so-called **pioneer species**. These slowly erode the rock as their hyphae penetrate tiny cracks and absorb minerals. Debris and other organic material become trapped in these cracks, providing the basis of a soil. Mosses are next to colonise the area, arriving by means of wind-blown spores.
They trap water, and their own dead remains build up the organic content of the soil.
Shallow-rooted grasses and herbs are able to establish themselves as a result of the improved soil conditions.
As the soil builds up, deep-rooted shrubs appear.
Over a much longer time scale, these eventually give way to trees such as beech or oak, so reaching the climax community.

Although we have looked at succession in terms of vegetation in this example, it is important to remember that the whole community, including animal species, is involved in succession.

A number of ***trends*** develop as succession proceeds.

- The soil develops – it increases in depth, has greater organic content, and different layers can be identified as it becomes a mature soil.
- The height and biomass of the vegetation increases.
- The primary production increases with soil development.
- Species diversity increases, from simple communities of early succession to richer communities of late succession.
- The number of food chains increases.
- Changes in height and density of vegetation provide a greater variety of microhabitats.
- The community becomes more stable.
  In the early stages of a succession, populations rapidly replace one another. The climax community is stable and dominated by long-lived plants.

Succession from cleared ground to oak hornbeam forest over 150 years, in south Poland



Changes in vegetation during succession

Secondary succession

Secondary succession occurs at sites that have previously supported a community.
It occurs after a major environmental disturbance disrupts a previous succession.
Forest fires, the ploughing of grassland and road building are examples.

Following such major disruption, the process of succession will begin again in the new environmental conditions.
After a fire, many species of plant will disappear.
But some seeds will remain viable in the soil and will germinate.
Pioneer species from adjacent sites will be quick to colonise by means of their seeds.
Without periodic fires, the prairies of North America and the African grasslands would become dominated by woody vegetation.

Secondary succession following a forest fire

The building of motorways is clearly disruptive to communities.
However, motorway verges soon start to undergo secondary succession.
Soon colonised by herbaceous vegetation, with time young shrubs such as hawthorn and trees like sycamore and beech establish themselves.
Relatively undisturbed by humans, motorway verges have become important reservoirs for our native wildlife.

Motorway verges are important havens for wildlife

Deflected succession

Succession does not always proceed through to the climax community.
The process is halted, often by the management practices of humans and through conservation measures.
This is known as **deflected succession**.

Conservation is not a question of just leaving the environment untouched, otherwise the result would be a small range of climax communities.

Grazing by sheep prevents grassland such as the South Downs from being colonised by scrub and developing into woodland.

Heather moorland is managed by controlled burning.
This maintains the heather and stops the succession to woodland.
Fire kills any saplings but not heather which grows from the roots after a few weeks.

Hedgerows and field margins are important habitats for conserving diversity in farmland (see page 230).

Woodland can be maintained by removing conifer plantations and replacing them with broad-leaved native trees.
Thinning the trees allows light to penetrate the canopy encouraging the growth of shrub and herb layers.
Coppicing and pollarding allows timber to be harvested in a sustainable way.

Deflected succession: sheep grazing

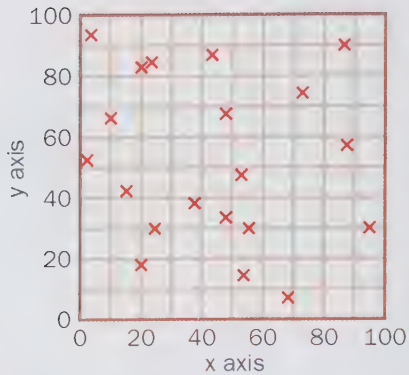
Wetlands can be managed by periodic dredging to prevent silting up and succession.

Even mowing and weeding the lawn preserves grass as a result of deflected succession.
If you were to study an unmown lawn over a period of 10 years, you would notice changes in species composition as herbs and shrubs invade it.

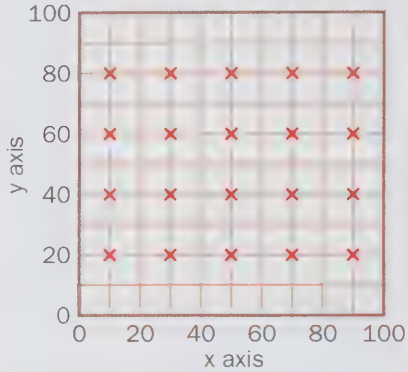
Sampling

Ecologists often need to estimate the abundance and distribution of animals and plants.
They may need to know if a population is changing, perhaps in order to conserve the species or monitor the level of a pest species.
It is not usually possible to count all the individuals in a particular population.
Instead, samples are taken which will hopefully be representative of the population as a whole.

There are two main types of sampling strategy: **random** and **systematic**.
To take a random sample, a grid is used to divide up the area.
Then a random numbers table, or a calculator with a random number key, is used to give coordinates.
The sample is taken where the coordinates intersect.
In practice, it is easier to draw a grid on paper and then space out the coordinates.
Systematic sampling is better if you are looking for a pattern, for instance the effects on the vegetation of a change in soil type.
A grid is again used to divide the area up into sampling units.
But this time the sampling points are taken at regular intervals.



Random sampling



Systematic sampling

Transects

Transects are commonly used for systematic sampling.
The samples are taken along a line laid out across the sample area.
Transects are useful for recording changes in the abundance and distribution of species where some sort of transition occurs.
For instance, in the zonation of species along a rocky shore, or in the succession that can be highlighted along sand dunes.
There are two common types of transects. The **line transect** involves laying a tape across the sample area and any species that touch the tape are recorded. This is a quick technique which is useful for distances over say 20 m.
The **belt transect** involves laying down a **quadrat** at intervals along the tape and estimating the species found within it.


A frame quadrat in use

Quadrats

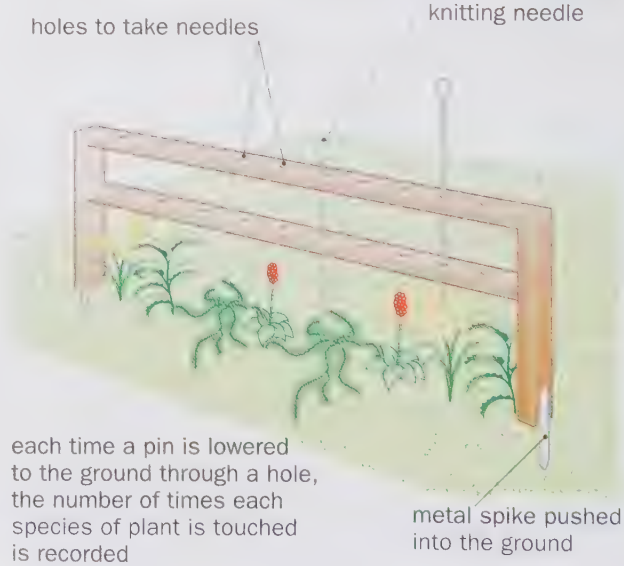
Quadrats are often used in sampling plant communities and are traditionally used in rocky shore studies, since many of the animals are slow-moving and stay within the quadrat.

A **frame quadrat** is a square, the most common size being $0.25\,m^2$. It can be made out of wood or bought commercially. The quadrat is laid down at sampling points within the sample area, or at points along a transect, and the organisms within it are estimated, often as **percentage cover** (the percentage of the area within the quadrat that the species covers).

A **point quadrat** was devised to give a more objective assessment of cover. Ten pins are dropped through a low-standing frame onto the vegetation. As each pin touches a plant of a particular species a ***hit*** is recorded.

$$\%\ \text{cover} = \frac{\text{hits}}{\text{hits} + \text{misses}} \times 100$$

So if the point quadrat is used 15 times and the total number of hits for one particular species is 20, the % cover $= \frac{20}{150} \times 100 = 13.3\%$

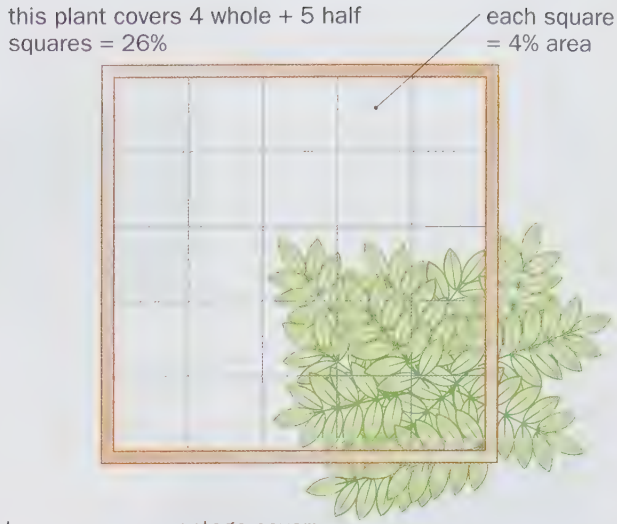


A point quadrat

Measures of abundance

Four measures of abundance are commonly used.

- **Density**: the mean number of individuals per unit area.
- **Frequency**: the number or percentage of sampling units in which a particular species occurs.
- **Biomass**: the dry weight of plants or animals in a certain area at a certain time, often referred to as **standing crop**.
- **Percentage cover**: the percentage of the ground covered by a species within the sampling unit.
  This is commonly used for estimating plants and does away with the need to count all the individuals.



to measure percentage cover:

i) lay a frame quadrat over the selected area
ii) count the number of squares occupied by the plant species in question
iii) count those squares that are partially occupied
iv) estimate how many full squares this would represent and add it to the total

Sampling animal populations

Animals are more difficult to sample than plants.
For one thing, many won't stay inside a quadrat to be counted!
Many hide during the day and only come out at night.
So some form of net or trap must be used to sample them.

A technique called **mark-release-recapture** (**MRR**) can be used to work out estimates of animal populations.
The population size is then estimated using the **Lincoln Index**:

$$P = \frac{M \times S}{R}$$

P = the estimate of the population size,
M = the number of animals captured, marked and released in the first sample,
S = the number of animals captured in the second sample,
R = the number of marked animals recaptured in the second sample.

mark-release-capture

catch a sample of the animals you want to study and count them

↓

mark each one in a way that will not harm them or make them more noticeable to predators

↓

release your sample back into their habitat and allow 1 or 2 days for them to redistribute into their own population

↓

take a second sample and count the total numbers along with the number marked from the first sample

Maths skills

20 ground beetles were caught in a pitfall trap.
Each was marked with a small dot of paint and when it was dry they were released. After 2 days, a second sample of 18 beetles was taken.
Of these, 6 were marked individuals from the first catch.
Estimate the total population of beetles.

Number in first catch = 20
Number in second catch = 18
Number of marked individuals in second catch = 6

$$\text{Total population} = \frac{20 \times 18}{6} = 60$$

When using the Lincoln Index, we make a number of assumptions.

- The marked individuals must redistribute themselves randomly amongst the unmarked individuals.
- The marks must not come off between marking and recapture.
- The marks must not affect the behaviour of the animals nor make them more noticeable to predators.
- Being caught in the first sample must not increase or decrease the chances of capture in the second sample.
- Ideally, there should be no movement of individuals into or out of the population during the exercise. Neither should any births or deaths occur in the population.

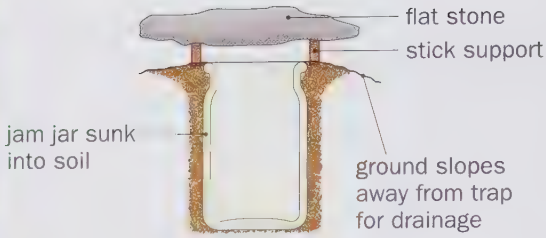
How would the disappearance of a mark or the death of some individuals affect the population estimate?

Marking a bee as part of a mark-release-recapture exercise to estimate the number of bees in an area of woodland

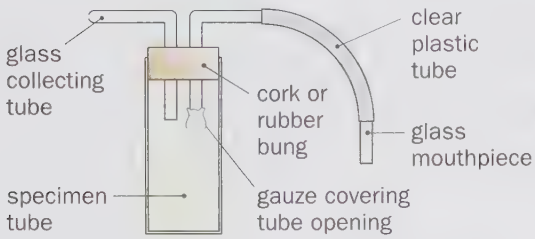
Collecting invertebrates

Butterfly net: used to catch flying insects. Short handle for ease of use. Large mesh bag can be folded over frame to prevent catch escaping.

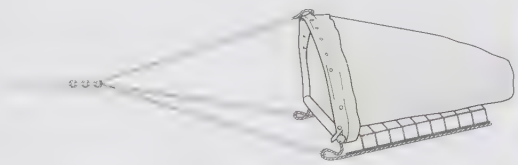
Sweep net: used to catch invertebrates in low-growing vegetation.
Long handle, robust frame with a small mesh bag.
The net is brushed through vegetation, dislodging animals that fall into the bag.



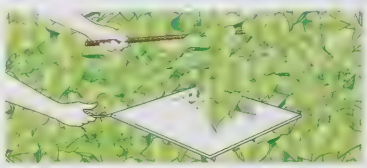
Pitfall trap: collects invertebrates that are active on the soil surface or in leaf litter.
Basically a jam jar sunk into the soil to ground level.
A piece of wood or stone keeps the rain out. Pitfalls are cheap and easy to use but the catch often reflects the activity of a particular species as well as its abundance.



Pooter: tube for sucking up small insects, especially useful for insects in cracks and crevices. Small invertebrates are drawn into the collecting tube by sucking through the mouthpiece. A piece of gauze prevents the insect from ending up in your mouth!



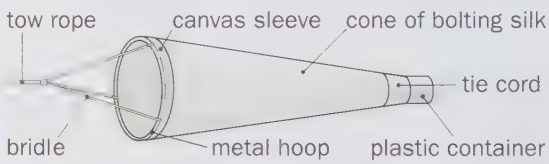
Dredge net: can be used to sample invertebrates on a pond bed or in grassland.
D-shaped frame mounted on steel runners supports a coarse meshed bag.
Towed over the bed of a pond or lake to sample bottom-living invertebrates, or hauled over the surface of grassland to dislodge insects.



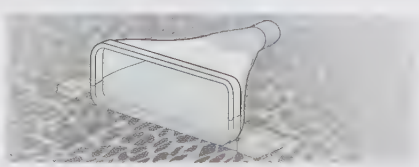
Beating tray: used to sample invertebrates in bushes and trees.
This can be as simple as a white plastic sheet spread out under a bush.
A branch is then shaken, dislodging the invertebrates which fall onto the sheet.
They can be collected from the sheet by means of a pooter.

Light traps: collect night-flying insects such as moths.
The insects attracted to the light hit the glass baffles and fall into the base of the trap.
Pieces of egg carton are put in the base for shelter and subsequent ease of handling.

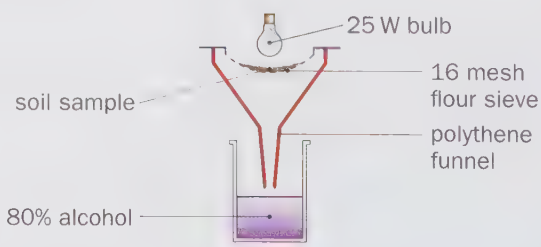
Pond net: collects freshwater invertebrates by 'kick sample' or sweeping.
Long handle, strong supported frame, rot-proof bag with 1 mm mesh.
Kick sampling dislodges invertebrates on stream bed, and the current sweeps them into the net bag.



Plankton net: used for sampling phytoplankton and zooplankton in marine and freshwater habitats.
Robust frame supports long conical net when full of water. Collecting jar holds filtered plankton.
Net is towed by a boat at 1–1.5 knots or anchored against the current in a stream.



Drift net: samples invertebrates drifting downstream.
Rectangular frame is secured to the river bed by metal stakes.
The invertebrates float into the bag on the current and are collected in a sample bottle at the end of the tapering bag.



Tullgren funnel: extracts small invertebrates from soil and leaf litter.
Easily made from a plastic funnel and a flour sieve.
The soil sample is placed on the gauze under a 25 W light bulb.
The invertebrates move down, away from the light and the heat, and slide down the funnel into a collecting jar.

Monitoring the physical environment

As part of an ecological study, you may need to measure physical and chemical factors in the environment, since these will influence the abundance and distribution of the organisms.

There are now a wide variety of battery-operated meters available for use in field conditions.
Electronic probes can be attached to data logging equipment so that readings can be recorded at regular intervals over a period of time.
For instance, measurements of temperature, oxygen concentration and pH in an upper shore rock pool over 24 hours in summer.

Temperature

Temperature directly affects an organism's metabolic processes.
Most living organisms have an optimum temperature range below which these processes tend to slow down.
This is particularly true of ectotherms. During the severe winter of 1962–63, when the sea actually froze, over 80% mortality was recorded in limpets.

Endotherms take steps to avoid extremes of temperature.
Swallows are summer migrants to the UK from South Africa, and Bewick swans are winter migrants to the UK from the Arctic Circle.
Some small mammals, like the dormouse, avoid severe winters by hibernating.

When recording temperature with an electronic field thermometer, it is important to note the time of day and location (aspect, altitude or depth).

Migrating snow geese

Light

Light is of great importance to photosynthesising plants, both the light intensity and the wavelength.
Light meters are available that measure light intensity, but the problem is that the level of illumination can fluctuate greatly, for example with cloud cover.
In ecological investigations it is probably more relevant to estimate the ***total*** amount of light falling on the vegetation during the course of the study.
For instance, if you were studying the conditions inside a wood, you may take a series of measurements at regular intervals and record them with a datalogger.

Light penetration in a wood will change during the year

pH (acidity and alkalinity)

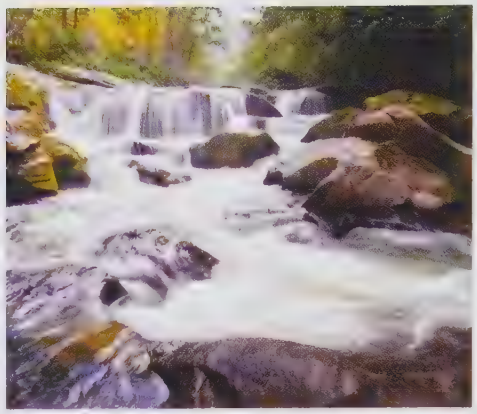
Aquatic plants and animals have differing pH requirements.
Some are tolerant to low pH, others are pH sensitive.
Hill streams with acidic water (pH below 5.7) are often completely lifeless.
The pH can be affected by a number of factors, including precipitation, type of bedrock and soil type.
But only extremes of soil pH will directly affect plant growth.
Usually pH has an indirect effect in that it affects the release of soil nutrients.
Many of these are soluble in acid conditions, so aluminium and manganese ions are mobilised in acid soils.
Calcifuges are plants such as heathers which thrive in acid, calcium-deficient conditions.
Calcicoles, on the other hand, are plants such as dog's mercury which prefer alkaline soils with plenty of calcium.
We shall look at the effects of extremes of pH on ecosystems in the next chapter.
Universal indicator can be used to test soil pH over a range of 4.0 to 11.0.
However, pH meters are usually more convenient and reliable for estimating the pH of the soil, seawater or freshwater.

Using a pH meter

Oxygen

Oxygen concentrations vary very little in the atmosphere.
However, oxygen levels in aquatic ecosystems can vary considerably.

Oxygen concentration is probably the most important element in overall water quality.
Most organisms are ***aerobic*** – they require oxygen.
But if organic waste, such as sewage, gets into a freshwater system, oxygen depletion can occur.
Bacteria break down the organic waste. As they do so, the increased bacterial respiration imposes a high oxygen demand on the waters.
The consequent low level of dissolved oxygen can have a detrimental effect upon the plant and animal life.
We will look at organic pollution in more detail in the next chapter.
Electronic oxygen meters have tended to be the least reliable of the environmental meters in the past.
They are much improved nowadays but still require careful calibration.



A rapid flowing upper hill stream

Conductivity

The conductivity of a water sample refers to its ability to carry an electrical current. Since electricity is carried in solutions by migrating ions, the conductivity of a solution indicates the level of dissolved salts.
The conductivity of pure water is zero, so adding any ions will increase the conductivity.

Conductivity measurements do not differentiate between salts.
So if you need to estimate chloride, for instance, you need to carry out a separate test (a silver nitrate titration).
For most ecologists, conductivity measurements are good enough.
The increase in salinity in an upper shore rock pool which has been isolated for a number of days can be shown using conductivity data.
Conductivity meters are accurate and very reliable.
They are also easy to use in the field.

Testing a water sample

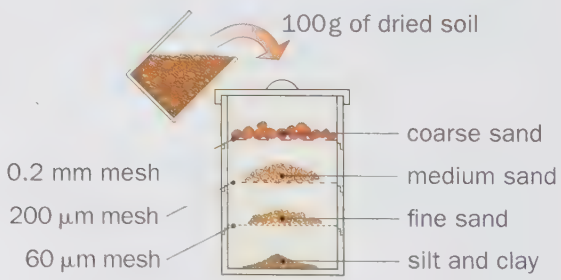
Soil type

Soil can be sampled simply by digging to show a **soil profile** or by using a **soil auger** to take a core sample.
Sieves with different mesh sizes can be used to categorise the various soil components, as shown in the diagram.
Depending upon your particular investigation, you may need to estimate the water content, organic content, temperature or pH of the soil.
For instance, samples taken along a line transect across sand dunes will show changes in organic content, chloride and pH with succession.
To measure soil water content, you need to dry your sample in an oven at 110 °C overnight. Then work out this equation:

$$\%\ \text{soil water} = \frac{\text{weight loss on drying}}{\text{weight of fresh soil}} \times 100$$

To measure organic content, you take the dry soil sample and heat it in a crucible to red heat for 15 minutes to incinerate all the organic matter.
Then work out this equation:

$$\%\ \text{organic matter in dry soil} = \frac{\text{weight loss on burning}}{\text{weight of dry soil}} \times 100$$



Separating soil compounds by sieving

Maths skills

Statistics for fieldwork

Ecologists often have to collect data to give them information about the distribution of organisms in a particular habitat. This can involve the use of various sampling techniques (see page 416).
After sampling there will probably be a large amount of data, such as the number of a particular species of seaweed.
It is a good idea to summarise the data into a more manageable form as this will make it easier to identify trends. This can be done fairly easily by any of three commonly used methods:

- The **mean** is often known as the average and is found by adding up all the values that you are dealing with and dividing the total by the number of values.
  The mean is shown by the symbol $\bar{x}$.
  The mean is the most commonly given in data summary as it can be used for further statistical analysis.
  However, one or two exceptional values can have a considerable effect on the mean and can make it unrepresentative.
- The **mode** is the most frequently occurring value in a set of values. In a series of numbers the mode is the most frequently occurring number. In a series of classes the mode is the most common class. The mode is not affected by extreme values but cannot be used for further statistical work.
- The **median** is the central value in a series when all the values are arranged in order. If the series has an even number of values then the median is the mid-point between the two centrally placed values. The median is unaffected by extreme values but cannot be used for further statistical work.

Sample size

How do you choose how many samples to take in order to achieve a valid result?
For instance, if you are sampling weeds on a playing field using quadrats, how many quadrat samples will you take?
Ideally the more quadrat samples you take, the more reliable the results.
However, taking hundreds of quadrat samples would be laborious and time-consuming.
So we need to take a common-sense approach and choose a sample size that will ensure all the common species have been identified, and further sampling will not merit the time and effort required.
In reality 10–15 quadrat samples will yield valid results.
Good scientists make sure that their data is ***reliable*** and ***valid***.
Reliable evidence should be ***repeatable*** so that if someone else collected the data again it would be the same (or similar).
So you can ***trust*** reliable data. It is then valid evidence only if it also measures what you intended to find out about.
Scientists need reliable and valid evidence in order to draw firm conclusions.

Mean

Data: 3, 4, 4, 4, 6, 6, 9

Working:

$$\frac{3+4+4+4+6+6+9}{7}$$

$$= \frac{36}{7} = 5.1$$

$$\bar{x} = 5.1$$

Mode

Data: 3, 4, 4, 4, 6, 6, 9

Mode: most frequently occurring number = 4

Data:

Class (number of springtails per sampling unit)	Frequency
0–19	0
20–39	5
40–59	9
60–79	13
80–99	7
100–119	2
120–139	1

Mode: most frequently occurring class = 60–79

Median

Data: 3, 4, 4, 4, 6, 6, 9
Median: central value = 4
Data: 3, 3, 4, 6, 8, 9
Median: mid-point between the two central values = 5

Calculating the standard deviation

The standard deviation enables us to measure the spread of data around its mean. This is important since the greater the variation in or spread of the data, the less useful is the mean as a summary of it.

Method

1 Tabulate the values (x) and their squares (x^2).
Add these values (Σx and Σx^2).

2 Find the mean of all the values of x ($\bar{x}$) and square it ($\bar{x}^2$)

3 Calculate the standard deviation using the formula:

$$\sigma = \sqrt{\left(\frac{\Sigma x^2}{n} - \bar{x}^2\right)}$$

where σ = standard deviation
Σ = the sum of
n = the number of values
$\bar{x}$ = the mean of the values

The larger the standard deviation, the greater the spread of data around the mean. Standard deviation is the best measure of this spread because it takes into account all the values under consideration.

Example: Sycamore leaf length (cm)

x	x^2
5	25
3	9
10	100
13	169
11	1221
12	144
17	289
6	36
6	36
3	9
2	4
$\Sigma x = 88$	$\Sigma x^2 = 942$

mean $\bar{x} = \frac{88}{11} = 8$ and $\bar{x}^2 = 64$

$n = 11$

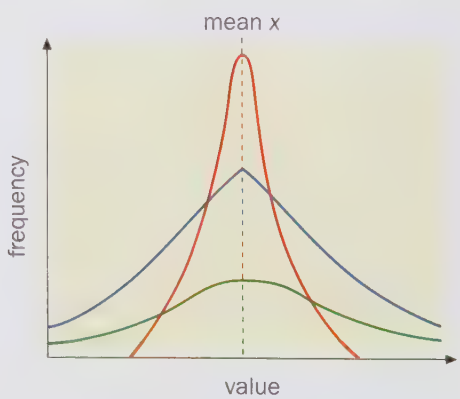
$$\sigma = \sqrt{\left(\frac{\Sigma x^2}{n} - \bar{x}^2\right)}$$

$$= \sqrt{\left(\frac{942}{11} - 64\right)}$$

$$= 4.7$$

This figure 4.7 is the standard deviation of the data from the mean. Thus the true mean lies within the range 8 ± 4.7.

There are more statistical tests relevant to fieldwork on pages 495–7.



The standard deviation is the variation of the sample, represented by the width of the normal distribution curve

Biology at work: Bolivian savanna interactions

The Beni Biological Station (BBS) in Northern Bolivia is an area consisting of representative samples of tropical forest, savanna and swamp, and is noted for its high **biodiversity**. In 1986, the BBS became the world's first Man and Biosphere Reserve as part of a 'Debt for Nature' swap.

A view of typical savanna vegetation in northern Bolivia

The BBS has 14 characteristic vegetation types and over 2000 species of vascular plants within the reserve. The fauna is made up of at least 100 species of mammal, about 500 species of bird, 45 species of amphibian and 200 species of fish. Many of these are endangered and the subject of research.

Neotropical savannas are under as much threat as tropical rainforests due to the increase in heavy cattle grazing and the associated annual burning of the scrub during the dry season. The irregular occurrence of fire is a natural, and quite beneficial, phenomenon of the savanna ecosystem.

With such high biodiversity, it is not surprising that species interact with each other.

		Species 2		
		positive (+)	negative (−)	no interaction
Species 1	positive (+)	mutualism symbiosis	predation parasitism	commensalism phoresy
	negative (−)	predation parasitism	competition	
	no interaction	commensalism phoresy		ammensalism

Classification of species interactions

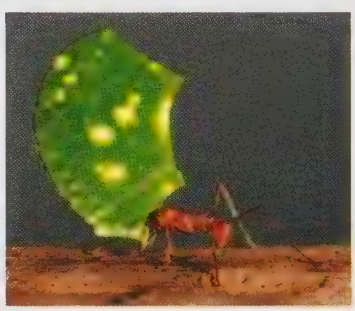
- **Mutualism** – both species benefit from the association. Symbiosis is a specialised form, which involves some form of physiological interdependence between two species.
- **Commensalism** – one species benefits whilst the other is unaffected. Phoresy is a specialised form, which involves one species benefiting from being transported by another that is unaffected.
- **Predation** – one species eats another species, so one species benefits whilst the other loses. Parasitism is a specialised form of predation where the host is harmed but is not normally killed.
- **Competition** – both species suffer from this interaction.

Umbrella tree and ants – the hollow stems and branches of the tree are inhabited by a fierce species of ant (*Azteca* sp.). These provide protection for the plant by killing or driving away predators. In return, the ants gain access to edible bodies on the plant and leaf nectaries. This is an example of mutualism.

Most bromeliads are epiphytic and grow on the branches of other trees

Termites and bacteria – termites are primitive social insects, which feed mainly on the cellulose component of plant material. They do not contain the enzymes necessary to do this but rely on bacteria found in their gut. In return, the bacteria gain shelter and access to a supply of sugar. This is an example of symbiosis similar to that found in ruminants.

Bromeliads and their fauna – some bromeliad varieties collect water in their tight, over-lapping leaf axils. These trap leaves and other detritus, forming food-rich, temporary aquatic ecosystems. These are inhabitated by a diverse range of competing invertebrate species, which may be eaten by frogs and reptiles.



Leafcutter ants and fungus – the most conspicuous ant species in the savanna forms large columns of individual ants, each carrying a leaf fragment several times its own size. These are chewed into a paste, which forms the substrate to grow fungus, on which the ants live, another example of mutualism.

Bats and flowers – there are 106 bat species in Bolivia with 46 being found in the BBS. These include frog- and fish-eating bats, vampire bats and those like the short-tailed leaf nosed bats (*Carollia* sp.) which pollinate the savanna tree (*Pseudobombax* sp.). This is another example of mutualism.

Pseudobombax sp.

The future of the savanna depends upon a deep understanding and knowledge of species interactions and the role they play in complex and intricate ecosystems. Without this it would be very difficult to assess the impact of human activity and hence propose appropriate management and conservation strategies.

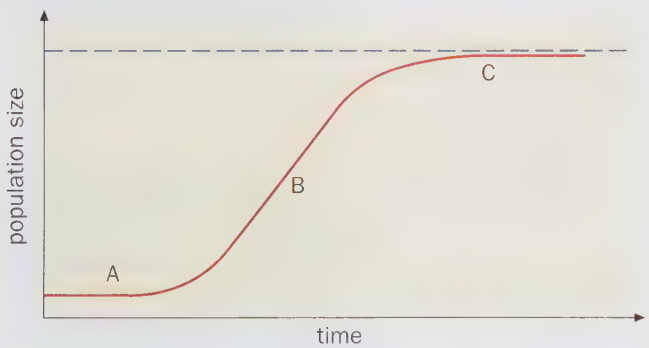
Summary

- A population is a group of individuals of the same species living in the same habitat at the same time.
- The numbers of individuals in a population are increased by births and immigration, and decreased by deaths and emigration.
- The size of a population at a particular time is determined by biotic potential, environmental resistance and the carrying capacity.
- Population changes may be density-dependent due to biotic factors such as competition for food, or density-independent due to biotic or abiotic factors such as adverse weather.
- Many populations show a pattern of growth that follows an S-shaped growth curve.
- Competition may be intraspecific between organisms of the same species, or interspecific between organisms of different species.
- If species compete for the same niche, it can lead to the exclusion of a species.
- Both predator and prey species have evolved adaptations to enable them to survive.
- Predator–prey cycles show the relationships between the two populations.
- Biological control is the use of predators, parasites or pathogens to control the numbers of a pest.
- Succession is the change in the structure and species composition of a community over time.
- Sampling can be random or systematic. Quadrats and transects are common sampling strategies.

Questions

1 Explain what is meant by the following terms and give an example in each case:
a) a density-dependent factor,
b) a density-independent factor,
c) intraspecific competition,
d) interspecific competition.

2 a) What is the name for this type of growth curve?
b) What does the dotted line represent?



This growth curve can be used to show the growth of a bacterial population and a rabbit population.
c) i) Describe and explain the growth of the bacteria at points A, B and C.
ii) Describe and explain the growth of the rabbit population at points A, B and C.

3 A light trap was used to sample a particular species of moth. In the first sample, 30 moths were caught and each one was marked and released. The next night another sample produced 24 moths, of which 6 were marked from the previous catch.
a) Estimate the total population of moths.
b) Why is it important that
i) a suitable period of time passes before the second catch is taken,
ii) the mark is not toxic nor does it make the moth conspicuous,
iii) the mark does not rub off too soon?

4 Ten identical plots of land were cleared of weeds and then sown with pea seeds. After sowing, nine of the plots were kept free of weeds for different lengths of time. After 9 weeks, all the plants were harvested from each plot and weighed. The results are shown in the table.

Period plot kept weed-free (weeks)	Yield at harvest (arbitrary units)	
	Pea plants	Weeds
0	4	80
2	37	44
4	58	24
6	72	12
8	79	4

a) Plot the data in a suitable form on graph paper.
b) What conclusion can you draw about the competition between pea plants and weeds?
c) How could you estimate the total mass of weeds growing in a large field?
d) Design an experiment to test the prediction that pea plants grown at a high density produce fewer peas per plant than those grown at a low density.

5 a) What is meant by the term 'succession'?
b) How is succession different from zonation?

c) What happens to the following during a succession sequence:
   i) plant height and biomass,
   ii) species diversity,
   ii) primary productivity,
   iv) community stability?

d) Explain what is meant by deflected succession and give examples based on agricultural practices.

6 The table shows the populations of lynx and snowshoe hares in Canada between 1885 and 1895.

a) Explain the low figure for hares in 1886.

b) How long did it take the hares to regain their original density?

c) When the hares reached this peak, what happened to the lynx population? Explain your answer.

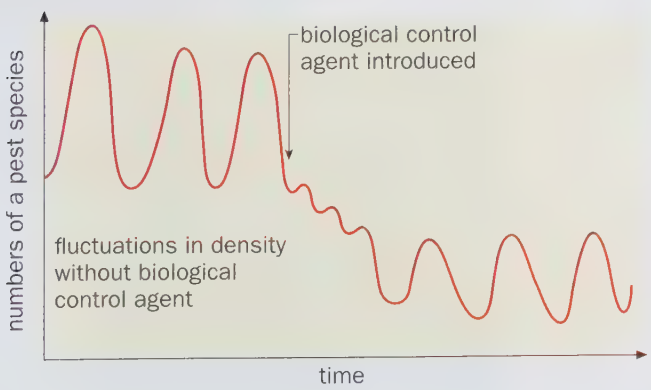
d) Explain the high figure for the lynx population in 1886.

e) Explain the high figure for the hare population in 1890.

Year	Lynx population	Hare population
1885	70000	135000
1886	80000	14000
1887	35000	90000
1888	10000	35000
1889	7500	20000
1890	7500	50000
1891	10000	55000
1892	15000	65000
1893	25000	60000
1894	40000	80000
1895	55000	135000

7 a) Explain what is meant by 'biological control'.

b) The graph shows how biological control can reduce the numbers of a pest species. Explain why the pest numbers fluctuated before the biological control agent was introduced.



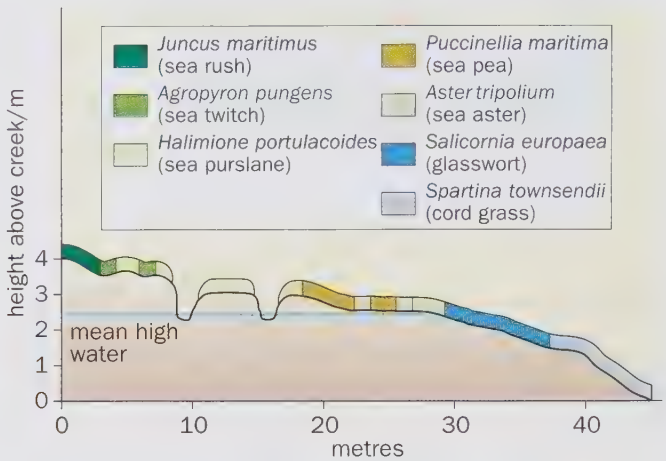
c) Suggest two features that a successful biological control agent should have.

d) Explain the advantages and disadvantages of using biological control, rather than chemical, to control pests.

8 A group of students investigated the distribution of plants across a salt marsh. The diagram shows the results.

a) Describe the techniques that the students could use to obtain the data in the diagram.

b) The distribution of plants in the saltmarsh is governed mainly by abiotic factors. Suggest two abiotic factors that could restrict the distribution of sea rush and cord grass in this habitat and explain how each factor would have its effect.



9 The table shows the results of investigations of several ponds in mid-Wales, UK.

a) Describe the relationship between the pH of these ponds and the numbers of invertebrate animal species.

b) Mere Pool has the greatest species diversity. Why do you think this is?

c) Which pond would you expect to be least stable? Explain your answer.

Pond	pH of pond water	Number of plant species	Number of invertebrate species
Mawn Pool	4.4	8	4
Rhulen Hill	4.8	11	5
Llanbadan	5.7	16	9
Mere Pool	6.6	23	19
Beilibedw	8.1	21	14

25 Human influences on ecosystems

Humans have many influences on ecosystems, one of the most obvious being pollution.
Pollution is defined as
'the release of substances or energy into the environment by man, in quantities harmful to plant or animal life, and which causes damage to structure or amenity'.

But a simpler and perhaps more appropriate definition could be 'the wrong amount of the wrong substance in the wrong place at the wrong time'!
As you will see, some pollutants are biodegradable, for example sewage, whilst others persist in the environment, such as broad-spectrum pesticides, and tend to accumulate along food chains.

Sewage effluent being discharged

Foaming effluent polluting a river

Deforestation

Forests help to maintain the balance of carbon dioxide (CO_2) and oxygen in the atmosphere.
This is important, because carbon dioxide is a 'greenhouse gas'.
It absorbs outgoing radiation and accumulation leads to global warming.

Forests also act as 'stores of water', with their canopies slowing down both the rate of evaporation and the rate at which water reaches the soil.
So deforestation means more water reaches the soil and more quickly.
With no roots to bind the soil together, soil erosion occurs.
This decreases the overall productivity of an area because essential nutrients are washed out of the soil (**leaching**).

Factory chimneys discharging

Forests conserve soil because the decomposition of their leaves adds to the organic content (**humus**) of the soil, although in the case of tropical rainforests this is less true.

High temperatures and high rainfall lead to high productivity, so the forest grows quickly.
As a result, most of the nutrients are located in the biomass, so chopping down trees essentially removes the nutrients from the forest.

Landfill site

So what are the reasons for the large scale clearance of forests?

- The world demand for tropical hardwoods as building materials.
- The demand for paper for newsprint, photocopiers, printers and office consumption.
- The clearing of land for farms, cattle ranches and plantations.
- The building of new road networks through the region.
- To provide firewood and charcoal as fuels.

Felling of rainforest trees

The greenhouse effect

There are a number of 'greenhouse gases' in the atmosphere. Some of these gases such as carbon dioxide, water vapour and methane are naturally occurring, whereas others are man-made, for example **chlorofluorocarbons** (**CFCs**) and nitrogen oxides (NOx).

Greenhouse gases allow solar energy to pass through the atmosphere to warm the Earth's surface up. But when the Earth radiates the heat energy back into space, some of it is absorbed and trapped by the greenhouse gases, causing the air to warm up.

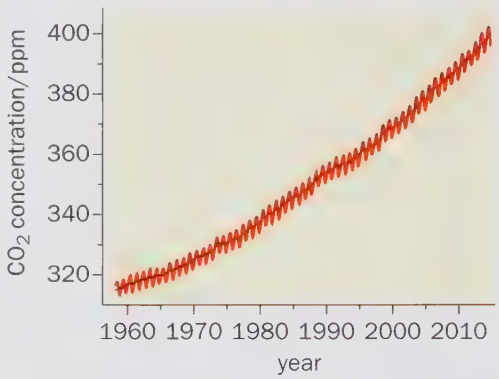
It is important to understand that the **greenhouse effect** is a ***natural*** process, and that without it the average temperature on Earth would be about −17 °C.
However, over the past 100 years there has been a build-up of these gases, which has increased the greenhouse effect.

- There has been an increase in the amount of fossil fuels being burnt by power stations and for domestic heating and transport. About 50% of the greenhouse effect is thought to be caused by carbon dioxide derived from these sources.
- As you have seen, deforestation has resulted in large areas of land being cleared and the trees being burnt. So there are fewer trees to take up carbon dioxide for photosynthesis.
- Methane has increased from a number of sources.
  There has been an expansion in rice growing and cattle rearing. Methane is belched out from the stomachs of cattle and it is also released from rice crops, as a result of the anaerobic conditions in paddy fields.
  Another source of methane is rotting material in landfill sites.
- CFCs have been used as aerosol propellants and as coolants in refrigerators and freezers. Although only present in small amounts, CFCs are many times more active than carbon dioxide as a greenhouse gas.
- Nitrogen dioxide (NO_2), nitric oxide (NO) and nitrous oxide (N_2O), together termed NOx, are emitted from vehicle exhausts.

Of all the greenhouse gases, the most significant increase has been in carbon dioxide levels, which have risen by 10% over the past 30 years.

Burning rainforest

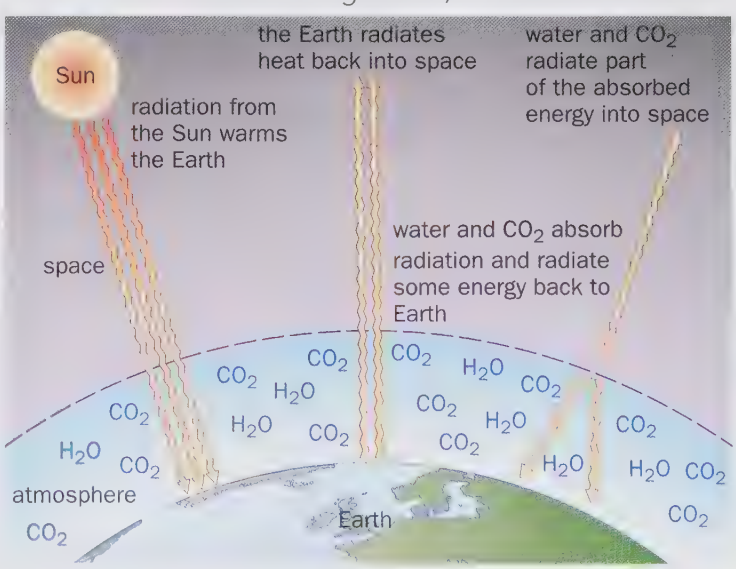
Industrial pollution



Atmospheric CO_2 concentrations (in parts per million) recorded at the Mauna Loa monitoring station, Hawaii

Global warming

A possible consequence of global warming is a rise in sea levels as a result of melting glaciers and the thermal expansion of water.
This would cause flooding of low-lying areas and coastal erosion.
There could also be a change in wind patterns and distribution of rainfall, leading to more extremes in the weather.
If the Earth was to slowly warm up, there would be a shift in climate belts, so the production of grain could be affected. It could cause massive reductions in the grain crops of North America and Central Asia.
This could affect the pattern of world food production, with obvious economic and political consequences.



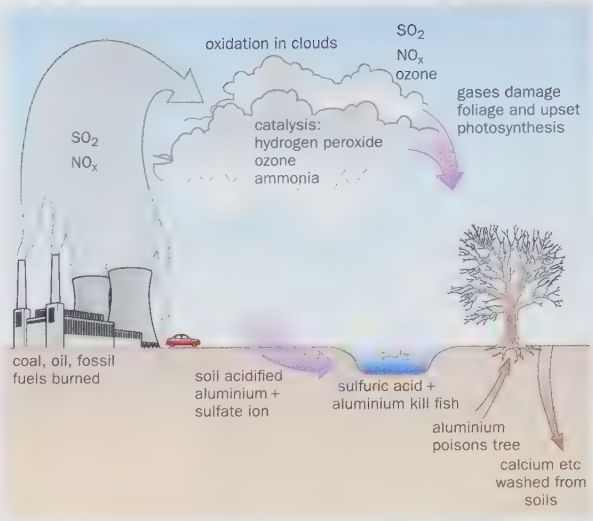
The greenhouse effect

Acid rain

Rain water is naturally slightly acidic, because it reacts with carbon dioxide in the air producing carbonic acid with a pH of about 5.6. **Acid rain** is classed as rain with a pH below 5.6. Europe and North America have rain that is ten times more acidic than this with a pH value of 4.0.

The main pollutant responsible for acidification is sulfur dioxide (SO_2). Although there are natural sources of sulfur dioxide such as volcanoes, most of it comes from the burning of fossil fuels, mainly as emissions from power stations. Various oxides of nitrogen are given off from vehicle exhausts. These gases combine with water vapour in the atmosphere to form sulfuric acid and nitric acid.

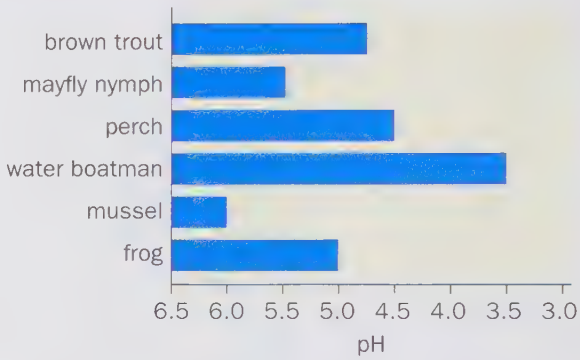
Acid rain is no respecter of boundaries. The polluting gases can ride the clouds for days and be carried long distances before coming down as acid rain. Britain is by far the greatest contributor to acid rain in Norway. Similarly, the USA is responsible for much of Canada's acid rain problem.



Effects of acid rain

Research has shown that the acidity of forest soils has increased five- to ten-fold over the past 20–50 years across vast areas of Europe and North America. The effects may be reduced if the soil is alkaline with a high calcium and magnesium content, as it will neutralise the effect of the acid to some extent.
Many of the soils of northern Europe are thin and cover granite. The low calcium content means the buffer capacity of these soils is low.

- The acid leaches out important minerals from the soil, such as calcium, magnesium and potassium, which are needed for plant growth.
- Toxic minerals like aluminium are mobilised, finding their way into freshwater systems. Here they become deposited on the gills of fish. The fish then produce mucus, which clogs the gills, and they die.
- Many Scottish lochs are now completely fishless.
- Acid rain causes extensive damage to conifers. Thinning of the crown, shedding of leaves, damage to root hairs and decreased resistance to drought and frost are all symptoms of acid rain.
- Decomposition in the soil is inhibited by the acid.
- Acidified lakes look crystal clear, devoid of life except for a luxuriant growth of moss and algae.



Different aquatic organisms have varying sensitivities to higher acidity (low pH)

The clean up

The technology exists to combat acid rain.

- Chemical plants can be installed to remove the sulfur from emissions before they reach the atmosphere.
- Low-sulfur fuels can be used. Crushing coal and washing it with a solvent can reduce the sulfur content.
- Flue gas desulfurisation removes the sulfur from power station chimneys by bombarding the waste gases with jets of wet powdered limestone. The acid gases are neutralised to form a sludge.
- Catalytic converters can be fitted to reduce nitrogen oxides in the exhaust fumes of cars.

These solutions are expensive but they are gradually being introduced as the cost of the effects of acid rain start to outweigh the cost of the solutions.

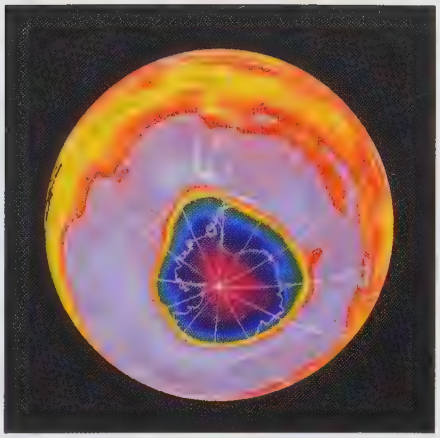
Ozone depletion

The **ozone layer** is present some 10–45 km up in the stratosphere.
It is formed by the effects of ultraviolet (UV) radiation on oxygen.
The ozone layer acts as a screen, preventing much of the damaging UV light from reaching Earth's surface.

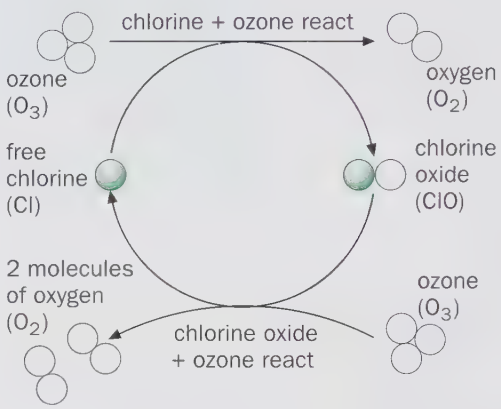
In a 1985, a large 'hole' was discovered in the ozone layer over the Antarctic. It was found that the ozone layer had thinned by as much as 67% in places.
Less ozone in the stratosphere allows more UV light through.
UV radiation is known to be associated with an increased incidence of skin cancer, but the use of blocking creams and wearing a hat can reduce this risk.

The cause of this ozone depletion is a group of chemicals called **CFCs**.
CFCs also act as greenhouse gases.
CFCs are remarkably inert gases that rise unchanged into the stratosphere where they are converted by UV light into free chlorine atoms.
The chlorine reacts with the ozone breaking it down into oxygen.

In the past CFCs were used as propellants in aerosol cans and coolants in refrigerators and air-conditioning units.
However, their use is restricted now, and according to a recent UN study, the ozone layer is showing early signs of thickening after years of depletion.
The ozone hole that appears annually over the Antarctic has also stopped growing bigger every year.
Scientists suggest that this is down to the political determination to phase out CFCs.



Measurements of column ozone in the Southern Hemisphere showing ozone hole in the centre



How chlorine reacts to break down ozone

Lead

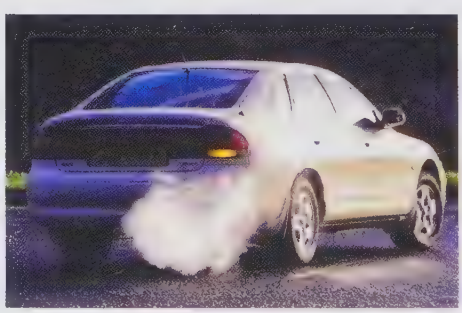
Lead has been known to be toxic for many years.
In the past, it was used in domestic water pipes, although this is not thought to have been a major hazard since lead is not easily absorbed from the gut.

Of far more concern is the lead that is emitted from vehicle exhausts.
Tetraethyl lead has been added to petrol as an anti-knock agent.
This makes the burning of petrol more efficient and raises its octane rating.
But the lead emitted in the exhaust fumes can be absorbed by the lungs.

The toxic effects of lead in the body include

- brain damage and mental retardation in children,
- other problems associated with the nervous system, such as convulsions,
- abnormalities in the digestive system such as intestinal colic,
- kidney malfunction.

In the UK, the use of unleaded petrol, leadless paints and the switch to copper or plastic pipes has helped to reduce the input of lead into the environment.
However, emissions of lead from vehicles are increasing on a global basis as economies in Asia develop and become more affluent.



Thermal pollution

Water is often taken from rivers and used as a coolant by industries.
The water that is returned to the river has a higher temperature than when it was taken out.
This discharge of warm water into rivers is called **thermal pollution**.
The warmer temperature decreases the solubility of oxygen, so lowering the amount of dissolved oxygen in the water.
Higher temperatures also increase the rate of metabolism of fish.
Valuable game fish, such as trout, cannot survive in water above 25 °C and will not reproduce in water warmer than 14 °C.
Coarser fish such as pike and carp, can tolerate temperatures as warm as 35 °C.
In fact, the warmer water enhances their growth rate.
The problems of thermal pollution include

- thermal shock, which can cause death to organisms in extreme cases,
- a decrease in oxygen levels, which affects organisms that live in well-aerated waters, and alters the food chains and food webs of a river,
- an increased susceptibility of aquatic organisms to disease, parasites and toxins.

Fish killed by thermal pollution

This is an example of **synergism**, where two pollutants act together producing an effect that is greater than the sum total of their separate effects. For example, increased water temperature makes:

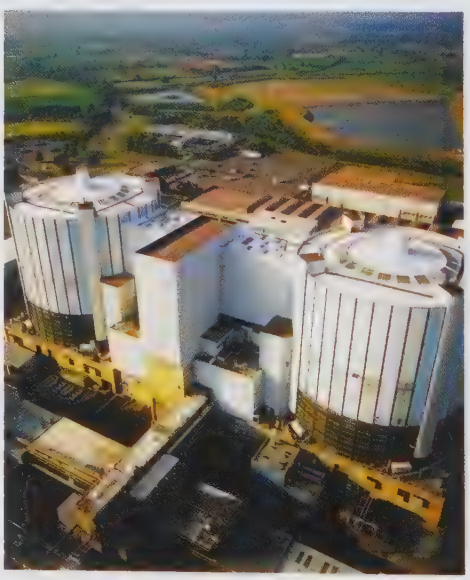
- fish more susceptible to the effects of heavy metals,
- an acceleration in the rate of eutrophication by encouraging the growth of bacteria,
- a change in species composition, as we have mentioned, where trout may be replaced by coarse fish.

Rugeley power station in Staffordshire, UK

Radiation

About 87% of the radiation that we are exposed to every day is ***natural*** radiation.
This comes from space as cosmic rays, or from rocks in the Earth's crust which contain radioactive materials.
About 12% comes from medical sources, such as the use of X-rays for diagnosis and from **radiotherapy** used in the treatment of cancers.
Only 1% of the radiation that we are exposed to comes from nuclear power stations or nuclear weapons.

As with most types of pollution, problems arise when too much of the pollutant is emitted in one place over a short period of time.
In 1986 there was an accident at the Chernobyl nuclear power station in south-west Russia.
A huge cloud of radioactive material was released into the atmosphere.
The winds blew the cloud across Europe, and areas like Poland and Scandinavia were showered with radioactive chemicals.
Upland areas of North Wales, Scotland and Cumbria in the UK, were also affected as the rain washed the radionuclides out of the sky.
Often the effects of radiation are more long-term in nature.
Some forms of radiation are known to act as mutagens, which induce genetic mutations.
The bombs dropped on Hiroshima and Nagasaki during the Second World War resulted in a high incidence of birth defects and cancers.



Nuclear power station

Organic pollution

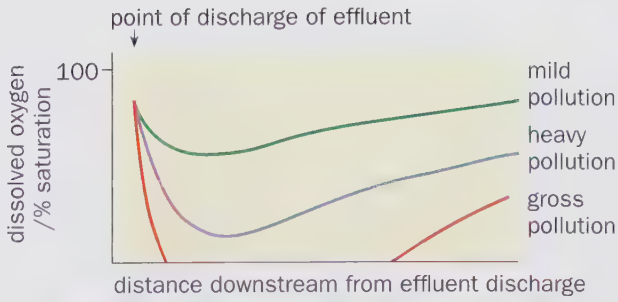
Organic pollution includes sewage, slurry from intensive livestock units, silage effluent (silage is stored grass to feed cattle in winter), washings from dairies and paper mill wastes.

Organic wastes are '***oxygen-requiring***' because they are decomposed by microorganisms which use up oxygen and reduce the oxygen level of the water.
The wastes act as food for large numbers of bacteria and these need oxygen to carry out respiration.

We say that the bacteria place a high **oxygen demand** upon the water.
The quality of a body of water can be measured by taking a sample and finding its **biochemical oxygen demand (BOD)**.
BOD is measured by taking a water sample of known volume and recording its dissolved oxygen content in $mg\,dm^{-3}$.
The sample is then incubated in the dark at 20 °C for 5 days.
The dissolved oxygen is then remeasured so that the amount used up by the effluent can be calculated over 5 days.
The ***lower*** the BOD, the fewer bacteria present, indicating ***less*** organic material in the water.

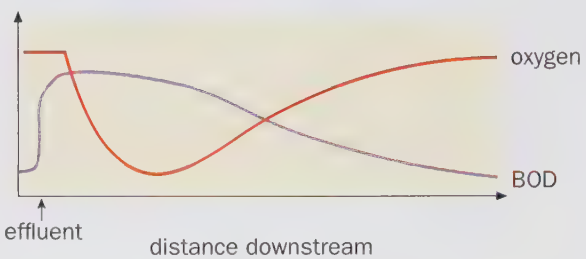
Whenever organic waste such as sewage gets into a river, there are changes in the river's chemical and biological components downstream from the point of discharge.

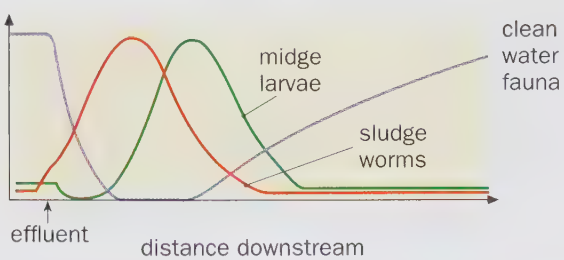
- Initially, the increased loading of organic matter provides food for bacteria and their numbers increase dramatically. Bacterial respiration means that a lot of dissolved oxygen is used up, creating a high BOD.
- Clean water invertebrates, such as stonefly and mayfly larvae, and fish, such as trout, swim away or are killed by the low level of oxygen.
- Pollution-tolerant species, such as sludge worms (*Tubifex*) and midge larvae (*Chironomus*), increase in numbers by feeding on the organic matter, and through reduced competition and predation.
- Eventually the bacterial population starts to decline as the organic matter is used up and protozoa begin to feed on them.
- Sewage fungus increases and then declines as the organic matter is used up.
- Further downstream, photosynthetic algae increase as light penetration increases, and nitrifying bacteria convert ammonium ions (NH_4^+) in the effluent into nitrates (NO_3^-).
- Eventually, pollution-intolerant species are found as the dissolved oxygen increases as a result of photosynthesis, and there is a reduction in the volume of organic material.
- Pollution-tolerant species decline due to increased predation and competition.

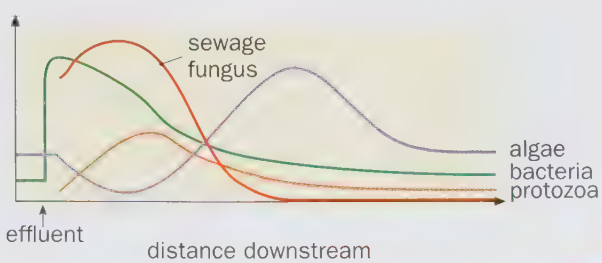


Stonefly larvae: pollution-intolerant species

Sludge worms: pollution-tolerant species

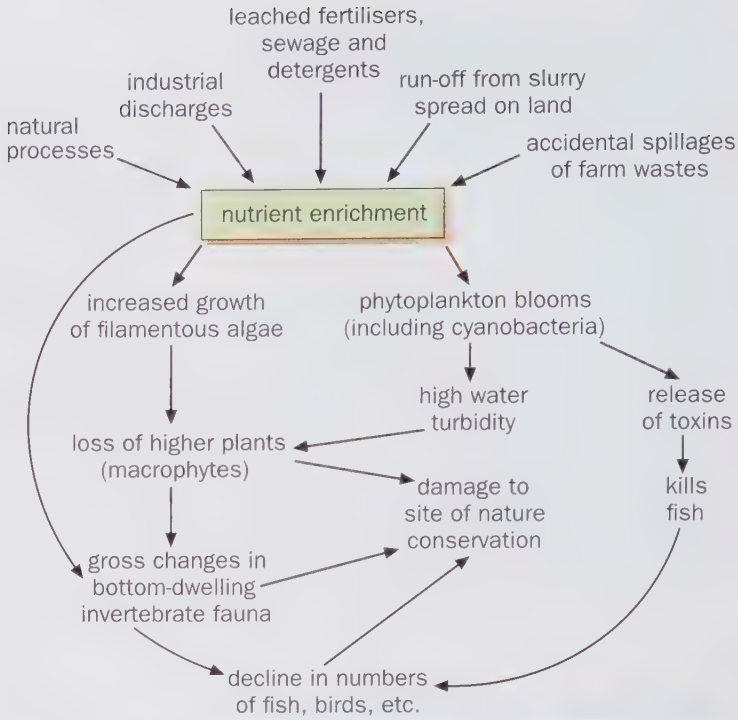






Eutrophication

Eutrophication is the naturally-occurring process of ***nutrient enrichment*** of freshwater and coastal waters. However, artificial enrichment of water bodies is occurring as a result of human activities, changing the biological communities found in our lakes, pond, canals and some rivers.



Causes of nutrient enrichment

Eutrophication can be accelerated by the following:

- increased leaching and run-off of nitrate-rich and phosphate-rich fertilisers from agricultural land,
- release of phosphate-containing detergents,
- run-off from slurry spread on agricultural land,
- drainage of washings from intensive livestock units,
- untreated sewage discharges,
- increased soil erosion as a result of deforestation.

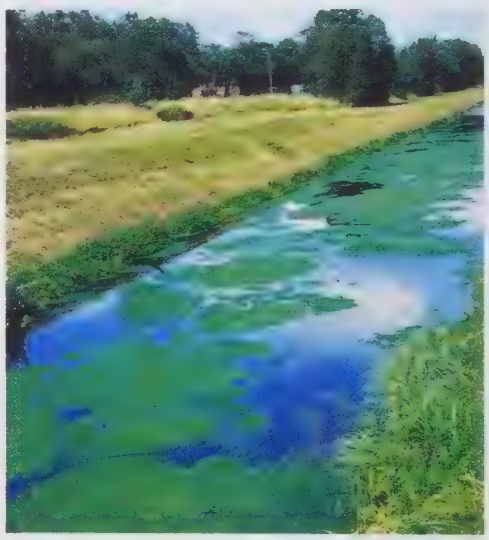
Eutrophication results in marked changes in plant and animal life.
The Norfolk Broads is a series of small, shallow lakes in East Anglia, which used to support a community with a rich flora and diverse fauna.
But over a period of 60 years, the waters have become heavily contaminated with phosphate (a common limiting factor in plant growth). This appears to be due mainly to sewage effluents as a result of the large tourist industry in the area.

The effects of eutrophication on aquatic ecosystems can be summarised as follows.

- Increased nitrate- and phosphate-loading causes massive increases in algae (microscopic plants). These form so-called **algal blooms**.
- Although the algae do release some oxygen from photosynthesis, their dense surface growth cuts down light penetration to the lower depths, reducing the numbers of large rooted plants (macrophytes).
- There is a general decrease in the diversity of species, not only the plant community but also the animals that rely on them for food and shelter.
- Dead algae sink to the bottom and are decomposed by aerobic bacteria. This uses up a lot of dissolved oxygen (producing a high BOD).
- Oxygen depletion means that many species of invertebrates and fish die. Many food chains will collapse.
- Turbidity increases. That is, the water becomes more cloudy and the rate of sedimentation increases. Less light penetrates for photosynthesis.

These effects may create a number of problems to humans:

- water removed for drinking may have an unacceptable taste or odour,
- the water may be harmful to health,
- its appeal to tourists and its value as a conservation area may decrease,
- increased vegetation may slow water flow and navigation by boats,
- important fisheries may be lost.

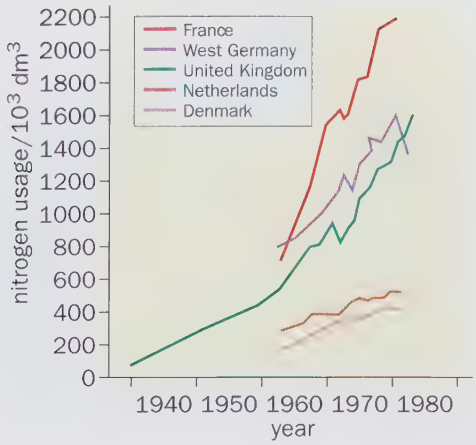


Algal bloom in Basingstoke canal following warm weather

Cutting water weeds in Basingstoke canal

Fertilisers

There has been a massive increase in the use of inorganic fertilisers over the past 50 years.
Prompted by the need for Britain to be self-sufficient in food during the Second World War, the application of fertilisers proved to be a cornerstone of the post-war boom in British agriculture.
The major plant nutrients that have contributed to this increase in crop yield are nitrogen and phosphorus, as nitrates and phosphates.

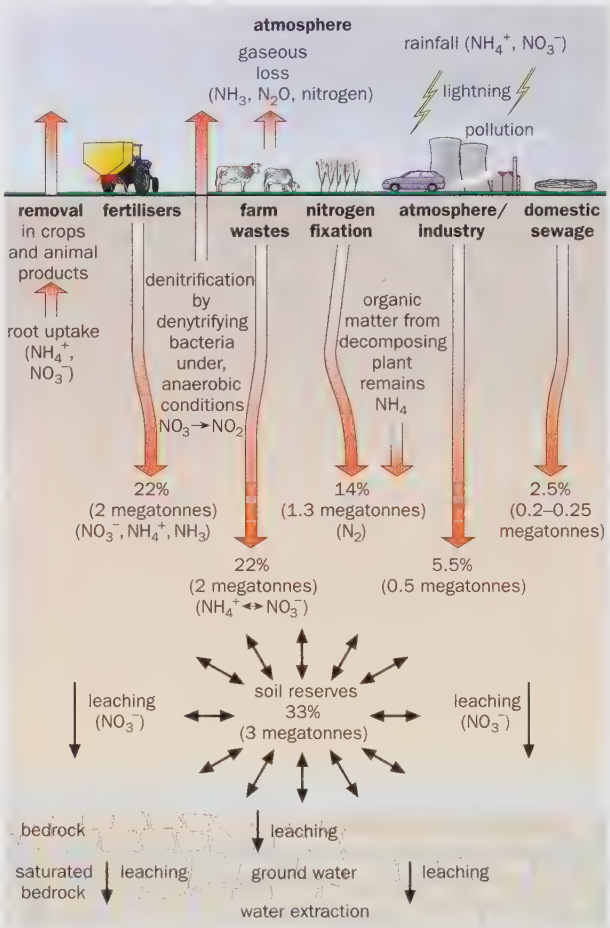


Fertiliser usage in western Europe

Not all of the fertiliser applied is taken up by crop plants.
A large proportion either washes off the soil surface (as run-off), or else trickles through the soil (a process called **leaching**) to eventually find its way into our waterways.
This can damage the environment by encouraging the growth of algae and so contribute to the process of eutrophication.

Fertilisers need to be added to the soil when the plant's demand for nutrients is greatest, that is when growth is rapid.
Fertilisers applied in the autumn may well be leached away because of slow plant growth and the likelihood of heavy rainfall.
Inorganic fertilisers such as ammonium nitrate are particularly soluble and prone to leaching if applied at the wrong time of year.
Over-application of fertilisers also leads to nutrient drainage.
This is detrimental to the environment, and the farmer also loses money if expensive fertilisers are washed out of the soil.

Once in the watercourses, the fertilisers do what they are supposed to do on the land, they stimulate plant growth.
They trigger the cycle of events that we have outlined in eutrophication. There is also particular concern about nitrates building up in the ground water.
The European Union (EU) limit on nitrate in water is 50 mg dm^{-3}.
Medical opinion is that 80 mg dm^{-3} poses no threat to human health, but water from lowland rivers in England often has in excess of 100 mg dm^{-3} and has to be diluted with low nitrate water before it is drinkable.



The leaching of nitrogen into ground water

High levels of nitrates in drinking water are known to be the cause of 'blue-baby syndrome'.
Bacteria in the baby's gut reduce the nitrate to nitrite.
The baby's haemoglobin picks up the nitrite in preference to oxygen, resulting in respiratory failure.
Only a handful of cases have occurred in Britain but even so, nursing mothers in agricultural areas of south-east England have been given bottled water because their drinking water exceeds the EU nitrate maximum.

A number of changes in farming strategy could reduce the current loss of nitrates to rivers and streams.

- Avoid over-use of nitrate fertiliser by matching application to crop needs.
- Avoid winter application when soils are wet and prone to leaching.
- Do not apply fertilisers too early in the spring or just before heavy rain is forecast.
- Split fertiliser applications: do not apply it all in one go.
- Use slow-release fertilisers, which give up their nitrogen gradually.
- Avoid ploughing up grassland, which releases large amounts of nitrogen.

Oil pollution

We tend to associate oil pollution with oil tanker disasters but intentional discharge can occur if a tanker takes on seawater to wash out its tanks, or for ballast after the cargo has been delivered.
During the Iraq occupation of Kuwait in 1991, Iraqi forces deliberately damaged nearly 900 Kuwaiti oil wells and hundreds of thousands of tonnes of oil spilled into the Arabian Gulf.
In April 2010, a huge explosion on the BP Deepwater Horizon oil rig resulted in 206 million gallons of oil spewing into the Gulf of Mexico. This was the worst environmental disaster faced by the USA.

The Amoco Cadiz sinks off the coast of Brittany, France

Effects on wildlife

We have become familiar with press photographs of oil-covered seabirds.

Oil pollution causes harm in the following ways.

- Oil coats the feathers of seabirds, reducing their buoyancy and insulation.
- If swallowed, oil causes intestinal irritation and can cause pneumonia.
- Mussels are unable to feed if coated with oil and it also affects their reproductive capacity.
- Oil destroys certain algae which are important primary producers in marine food webs.
- Little effect is experienced by fish because they just swim away!

Quite often, the most damaging effects of an oil spillage are a result of the actions of governments who, prompted by press coverage, need to be seen to be doing something about a disaster.

- Detergents and dispersants are often used, which are 10–100 times more toxic than the oil itself. Even worse, these kill the bacteria that feed on the oil and break it down naturally.
- Expensive inflatable booms have been used to limit the spread of oil slicks but these are useless in bad weather.
- Mechanical methods such as bulldozers can do great damage to intertidal organisms if used in an attempt to clear a beach.
- Attempts have been made to set the oil alight. In 1967, 22000 tonnes of bombs were used in an attempt to break up the wreck of the *Torrey Canyon* on the Seven Stones reef off the coast of Cornwall, UK, but it was only hit once!

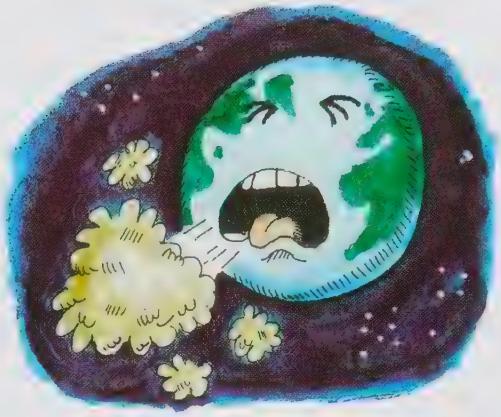
An oiled razorbill at Great Yarmouth

Let nature take its course

Current scientific opinion favours the decomposition of oil by natural bacterial populations.
The recovery time in temperate waters is 2–3 years (slower in polar regions and faster in the tropics, because degradation is temperature-dependent).
Growth of the oil-degrading bacteria may be limited by availability of nutrients (particularly nitrates and phosphates).
Bags of fertiliser can speed up bacterial growth and aid recovery.
Compound containing phosphorus and nitrogen can be sprayed onto oil-covered rocks to encourage bacterial growth.
In the wake of the Kuwaiti disaster, extensive growths of blue-green microbial mats were found over oil-covered areas of the coast.
The major constituent was a cyanobacterium, *Microcoleus*.
Cyanobacteria are photosynthetic and produce a sticky mucilage which traps the oil-degrading bacteria preventing them from being washed out to sea. Their photosynthetic partner also provides them with oxygen.

Burning oil wells in Kuwait

Bioaccumulation



Bioaccumulation is the build-up in body tissues of substances that are neither used (metabolised) nor excreted by cells. Many chemicals cannot be broken down and so they accumulate in the soil or in the aquatic environment.

Many of these chemicals are released into the environment as a result of human activities.
They include:

- a number of pesticides, such as Dieldrin, DDT and DDE,
- heavy metals, such as cadmium, mercury, tin and lead,
- industrial chemicals, such as polychlorinated biphenyls (PCBs).

Pesticides such as DDT have been used to combat malaria by controlling the mosquito **vector**.
They have also been used to control crop pests, and as a result have saved millions of people from starvation.
But because these sorts of chemicals do not readily break down, they persist in the environment and tend to accumulate in the body tissues of organisms.

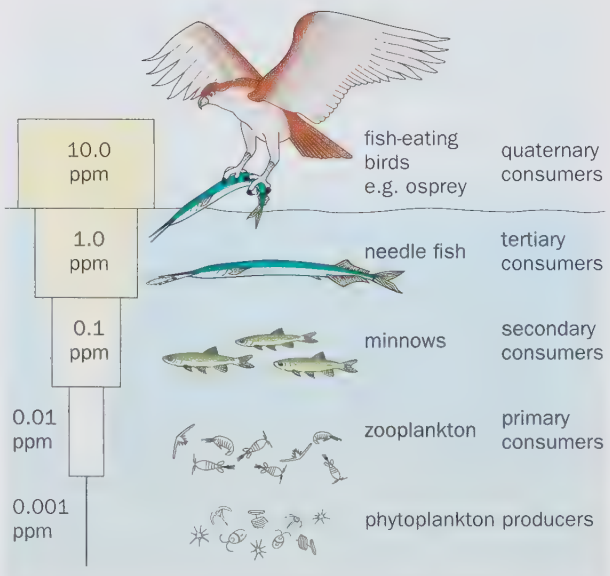
Pesticide spraying in south-east Asia

Rodents such as rats and mice are becoming resistant to many anticoagulant poisons that have been used to control their numbers.
As a result, these chemicals are building up in their body tissues.
This makes it more likely that predators such as the barn owl will become poisoned.

Bioaccumulation is considered to be most significant in marine and freshwater environments.
Many aquatic animals are filter feeders, passing vast quantities of water over their gills in order to extract food particles.
As a result, even if a pollutant is present in minute concentrations, over a period of time it can accumulate in large quantities.
The oyster, for example, has been shown to accumulate DDT to a level at least 70 000 times greater than that present in seawater.

Bioaccumulation need not simply refer to individual organisms. It also refers to the increasing concentrations of chemicals at successively higher trophic levels in food chains.
So each time an organism containing a pollutant is eaten by a predator, the pollutant is passed on and builds up in its tissues.
By consuming many prey, an animal can accumulate high concentrations of the pollutant, which can eventually prove toxic.

The diagram shows how a very low level of DDE pesticide at the producer level can become at least a thousand times more concentrated in the top carnivores.



DDE concentrations in a food chain

Legislation and stricter testing of pesticides has resulted in greater control of these toxic chemicals.
The use of alternative methods such as biological control has also reduced the hazard of persistent broad-spectrum pesticides.

Biology at work: Bioaccumulation of mercury

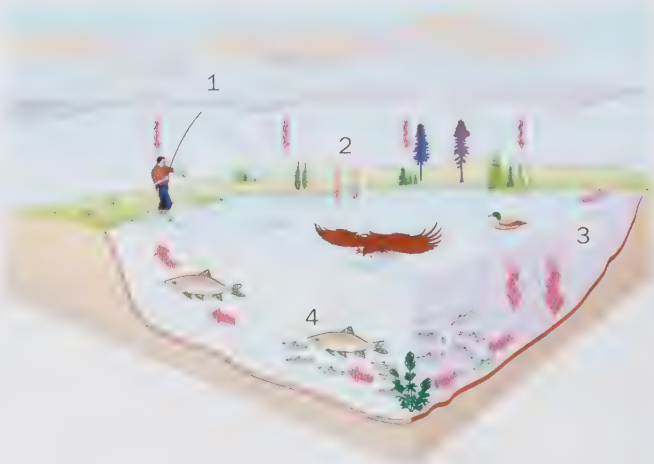
In the lakes of north-eastern USA, a water bird has become a symbol of conservation because of efforts to preserve its breeding grounds.
These efforts may be in vain because mercury poisoning is threatening the loon's survival, and the survival of other organisms, including humans which depend upon the same freshwater ecosystem.

A female loon on a nesting platform, Aziscohos Lake, Maine, USA

Bioaccumulation is the build-up in body tissues of substances that are not used by or excreted from cells.
Loons are bioaccumulators of mercury and because they appear at the top of the food chain mercury can reach high levels.
Mercury enters the atmosphere from activities such as waste incineration, coal burning and chlorine production.

Mercury in the food chain

In freshwater ecosystems bacteria convert inorganic mercury into the highly toxic methyl-mercury, an organic compound.
Invertebrates eat the bacteria and the methyl-mercury becomes more concentrated.
In turn, fish eat the invertebrates. This results in mercury becoming concentrated in the muscle tissue of the fish.
When humans eat contaminated fish the methyl-mercury is readily absorbed into the bloodstream.
From here it crosses into the brain and damages the central nervous system.
Typical symptoms of mercury poisoning include: irritability, tremors, tunnel vision and poor memory.
Mercury can also cross the placenta and damage the developing foetus in pregnant women.



1 Rain or snow washes mercury out of the atmosphere and deposits it on the land or in lakes and rivers.
2 Some of this mercury cycles back into the atmosphere from evaporation or forest fires and other disturbances.
3 In lakes and ponds, bacteria convert mercury into methyl-mercury. Highly acidic lakes which are warmer and higher in dissolved carbon produce more methyl-mercury.
4 Methyl-mercury enters the food chain and bioaccumulates from bacteria to invertebrates to fish to loons and humans

Why loons?

Loons are a good model for demonstrating the flow of mercury through an ecosystem because:

- they catch fish on the lakes where they also breed,
- they live for 20 years or more,
- they can eat a large quantity of large fish,
- they are tolerant to high levels of mercury whilst displaying a range of effects.

Monitoring involves taking regular blood and feather samples.
These reveal an increase in methyl-mercury levels from 1 ppm in Alaska in the west to 5.3 ppm in Maine in north-eastern USA.

These levels don't kill the loons outright but do have significant effects on their reproduction. They may lay fewer or even no eggs at all.
Their behaviour may change, meaning that they do not incubate eggs or take appropriate care of their chicks.
Chick embryos can be killed by only one-fifth of the level of mercury that causes observable effects in adults.

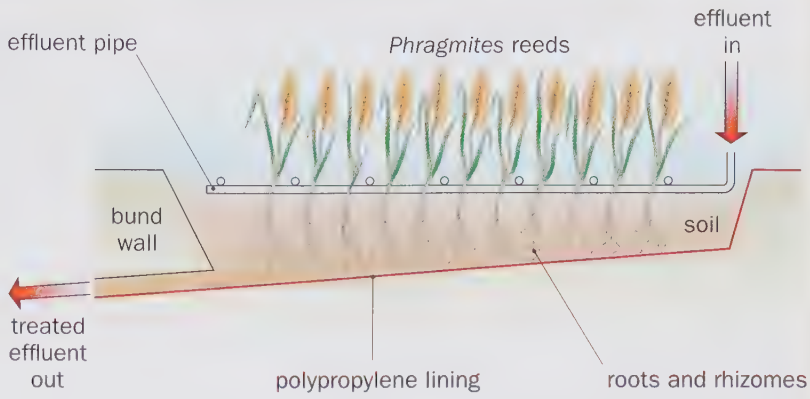
Releasing a loon after taking a blood sample

This research into excessive environmental mercury levels obviously has implications for human health.
In December 2011, the US Environmental Protection Agency (EPA) issued the first national standards for mercury pollution from power plants.
These standards are designed to prevent about 90% of the mercury from coal-burning power stations being emitted into the air.

Biology at work: Natural treatment of man-made waste

In several European countries, the common reed *Phragmites* is used in the treatment of domestic and industrial waste.

There is some concern, however, over their suitability to a northern European climate as they can freeze solid and stop working in severe winter weather.
Reed beds are also common in Australia where the danger of them freezing is very low.



A reed bed system

The roots and rhizomes (underground stems) of the reeds grow both vertically and horizontally through the soil, aiding the passage of the effluent.
Oxygen enters through the stomata of the leaves, passes down the hollow stems and out through the roots into the soil.
Reed beds are shallow and allow the effluent to keep moving, so maintaining oxygen levels.

Aerobic bacteria collect and degrade organic material, releasing harmless substances such as carbon dioxide, nitrogen and water.
The bacteria break down organic material in the effluent that would otherwise starve aquatic life of oxygen.
They also break down nitrogenous waste, and phosphates that can be absorbed onto the materials that the reeds grow in.

Some anaerobic decay also occurs in the less-well-oxygenated areas of the reed bed and this contributes to the removal of the pollution.


Some of the waste from chemical plants, such as this one, can be treated in reed bed systems

Where these beds have been used to treat domestic sewage, there has been an 80–90% reduction in the biochemical oxygen demand (BOD).
BOD is the amount of oxygen used up by microorganisms in a water sample kept in the dark at 20 °C for 5 days.
It reflects the amount of organic material in the water that is broken down by the microorganisms (high BOD results from a large amount of organic material).

Most reed beds in the UK are used as the final stage sewage treatment.
However, there are examples of reed beds being used to treat effluent from the chemical industry.
These beds contain a variety of microbes and remove chemicals such as phenol, methanol and heavy metals.


The traditional approach to treating effluent

Reed beds have low running costs and, unlike other treatment plants, are not unsightly.
However, they do require large areas of land and regular maintenance if they are to be effective.

Another drawback is that reed beds are not necessarily a long-term solution, only remaining viable for around 7–10 years before they need replacing.

Summary

- Human activity can have great influence on the environment at global and local levels.
- Deforestation means the loss of important habitats, soil erosion and a change in the balance of atmospheric gases.
- Increasing levels of carbon dioxide are causing the greenhouse effect and global warming.
- Emissions from power stations and vehicle exhausts result in acid rain.
- Chlorofluorocarbons are causing ozone depletion and exposing us to harmful ultraviolet radiation.
- Organic material, fertilisers, detergents, toxic chemicals and heat all contribute to water pollution.
- Eutrophication is the enrichment of nutrients that upsets the ecological balance of ponds and lakes.
- Oil pollution has dramatic effects on the wildlife of marine ecosystems.
- Some pesticides and toxic heavy metals accumulate along food chains.

Questions

1 a) Describe and explain the long-term effects of large-scale deforestation on the Earth's atmosphere.

b) The world-wide use of fossil fuels has increased rapidly during this century.

i) Give two reasons for the increase in the amount of fossil fuels used.

ii) Give two effects on the environment of this increase.

c) Power stations often use water in cooling processes and then discharge warm water into rivers. Describe how this warm water might affect organisms that live in the river.

2 Lichens have been found to be reliable indicator species for sulfur dioxide in the air. The table shows the percentage cover of a species of lichen at different distances from the centre of a large city.

Distance from city centre in (km)	Percentage lichen cover
5	3
8	22
11	45
16	75
19	75

a) Draw a graph to show the relationship between lichen cover and distance from the city centre.

b) What percentage cover of lichen would you expect at 10 km from the city centre?

c) i) Explain the trend in percentage lichen cover between 5 and 16 km from the city centre.

ii) Suggest why this trend is not continued between 16 and 19 km from the city centre.

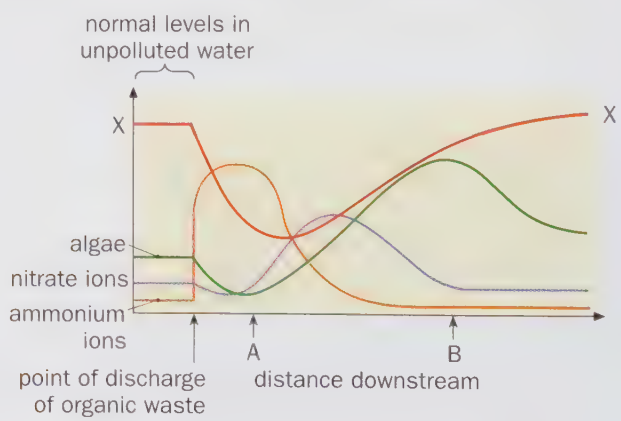
3 The table shows the soil characteristics of a deciduous woodland in 1994 and 1997. In the winter of 1995, 90% of the woodland was cleared.

a) Suggest what effect these changes will have had on the rate of decomposition. Explain your answer.

b) Suggest an explanation for the changes in the soil nutrient levels.

Soil characteristic	1994	1997
mean soil temperature (°C) – day	9.1	11.5
mean soil temperature (°C) – night	5.2	3.0
soil moisture content (%)	32.0	21.0
soil nitrogen ($mg\,kg^{-1}$)	13.6	2.3
soil potassium ($mg\,kg^{-1}$)	11.0	1.9
soil calcium ($mg\,kg^{-1}$)	15.3	4.8
pH	7.4	6.9

4 The graph shows some changes that occurred in a river following pollution by organic waste.



a) i) Suggest an abiotic factor represented by curve X.

ii) Name one organic pollutant which would result in these changes.

iii) State one difference in appearance between fresh samples of water taken from the river at points A and B.

b) i) Give two physiological characteristics you might expect organisms in the polluted water community to possess.
ii) Name a group of organisms you would probably find near the point of discharge of the pollutant.

c) Explain each of the following changes:
i) the increase in concentration of ammonium ions after the point of discharge,
ii) the subsequent increase in the concentration of nitrate ions.

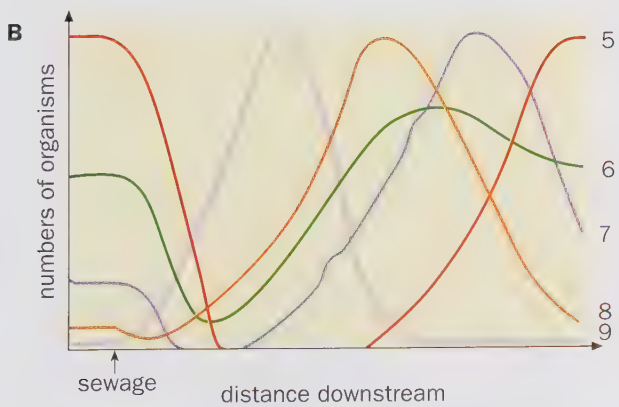
d) Give two possible reasons for the initial fall in the algal population after the discharge of organic waste.

e) Briefly explain the subsequent rise and fall in the algal population further downstream.

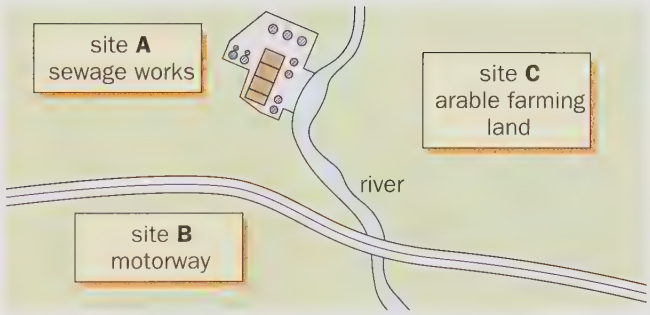
5 The graphs show the effect of untreated sewage on a stream.

a) Graph A shows physical and chemical effects (ammonia, nitrate, dissolved oxygen, suspended solids). Identify each of the lines 1–4, giving the reasons for your choice.

b) Graph B shows changes in the plants and animals (algae, midge larvae, stonefly larvae, sludge worms and 'water-lice'). Identify each of the lines 5–9, giving reasons for your choice.



6 The map shows a small area in the Midlands, UK. The sites indicated by letters **A**, **B** and **C** are sources of pollution. For each of the sites suggest



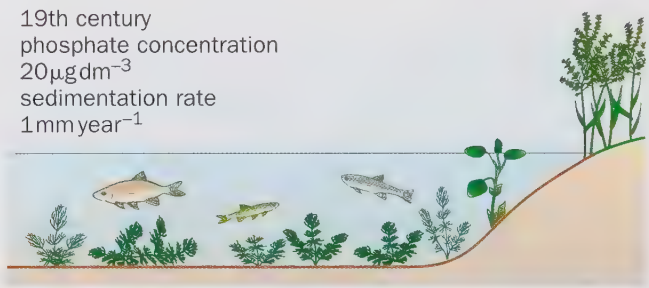
a) the main pollutants associated with each,

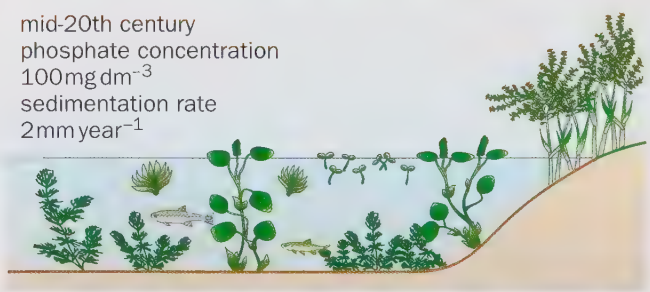
b) explain how these pollutants affect the living organisms inhabiting the site.

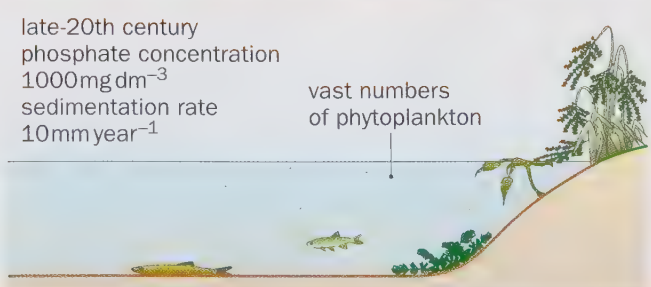
7 The Norfolk Broads, UK (large areas of freshwater) have become heavily contaminated with phosphate over the past 100 years. The drawings show the effect this has had on the community of plants and animals in the Broads.

a) Suggest one reason for the large increase in the phosphate concentration in the Broads over the past 100 years.

b) Explain the reasons for the changes to the community that occurred
i) by the mid-20th century,
ii) between the mid- to late-20th century.

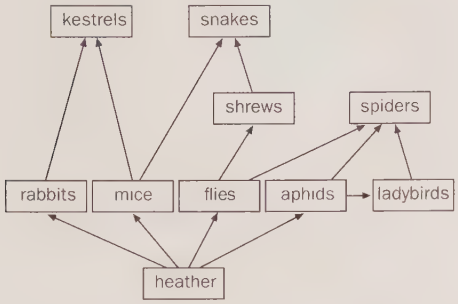






Energy and ecosystems

1 The diagram below represents a food web in a heathland ecosystem.



a) In this foodweb, name the following.
   i) The tertiary consumers [1]
   ii) The organism which has the largest population biomass [1]

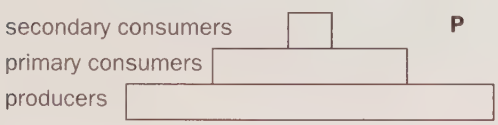
b) Give *two* reasons why all the light reaching the heather cannot be used in photosynthesis. [2]

c) The acidic conditions present in the soil of heathlands inhibit the growth of bacteria. Suggest *two* ways in which this ecosystem may be affected by having few bacteria in the soil. [2]

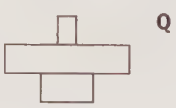
AQA (formerly NEAB) **[6]**

2 a) Define the following terms: habitat; niche; community; population; [4]

b) In the United Kingdom, deciduous trees lose their leaves in October or November and new leaf growth takes place during April to May of the following year.
Diagram P below shows a pyramid of biomass for a deciduous woodland (in the UK) in July.



   i) Explain how the biomass of the second trophic level would be determined. [4]
   ii) Explain why the biomass decreases at each trophic level. [2]
   iii) Diagram Q below represents a pyramid of biomass for the same woodland in January. This pyramid is drawn to the same scale as diagram P.



With reference to diagrams P and Q, explain the changes in biomass of each of the trophic levels in the woodland between July and January. [4]

OCR (formerly Camb) **[14]**

3 A farmer was interested in the productivity of an area of grassland.
The relationship between GPP (gross primary productivity), NPP (net primary productivity) and R (plant respiration) is

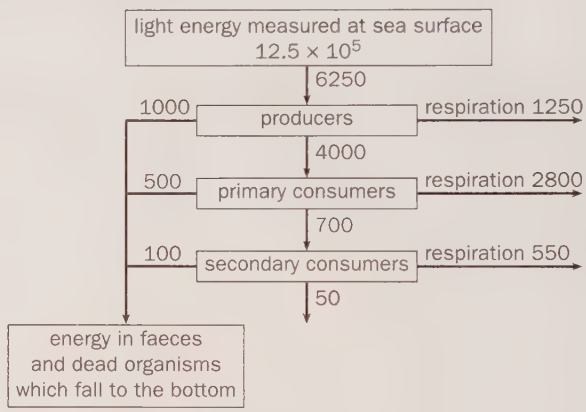
GPP − R = NPP

a) In one area of grassland GPP is 10 500 kJ m^{-2} $year^{-1}$. The efficiency of transfer of energy from GPP to NPP for this grassland in 45%. Calculate the values for NPP and R. Show your working. [2]

b) Productivity is expressed kJ m^{-2} $year^{-1}$. It is more useful to show productivity in this way than as measurements of biomass in the grassland. Suggest why. [2]

Edexcel **[4]**

4 The diagram below shows the flow of energy through a marine ecosystem. The units are kJ m^{-2} $year^{-1}$.



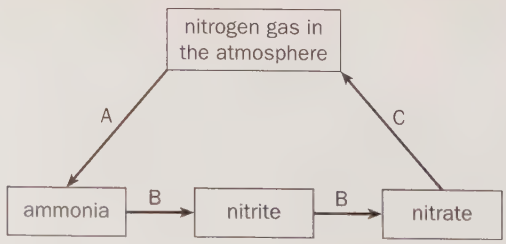
a) i) Calculate the percentage of the light energy at the sea surface which is converted into chemical energy in the producers. Show your working. [2]
   ii) The percentage of the light energy at the sea surface which is converted into chemical energy in the producers is very small. Give *two* reasons for this. [2]

b) Use the information in the diagram to explain why marine ecosystems such as this rarely have more than five trophic levels. [2]

c) What happens to the energy in faeces and dead organisms which fall to the bottom of the sea? [2]

[8]

5 The diagram below shows part of the nitrogen cycle.



a) Name the processes at A, B and C. [3]
b) Farmers drain their land to prevent waterlogging, and plough the soil before planting crops. Suggest how these practices will mean less process C taking place. [2]
c) Some farmers plant legume plants which have nodules containing nitrogen-fixing bacteria on their roots.
   i) Give an example of a legume plant that farmers may grow as a crop. [1]
   ii) Explain how nitrogen-fixing bacteria increase the growth of the legumes. [2]

[8]

Interactions between organisms

6 The following extract is from a newspaper article.

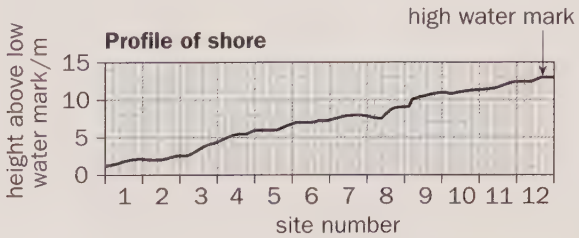
A common soil fungus, *Metarhizium anisopliae,* could become a valuable weapon in the fight against malaria. It kills the mosquitoes that carry the malaria-causing microorganism. As a result of a laboratory investigation, researchers have found that spraying with the fungus could help to prevent the spread of malaria.
A second investigation was then carried out.
Fungal spores were sprayed on walls of houses.
Scientists found that, when a mosquito came into contact with the spores, the spores germinated and the fungus grew slowly in the body of the mosquito.
The mosquito then died.
The use of the fungus in killing mosquitoes is an important discovery because many mosquitoes have developed resistance to chemical insecticides.
Those insecticides that are still effective, such as DDT, are now banned because they can accumulate in food chains.

Use information from the passage and your own knowledge to answer the following questions.

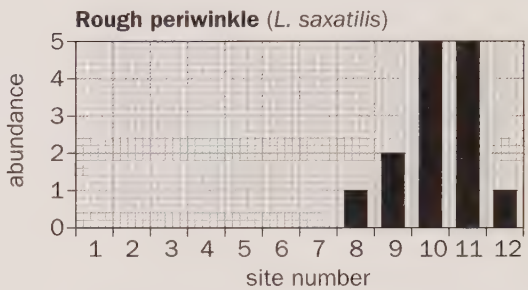
a) i) What is biological control? [2]
   ii) Evaluate the use of biological control to kill mosquitoes that transmit malaria. [4]
b) Explain why the scientists carried out the second investigation. [2]
c) Insecticides such as DDT can accumulate in food chains. Explain how. [3]
d) Scientists suggested that, as a result of these investigations, the fungus could be used to control mosquitoes. They thought it would be necessary to use an insecticide as well as the fungus for the first few weeks. Explain why. [2]

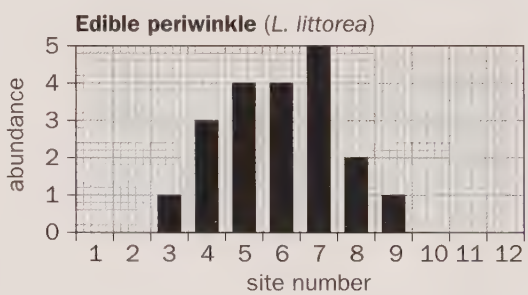
[13]

7 A survey was carried out on a rocky shore to determine the distribution of two species of marine mollusc, *Littorina saxatilis* (the rough periwinkle), and *L. littorea* (the common periwinkle).
Both species are primary consumers. A profile of the rocky shore is shown on the diagram below.
At low water mark, the shore is covered by sea water most of the time. The sea reaches high water mark twice each day.



The sites of sampling were 10 metres apart, starting at the low water mark. The distributions were assessed by means of an abundance scale with 5 representing the greatest abundance. The results are shown as bar charts in the diagrams below.





a) Compare the distribution and abundance of these two species on this rocky shore. [3]
b) Suggest which of the two species is likely to be more tolerant of desiccation. Explain your answer. [2]
c) Suggest *two* factors, other than desiccation, which might account for the difference in distribution of the two species. [2]

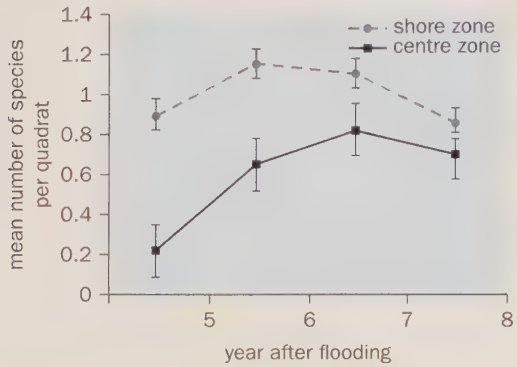
Edexcel (formerly London) **[7]**

8 An area of wet meadow was flooded to create a shallow (20 cm) artificial lake with an area of 35 ha. Each year ecologists recorded the number of plant species.

They divided the lake into two zones:

- Shore zone – within 50 m of shore
- Centre zone – more than 50 m from shore.

Thirty frame quadrats (each 0.5×0.5 m) were used at random within each zone to estimate the number of species.
The data shows the number of large plant species per quadrat in each of the zones 5–8 years after the flooding.
Error bars show ± standard deviation.



a) Describe how the ecologists would have decided where to place the quadrats in each zone. [3]

b) i) Describe the changes in the number of species between 5 and 8 years in the two zones. [3]

ii) What information does the standard deviation provide about the number of species in the two zones? [2]

c) Describe how succession is likely to proceed in this shallow lake over the next 100-year period. [5]

ZigZag Education **[13]**

Woodlice are small, invertebrate crustaceans that prefer damp conditions. They are common in uncultivated land, where they feed on dead plant matter.
An ecologist investigated the population of woodlice in a field that had not been cultivated for 10 years.
He used the mark-recapture technique.

a) Suggest how he marked the woodlice that he caught. [2]

The woodlice were caught using five pitfall traps.
These traps were sunk into the soil at 2 m intervals so the woodlice could walk by and fall into the traps. Results are given in the table below.

	Number of woodlice per trap					
	1	2	3	4	5	Total
woodlice marked and released near trap	2	0	15	4	12	33
marked woodlice captured after 24 hours	0	0	4	1	5	10
unmarked woodlice captured after 24 hours	4	2	12	5	14	37

b) i) Estimate the size of the woodlice population in the field. Show your working. [2]

ii) Give *two* reasons why this estimate of the population may be unreliable. [2]

iii) The traps caught different numbers of woodlice. Suggest *one* reason why. [1]

ZigZag Education **[7]**

Human influences on ecosystems

10 The carbon cycle describes the movement of carbon within an ecosystem.
In this cycle, carbon neutral processes do not change the concentration of carbon dioxide in the atmosphere.
The table below shows the main sources and combustion products of some fuels.

Fuel	Main sources	Main combustion products
biodiesel	oils from crops such as soya beans, rape seeds, palm seeds	carbon dioxide and water vapour
ethanol	fermented sugars from crops such as sugar cane, sugar beet	carbon dioxide and water vapour
hydrogen	catalysis of methane from fossil deposits or biogas generation using waste biomass	water vapour
methane	extracted from fossil deposits or biogas generation using waste biomass	carbon dioxide and water vapour
propane	refining of crude oil from fossil deposits	carbon dioxide and water vapour

a) Using the information in the table, which of the following lists of four fuels, A–D, could be considered to be biofuels.
A biodiesel, ethanol, hydrogen, methane
B biodiesel, ethanol, hydrogen, propane
C biodiesel, ethanol, methane, propane
D biodiesel, hydrogen, methane, propane [1]

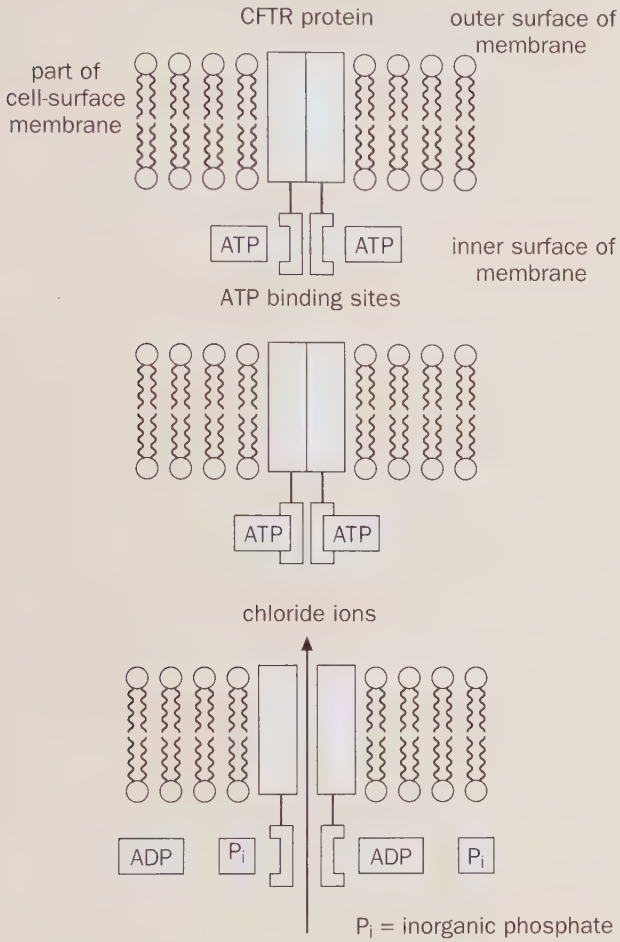
b) Large areas of land may need to be cleared in order to produce biofuels. This might involve deforestation.
Discuss why the production of biofuels may not be carbon neutral. [5]

c) Explain how the combustion products, from the burning of fuels, may lead to global warming. [4]

Edexcel **[10]**

Synoptic questions

1 Cystic fibrosis is a genetic disorder caused by a mutation in the gene which codes for a protein known as the CFTR protein. This protein is involved in the transport of chloride ions through the cell-surface membrane. The diagram below shows how the normal CFTR protein is believed to function in the cell-surface membrane.



a) i) Describe the sequence of events that takes place when ATP is present. [3]

ii) What is the function of ATP in this sequence of events? [1]

b) One symptom of cystic fibrosis is the production of very sticky, thick mucus which cannot easily be moved. This occurs particularly in the lungs, pancreas and testes.

Suggest an explanation for each of the following.

i) Many people affected by cystic fibrosis suffer from repeated lung infections. [1]

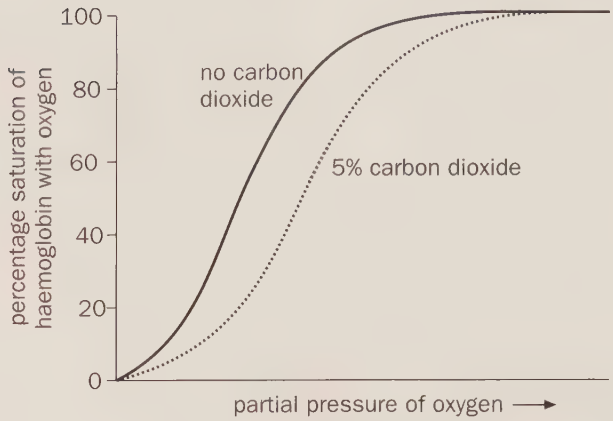
ii) Reduced ability to digest starch in the small intestine is common among people affected by cystic fibrosis. [1]

iii) 95% of males affected by cystic fibrosis are infertile. [1]

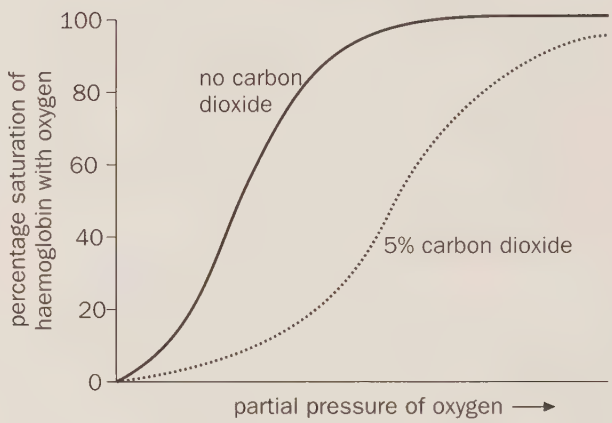
Edexcel (formerly London) **[7]**

2 a) Graph 1 below shows the dissociation curves for human oxyhaemoglobin in the absence of carbon dioxide and in the presence of 5% carbon dioxide. Graph 2 shows similar curves for crocodile oxyhaemoglobin.

graph 1: human haemoglobin



graph 2: crocodile haemoglobin

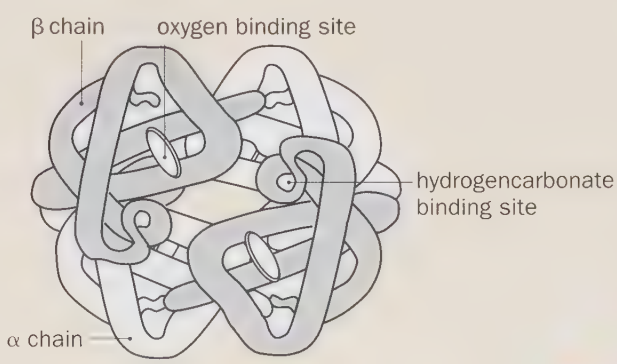


i) Use graph 1 to describe the effect of carbon dioxide on human haemoglobin. [1]

ii) Explain how this effect enables respiring tissues to obtain oxygen. [1]

iii) Crocodiles are able to stay under water longer than humans. Explain how the different effect of carbon dioxide on the dissociation of their oxyhaemoglobin helps them to do this. [2]

b) The diagram below shows the structure of a molecule of crocodile haemoglobin.



What is the evidence that this protein has
i) a tertiary structure, and ii) a quaternary structure? [2]

c) In terms of molecular shape, suggest how the presence of hydrogencarbonate binding sites might account for the amount of oxygen released by the crocodile haemoglobin. [2]

AQA (formerly AEB) **[8]**

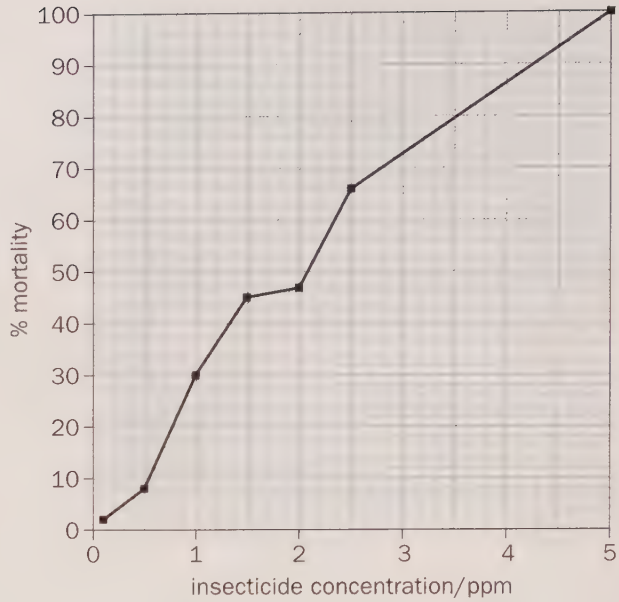
The Colorado beetle is a pest of potato crops. A soil bacterium, *Bacillus thuringiensis*, produces a substance called Bt which kills Colorado beetles but is harmless to humans. Scientists have isolated the gene for Bt production from bacteria and inserted it into potato plants so that the plant produces Bt in its leaf tissues.

a) i) What is a gene? [2]
ii) Suggest how the gene for Bt production could be isolated from the bacteria and inserted into cells of the potato plant. [4]

b) Bt can also be used as a spray. Colorado beetles may be killed if they ingest potato leaves which have been sprayed with Bt.
Suggest and explain *one* reason why using Bt-producing potato plants might increase the rate of evolution of Bt-resistance in the beetles compared with using Bt as a spray. [2]

AQA (formerly NEAB) **[8]**

The toxicity of certain substances can be determined by the use of an LD_{50} test. LD_{50} is defined as the concentration of a substance which results in the death of 50% of a population of test organisms in a given time period.
An investigation was carried out to test the effectiveness of a new insecticide on the larvae of the mosquito, *Anopheles* sp. Groups of larvae were treated with the insecticide at concentrations ranging from 0.1 to 5.0 parts per million (ppm) and the percentage mortality was calculated after 2 days. The results are shown in the graph below.



a) i) From the graph, find the LD_{50} for this insecticide. [1]
ii) Suggest and explain *one* long-term effect on mosquito populations of repeated use of this insecticide at concentrations lower than the LD_{50}. [2]

b) Suggest a suitable control for this investigation and explain why it is necessary. [2]

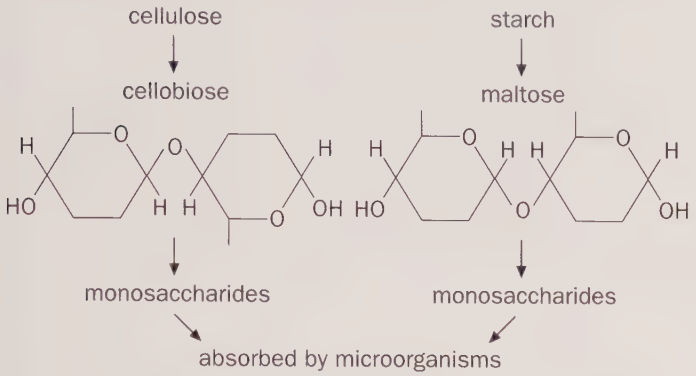
c) The larvae of mosquitoes live in freshwater pools. Further tests on the insecticide showed that it was soluble in water and chemically stable. Suggest *three* reasons why the insecticide might be unsuitable for general use. [3]

d) i) Some insecticides function as non-competitive inhibitors of enzyme activity. Explain how a non-competitive inhibitor works. [2]
ii) Suppose that an insecticide acted as an inhibitor of the enzyme acetylcholinesterase, which breaks down acetylcholine. Suggest what effects this may have on the body of an insect. [3]

e) Predators of mosquito larvae, such as the water boatman, *Notonecta* sp., are important natural control agents in areas such as rice fields in the southern United States.
i) State *two* advantages of the use of natural predators, rather than chemical insecticides, for the control of insect pests. [2]
ii) State *one* disadvantage of the use of natural predators, rather than chemical insecticides, for the control of insect pests. [1]

Edexcel (formerly London) **[16]**

5 a) Microorganisms present in a rabbit's gut are able to digest carbohydrates in the plant material that they eat. The diagram below shows the biochemical pathways by which cellulose and starch are digested in the gut of a rabbit.



i) Describe how a molecule of cellulose differs from a molecule of starch. [1]

ii) Draw a diagram to show the molecules produced by digestion of cellobiose. [2]

iii) Cellobiose and maltose are both disaccharides. Explain why amylase enzymes produced by the rabbit are unable to digest cellobiose. [3]

b) One way in which rabbits cause considerable damage to agricultural land is by competing for plant material that would normally be eaten by domestic animals.
Table 1 below shows some features of the energy budgets of rabbits and cattle living under the same environmental conditions. All figures are kilojoules per day per kilogram of body mass.

Feature	Rabbits	Cattle
Energy consumed in food	1272	424
Energy lost as heat	567	311
Energy gained in body mass	68	17

i) What is the purpose of giving these figures per kilogram of body mass? [1]

ii) Explain the difference in the figures for the amount of energy lost as heat. [2]

iii) Use information given in the diagram for a) to explain why all the energy consumed in food cannot be converted to body mass or is lost as heat. [2]

c) Rabbits were introduced to Australia in the middle of the last century. Their population grew rapidly and they are now major agricultural pests. Table 2 below compares some features concerned with heat loss in cattle and rabbits at a temperature of 30 °C.

Feature	Cattle	Rabbits
Percentage of body heat lost by evaporation	81.0	17.0
Core temperature of the body	38.2	39.3

Use information given in parts b) and c) of this question to explain each of the following.

i) How evaporation helps cattle to maintain a constant body temperature. [2]

ii) The main way in which a rabbit would lose heat at an environmental temperature of 30 °C. [2]

iii) Why rabbits are major agricultural pests in Australia. [2]

iv) Why rabbits are better able to survive than cattle in the hot, dry conditions found in many parts of Australia. [3]

AQA **[20]**

6 ATP can be considered as a temporary energy store. It supplies energy to cells for a range of processes. During aerobic respiration ATP is mainly produced in mitochondria by oxidative phosphorylation. In photosynthesis it is produced in chloroplasts during the light-dependent reaction.

a) Describe the similarities and differences in the ways in which ATP is produced in respiration and photosynthesis.

i) similarities

ii) differences [6]

b) Describe how ATP is used in processes within cells. [6]

AQA (formerly NEAB) **[12]**

7 There are wolves in many European countries. Scientists investigated the genetic diversity of these wolves. They collected samples of DNA from the mitochondria of wolves from different countries. For each sample they identified which haplotypes were present in the DNA. A haplotype is a particular sequence of bases in DNA. Mutations can produce new haplotypes.

Country	Number of wolves sampled	Number of different haplotypes in mitochondrial DNA
Spain	84	3
Portugal	19	2
Italy	101	1
France	7	1
Bulgaria	29	6
Sweden	93	1

The scientists wanted to find out whether one of the haplotypes in the Portuguese wolves was the same as one of those in the Spanish wolves. They used a restriction endonuclease, electrophoresis and a labelled DNA probe.

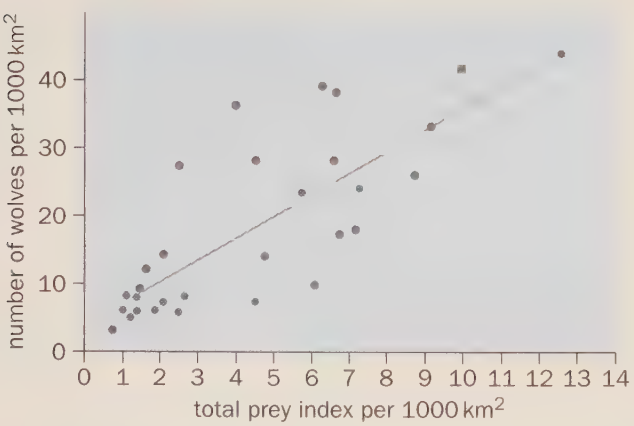
a) For what purpose did they use
   i) the restriction endonuclease? [1]
   ii) electrophoresis? [1]

b) The labelled DNA probe could be used to find out whether the haplotypes were the same. Explain why. [2]

c) Two hundred years ago there were many wolves in Italy. By the 1970s there were fewer than 100 wolves left. Since 1980, wolves have increased in number and have spread to France.
   i) Use this information to explain the number of haplotypes in the Italian wolves. [2]
   ii) Suggest an explanation for the number of haplotypes in the wolves that have spread to France. [1]

d) The scientists analysed the DNA on the Y chromosome and the DNA in the mitochondria of the Swedish wolves. They concluded that the Swedish wolf population descended from one male wolf from Finland and one female wolf from Russia.
   i) Explain why DNA on the Y chromosome helped them to reach this conclusion. [1]
   ii) Suggest why DNA in the mitochondria helped them to reach this conclusion. [1]

Wolves eat different mammals. An ecologist investigated factors that affect wolf numbers in North America. He collected data from different field studies carried out in different places. The graph shows his results.



e) i) The wolf numbers are given per unit area. Explain why. [2]
   ii) The ecologist calculated the total prey index for each of the places that had been studied. In order to do this, he gave each prey species a value based on how much food was available to wolves from the prey animal concerned. He called this value the prey index. The ecologist considered that the prey index gave a better idea of the food available than the prey biomass in kg. Suggest why the prey index gives a better idea of food available. [2]

f) The ecologist calculated the total prey index by combining the prey indices and the total number of animals of each species present in 1000 km². He plotted this information on the graph. What does the graph suggest about the factors that determine wolf numbers in North America? Explain your answer. [2]

AQA **[15]**

The soils in wet, marshy lands usually have anaerobic conditions that inhibit decomposition. As a result of this, dense layers of semi-decayed organic matter, known as marshland peat, build up. The table below shows some of the components of marshland peat.

Component	Chemical nature	Main source
cutin	polymer of organic acids linked by ester bonds	waxy layers of leaves and fruits
lignin	polymers of phenyl propene	
hemicellulose	branched polysaccharide monomers include hexoses and pentoses linked by glycosidic bonds	cell walls of all plant cells
cellulose		cell walls of all plant cells

a) Describe the chemical nature of cellulose. [3]

b) Name a plant tissue that could be the main source of the lignin found in marshland peat. [1]

c) All of the components shown in the table are organic carbon compounds. Describe the role of microorganisms in the recycling of the carbon from these compounds. [3]

d) Landscapes rich in peat act as carbon sinks. However, during recent decades, some countries have been draining and clearing marshy peatlands to grow crops, such as palms, to produce biofuels. During this clearance and drainage, the rate of decomposition in the peat increases and the organic debris is burnt. This change of use of the peatlands has turned carbon sinks into carbon sources.

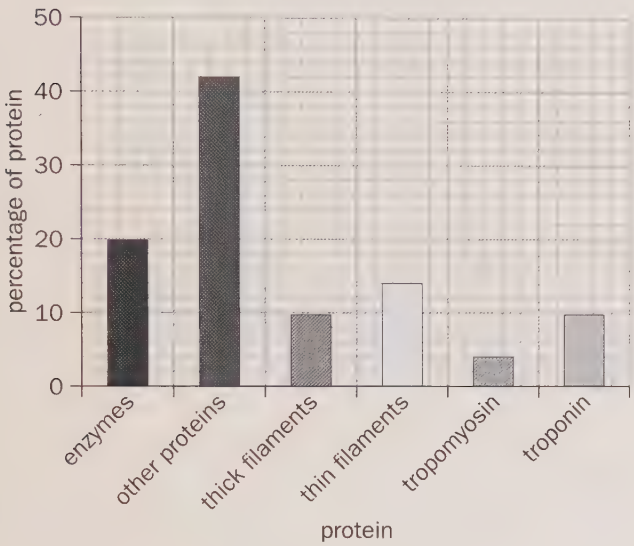
i) Suggest *one* reason why some countries may decide to drain their marshy peatlands for the production of biofuels. [1]

ii) Biofuels are considered to be carbon neutral. Suggest why the continued draining and clearance of peatlands may contribute towards global warming even though these peatlands may be used to produce biofuels. [5]

Edexcel **[13]**

9 Skeletal muscle and cardiac muscle have some of the same proteins.

a) The percentages of the proteins found in cardiac muscle are shown in the bar chart below.

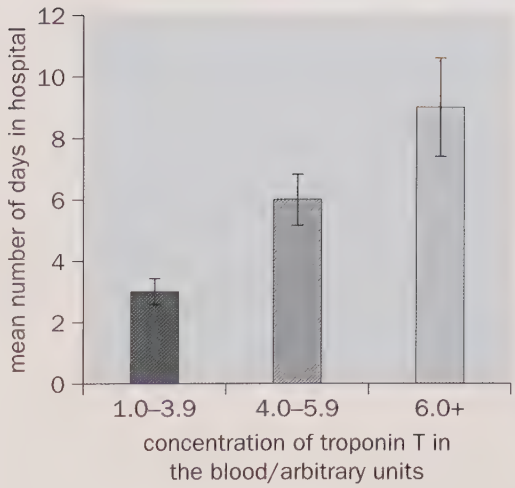


i) Using the information in the bar chart, give the percentage of protein that is actin and the percentage that is myosin. [2]

ii) Describe how calcium ions affect troponin as a skeletal muscle fibre contracts. [2]

iii) Some of the 'other proteins' shown in the bar chart are found in the sinoatrial node (SAN). State the location of the SAN in the heart. [1]

b) Troponin T is found in cardiac muscle cells. It can leak into the blood if the heart is damaged as a result of cardiovascular disease (CVD). Testing for troponin T in blood can be used to study patients with CVD.
The graph below shows the concentration of troponin T in the blood of patients with CVD. The graph also shows the mean number of days and the range of time spent in hospital.



i) Suggest a conclusion that a doctor could draw from these data. [1]

ii) Comment on the validity of the doctor's conclusion. [2]

Edexcel **[8]**

10 a) In the UK in 2009, there was a major outbreak of a type of influenza known as 'swine flu.' 'Swine flu' was caused by a new strain of the influenza virus.
Explain why the influenza virus is usually described as a pathogen rather than a parasite. [3]

b) When an individual is infected with a virus, an immune response is triggered.

i) Define the term *immune response*. [2]

ii) One type of cell involved in an immune response is a plasma cell, which releases antibodies. Plasma cells contain RNA. Outline the roles of RNA in plasma cells.
In your answer you should give an account of the different roles of RNA. [6]

iii) Outline *two* ways in which antibodies reduce the threat from pathogens. [4]

c) i) In an attempt to reduce the consequences of further outbreaks of influenza, the government encourages immunisation of key groups of people, such as the elderly and children that have another risk factor.
Suggest *two* other groups who should be immunised and explain why immunisation for them would be particularly important. [4]

ii) Immunisation of large numbers of people costs the UK government a lot of money. Other than the direct effects on health or reducing the number of deaths, suggest a reason why spending a large amount of money on immunisation is considered worthwhile. [1]

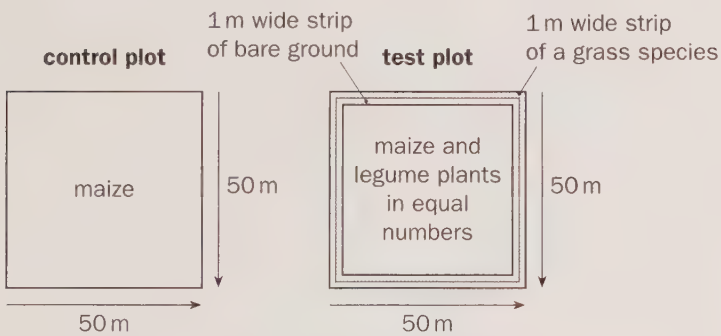
iii) Much of the money spent on immunisation programmes is used to publicise the health benefits of immunisation. Despite this, some individuals are reluctant to have the immunisation.
Give *one* reason why, despite being aware of the immunisation programme, some people choose not to be immunised. [1]

OCR **[21]**

11 **Push-pull** stimuli can be used together as part of a pest control system.

- A push stimulus drives the pest away from the crop plant.
- A pull stimulus attracts the pest towards a different species of plant

Stemborers are insect pests that feed on maize plants. Scientists investigated the effect of **push-pull** stimuli on the control of these pests.
For this investigation, the scientists divided a large field into plots measuring 50 m × 50 m. They then designated each plot as a control plot or a test plot.
The diagram below shows what they planted in each type of plot.



The legumes planted with the maize drive stemborers away.
The grass species attracts stemborers.
The table below shows the results of the investigation.

Plots	Mean percentage damage to maize plants	Mean maize grain yield (tonnes per hectare)*	Mean production costs per farmer ($ per hectare)*	Mean total income for farmer ($ per hectare)*
control	29.6	1.5 (±0.2)	250 (±0.7)	329 (±5.9)
test	6.7	3.7 (±0.3)	278 (±1.1)	679 (±10.2)

*(± standard deviation)

a) In the test plot of land, identify the push stimulus and the pull stimulus. [1]

b) When measuring the mean percentage damage to maize plants, 60 plants from each test plot were selected at random and examined.
Describe how the maize plants could be selected at random. [3]

c) In the test plot, bare ground was left between the maize and the grass species.
Suggest an explanation why. [2]

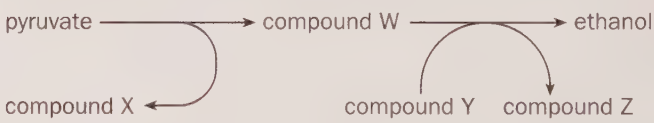
d) The legume plants have nodules containing nitrogen-fixing bacteria on their roots.
Explain how nitrogen-fixing bacteria could increase the growth of the maize. [2]

e) A year after this investigation, the government of one country decided that their farmers should use these **push-pull** stimuli.
How do these data support this decision? [3]

AQA **[11]**

12 a) Glycolysis is the initial stage of cellular respiration.
i) State precisely where in the cell glycolysis occurs. [1]
ii) Outline the process of glycolysis. [4]

b) Yeast cells can carry out anaerobic respiration.
Figure 1 outlines the process of anaerobic respiration in yeast.



Identify the compounds W, X, Y and Z. [4]

In South-East Asia the main source of commercial sugar is the palm, *Borassus flabellifer*. Sap of this species has high sugar content. Yeasts and bacteria, however, can contaminate the sap as it is collected and ferment the sugar, producing ethanol.
This contamination makes it less suitable as a source of sugar.
A study was carried out to investigate the effect of three treatments traditionally used to reduce fermentation during the collection of the sap.
The sap is treated in one of the following ways:

- with a weak alkaline solution (treatment A),
- with bark from the tree *Valeria copallifera* (treatment V),
- with bark from the tree *Careya arborea* (treatment C).

The sap was collected from the palm trees over a 60-hour period. Samples of the collected sap were taken at 15 hour intervals. In each sample, the concentration of alcohol and the number of bacteria were recorded.

The results are shown in the table.

Treatment	Sample time (hours)	Alcohol concentration (%)	Number of bacteria (10^6 cm^{-3})
control (no treatment)	15	0.2	19
	30	3.5	800
	45	5.2	2200
	60	2.6	3400
A	15	0.0	3
	30	0.1	4
	45	0.2	5
	60	0.3	7
V	15	0.2	110
	30	1.1	2900
	45	1.2	2400
	60	1.8	2000
C	15	0.4	230
	30	1.1	160
	45	1.3	3
	60	3.6	40

c) i) With reference to the data in the table, describe the effect of the different treatments on the alcohol concentration of the treated samples compared with the control samples. [2]

ii) Suggest a reason for the difference in alcohol concentration at 60 hours between the two bark treatments V and C. [1]

iii) To be used as a source of commercial sugar, the sap needs to be as uncontaminated as possible.
Suggest, with a reason, which of the treatments shown in the table would be best for use with sap so that it is suitable as a source of commercial sugar. [2]

OCR **[14]**

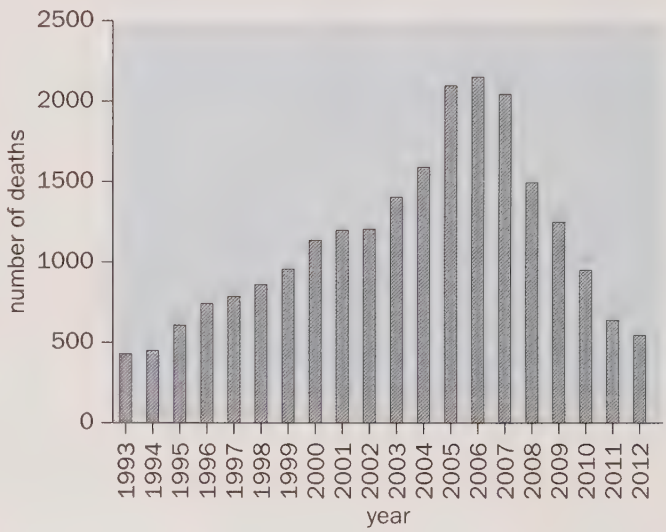
13 a) *Clostridium difficile* is a bacterium that is present in the gut of up to 3% of healthy adults and 66% of healthy infants.

i) *C. difficile* rarely causes problems, either in healthy adults or in infants. This is because its numbers are kept low by competition with harmless bacteria that normally live in the intestine.
Use this information to explain why some patients treated with antibiotics can be affected by *C. difficile*. [2]

ii) Suggest why elderly people are more likely to be affected by *C. difficile*. [1]

b) The antibiotic methicillin inhibits the enzyme transpeptidase. This enzyme is used by some bacteria to join monomers together during cell wall formation. Methicillin has a similar structure to these monomers. Use this information to explain how methicillin inhibits the enzyme transpeptidase. [2]

c) MRSA is a variety of *Staphylococcus aureus*. It is difficult to treat infections caused by this bacterium because it is resistant to methicillin and to some other antibiotics. As a result, some patients who are already very ill may die if they become infected with MRSA.
The graph shows the number of deaths in England and Wales between 1993 and 2012 caused by MRSA.



i) It may be difficult to identify MRSA as the actual cause of death. Explain why. [1]

ii) Describe the change in the number of deaths caused by MRSA in England and Wales during the period shown in the graph. [2]

d) Describe how gene transmission and selection have increased the difficulty of treating bacterial infections with antibiotics. [6]

e) Suggest why there are fewer deaths from MRSA in recent years. [1]

AQA **[15]**

Revision skills

When you revise, you need to balance your time between:

- learning your notes
- practising past paper questions (for this you can use the eight Further questions sections and the Synoptic questions in this book).

The next four pages concentrate mainly on how you can learn your notes effectively so that you have a good knowledge and understanding of biology when you go into the exam room.

Before you start

- Get a copy of the specification and any supporting material from your teacher or the Awarding Body.
- Be clear about which topics you need to revise for the exam you are about to take.
  The website at www.oxfordsecondary.co.uk/advancedforyou tells you exactly which parts of this book you need for your exam specification.
- Work out which are your strong topics and which you will need to spend more time on. One way of doing this is to look at tests or exams that you have done in the past to identify any weaknesses. Use your test scores to help you make a judgement.

Some helpful ideas

1 **Work out your best way of learning.** Some people learn best from diagrams/videos whilst some prefer listening (perhaps to taped notes) and making up rhymes and phrases. Others prefer doing something active with the information like answering questions or making a poster on a topic.

   If you know which way you prefer, then this will help you to get the most out of your revision. Also a ***variety*** of learning techniques can help.

2 **Test yourself!** Just reading through notes will not make them stick. Get someone to test you on a section of work or write down some questions testing your knowledge of the topic. Past paper questions are readily available from exam board websites, but make sure you select ones that are relevant.

3 **Teach others.** If you can find someone to teach a topic to (friends or family?) then this is sometimes the best way to learn. You and some friends might take it in turns to present a topic to each other.

4 **Make sure that you understand the work.** It is unlikely that you will remember much biology if you do not understand it!
   Having said that, there are still things that you need to learn by heart. For example, if you don't know that a triglyceride is made up of three fatty acid molecules and a molecule of glycerol, there is no way to work that out from first principles. So know your basic facts!
   But don't waste your time learning data that will be given in the exam. If you aren't sure, ask your teacher for guidance.

Teach others!

Some revision techniques

1 Get an overall view of a topic first.
Before you start revising a topic, quickly read through the whole topic so that you have a general understanding of it and of how the different bits of it connect with each other. A good place to start is with the summary shown at the end of each chapter.

2 Highlight key words and phrases in your notes using highlighter pens in different colours.

3 Make notes.
Rewriting and condensing notes is a good and active way of reading and then understanding what you need to learn.

4 Make yourself 'flash cards'.
These have questions on one side and answers with notes on the other. Test yourself by trying to answer the questions before checking with the notes. Add new cards to your pack as you reach new revision topics, and remove cards you are consistently getting correct to keep the pack size manageable.

5 Visualisation.
When you are memorising material, always try to get a bold and bright picture in your mind that will help you to remember.
It helps if the picture is something which is very important to you because it is easily remembered (football team? rock group?).
Sometimes imagining something outrageous that can be connected to the fact may help.

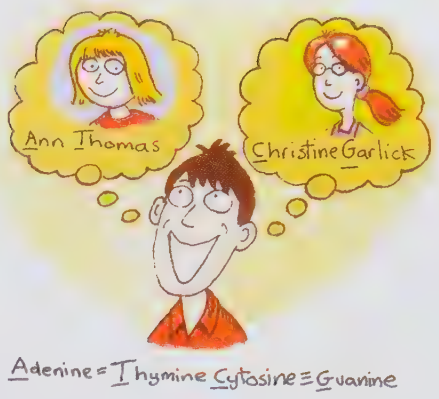
6 A poster.
You can summarise a topic with a poster which you can put on the wall of your bedroom. Include important words and phrases in large, bold letters. If you are a *visual* person then include bright, colourful diagrams which illustrate the ideas.

7 A mind map.
This is a poster which summarises a topic by showing the links between the different concepts that make it up. Making a mind map forces you to think about a topic and will help your understanding of it. The more often you redraw and enhance the mind map the better your recall and understanding will be.

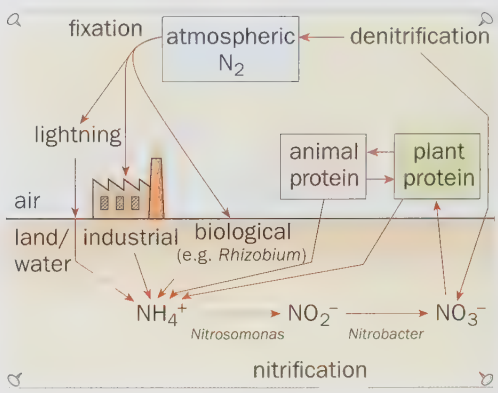
8 Use rhymes and phrases.
Some people are good at rhymes or raps and this can be a way of memorising work. Phrases such as **R**ichard **o**f **Y**ork **g**ave **b**attle **i**n **v**ain, which is used to remember the colours of the spectrum (red, orange, yellow, green, and so on), can be useful.

9 Revision apps.
There are many revision applications for smart phones, tablets and PCs available. These can have high levels of animation, interactivity and testing. Used frequently, for short periods of time, they can be very effective. For example, a flash card app can be used for 10 minutes each day on the bus to college.

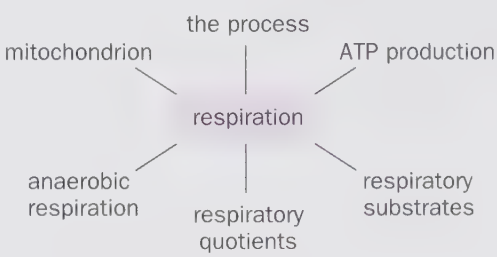
10 Practice calculations.
In biology these often appear in questions on energy flow and genetic crosses in tests of significance (see page 380). You can learn the techniques involved in these calculations as follows.



Use visualisation



Make a poster



A mind map

Find a worked example to look through in your notes or book.
Make sure you understand the logical steps between lines in the calculation.
Then cover the answer and try to solve the problem yourself.
If you get the answer wrong, this method can show you straight away where you went wrong. Or if you get stuck, you can reveal the next line, and then carry on. You should then try some other problems from scratch.

Organising revision

Do you have difficulty starting revision and feel that there is so much to do that you will never complete it?
Do you constantly put off revising and find other things to do?

There are ways in which you can help yourself.

1 **Think about the positive effects of starting.**
When you have finished a session you will feel good that you have made progress and will feel less anxious about not getting enough work done.

2 **Think about the negative effects of delaying.**
What will happen and who will be affected if you put off starting?

3 **Give yourself rewards.**
Think of things with which you can reward yourself at the end of a session, such as a cup of coffee or listening to some favourite music.

4 **Get help!**
Think of ways to involve friends, family and fellow students which will make revision easier and more enjoyable.

Make a revision timetable

Some recent research suggests that some students do better at Advanced level because they:

- revise topics throughout the whole course,
- start their exam revision earlier,
- use better techniques for learning work such as testing themselves, rather than just reading their notes,
- get help from others rather than working alone,
- have a planned exam revision timetable which includes working on their weaknesses.

You can use these ideas to help you plan a **revision timetable**.

1 Start your revision a long time before your exams (at least 8 weeks). Plan to spend quite a lot of the last 2 weeks before your exam on revising your weaker topics again.

2 Note down when you will cover each topic and stick to this!

3 Spend more time on your weaker areas. Try to get extra help on them from your teacher. (Don't just do the things you are good at already!)

4 Give yourself enough time to do past questions.

5 Arrange some revision periods to work with someone who can help.

6 Do some social activities in between revision sessions, so that you don't go completely crazy!

Reward yourself AT THE END of a planned revision session!

Start your revision early

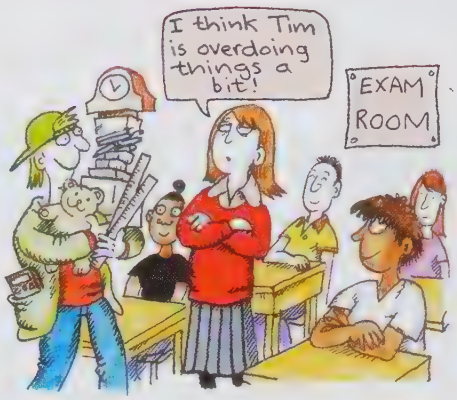

Do some social activities between revision sessions

Exam technique

Before the exam

1. Make sure that you carefully check the dates and times of all your exams, so that you are not late!
2. Make sure that you know which type of paper (such as multiple-choice, short answer) is on which day, and which topics are being examined on each paper.
3. Make sure that you know how many questions you have to do on each paper, how long it is and whether you will get a choice of question. Plan how long you will spend on each question on a paper.
4. Make sure that you are familiar with the chemical formulae of the compounds named in your specification.
5. On the night before the exam, it may help you to steady your nerves by briefly looking through your notes. But don't do too much!
6. Make sure that you get a good night's rest before your exam.

On the day of the exam



1. Aim to arrive early at the exam and try to get into the room as early as possible. This will help you to settle your nerves and give you time to prepare.
2. Don't eat too much or too little food, before an exam.
3. Make sure that you are properly equipped with pens and pencils (and spares in case they break), a rubber, ruler, calculator (check the batteries) and a watch. Don't take a phone or any other barred equipment into the exam room.

During the exam

1. Don't waste time when you get the paper! Fill in your details on the front of any sheets of paper or answer booklets that you are going to use. Read the instructions on the front page of the exam.
2. Read each question very carefully and underline important parts. If you have a choice on which questions to attempt, then read ***all*** of the questions on the paper. It is generally the case that as a question progresses, parts become more demanding and, within a paper, later questions are more demanding than earlier ones.
3. Don't dive into a question without reading all of it first.
4. Do not spend too long on any question! If you are stuck just leave some space so that you can go back to it later.
   It is easier to get 50% on all the questions than 100% on half of them.
5. Sometimes you may be stuck on one part of the question. Check to make sure that there are no later parts which you can do easily.
6. Write neatly and in short sentences that will be easier for the marker to understand. Try to be precise and detailed without writing too much, so that you don't waste time.

7 Make your diagrams clear and neat but do not spend a long time on them to make them perfect.

8 Check that you have done all the required questions – for example, sometimes people don't see that there is a question on the last page of the paper.

9 Check your answers. The first thing that you should check is whether an answer is much too long or too short.
You must also check units and significant figures.

Some hints on answering questions

1 If an exam paper shows that a question is worth 4 marks then put down 4 separate points in your answer.

2 Use correct scientific vocabulary as much as possible.

3 When you do calculation questions you must show your working – for example, there is often a mark for writing down the right equation which you will get even if you don't get the right final answer.

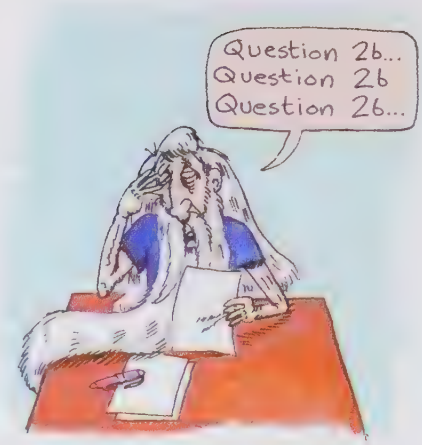
4 Don't forget units and to give the answer to the same number of significant figures as the numbers in the question.

5 In a multiple choice question narrow down your options by crossing out those answers which can't possibly be right. If you are then not sure make an educated guess!

6 When you are asked to draw a graph, label the axes (including units) and plan a sensible scale to fill most of the grid.

7 Words that are used in exam questions are chosen very carefully. Each word has a precise meaning which you will need to understand if you are to respond to the question with a relevant answer. Here are a few words that often occur in questions together with their meanings.

Briefly or Concisely:	**give short statements of the main points**
Compare:	**point out similarities**
Contrast:	**point out the differences**
Define:	**state the exact meaning of a word or phrase**
Describe:	**give a detailed, factual account**
Discuss:	**explain, then give two sides of the issue**
Explain or Account for:	**give reasons for how and why it is**
Evaluate:	**assess the effectiveness/validity of something**
Illustrate:	**use clear drawings and diagrams**
List:	**give a sequence of words**
Outline:	**give the main points**
State:	**present in brief, clear form**
Suggest:	**put forward ideas**
Summarise:	**give a concise, clear explanation of the main points, leaving out details and examples**



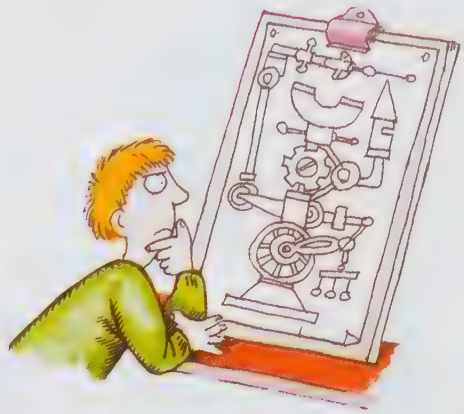
Don't spend too long on any one question

Use the correct scientific words

Practical skills

You should develop your practical skills in the following four areas:

1 **Planning**
2 **Implementing**
3 **Analysing and drawing conclusions**
4 **Evaluating**



15% of the marks in the written examinations will assess practical skills. You will find questions set in practical contexts. These can assess skills such as presenting data, identifying variables and evaluating methods. You will need to consider the quality of data (and the validity of conclusions drawn from them) in terms of margins of error, accuracy and precision. Remember that no measurements can be taken without some degree of uncertainty!

Practical skills that cannot be assessed in written examinations will be assessed by your teacher, and endorsed by your awarding body.

▶ Planning

Your awarding body will have a set of investigations you will need to carry out during the course and your teacher may include some extra ones to help you develop your skills. Before carrying out each practical you need to develop a plan. Research from textbooks or the internet can help you with experimental design.

Investigating the action of catalase

One investigation could be to study the effect of pH on the activity of the enzyme catalase. This enzyme catalyses the breakdown of hydrogen peroxide into water and oxygen gas. You need to plan your method ***in detail***.

When planning you should think about:

- What will you use as your source of the enzyme (potato works well)?
- What range of pHs will you investigate (this requires prior research)?
- Which product of the reaction will you measure?
- What apparatus will you need?
- How many measurements will you take (think about replicates)?
- Over what time period will you record (here you need to think not only biologically but also about the time available to you)?
- The identification of the **independent variable** (the variable you will vary between tests), the **dependent variable** (the variable you will measure to judge the effect of varying the independent variable), and control variables that will be kept constant in fair tests, for example temperature.

All of these points are crucial to the ***reliability*** of your evidence.
But you also need to show an understanding of the biology behind the practical, showing in your planning that you understand the theory behind factors that affect enzyme activity. This should be evident in areas like your choice of pHs.
It is also important that you show some evidence of ***risk assessment***, in this example the safety issue surrounds the corrosive nature of hydrogen peroxide.

Implementing

This skill looks at the way that you carry out investigations and you will need to be familiar with a variety of practical techniques. The manipulation of apparatus and following instructions will be assessed by your teacher. As well as the technique for measuring oxygen release due to the action of catalase, the techniques below might be useful, depending on the nature of your activity.

The photosynthometer is used to measure oxygen production from photosynthesising pondweed

The colorimeter could be used to measure the release of pigment from beetroot tissue exposed to different temperatures

Data logging can be a useful way to gather data, particularly over long periods of time or in very fast reactions

The key areas in this skill are:

- competence in assembling and operating the apparatus,
- taking detailed and accurate measurements, for example reading accurately from the meniscus in the burette,
- taking care to obtain reliable results (this is where replicates are vital),
- having assessed risks in the planning, do you take adequate precautions to minimise them, such as wearing safety specs?

This skill also focuses on the clear and logical **recording** of your measurements.
As most investigations will invariably involve a table.
The usual format involves putting the variable you have changed in the first column.
This is the independent variable and in the example would be the range of pHs.
The second column will contain your measurements of the dependent variable, in this case volume of oxygen produced.

Remember that one of the ways that you can show **precision** and reliability in your results is through this table, therefore make sure that all columns are clearly labelled, and that you have used appropriate units.

		Total oxygen produced (cm^3)				
pH	**Time (min)**	**1**	**2**	**3**	**4**	**5**
6	Attempt 1	2.5	4.8	7.1	9.4	11.6
	Attempt 2	2.3	5.2	8.2	9.0	12.6
	Attempt 3	2.6	5.3	9.0	12.0	15.0
	Average	**2.47**	**5.1**	**8.1**	**10.13**	**13.07**
7.2	Attempt 1	3.3	7.0	11.1	14.9	18.5
	Attempt 2	3.1	8.0	12.7	16.0	19.7
	Attempt 3	5.1	11.0	16.2	20.7	24.6
	Average	**3.83**	**8.67**	**13.33**	**17.2**	**20.93**
9	Attempt 1	3.2	7.0	10.0	13.4	16.2
	Attempt 2	0.9	3.4	6.4	9.4	12.4
	Attempt 3	3.4	7.0	11.0	15.0	18.4
	Average	**2.5**	**5.8**	**9.13**	**12.6**	**15.67**

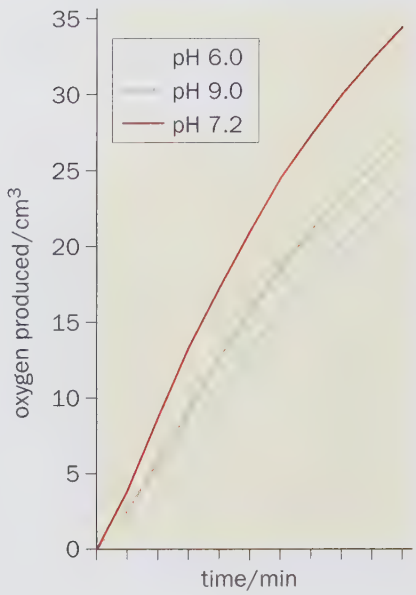
Analysing

Once you have a set of data, then your analysis begins. The skills you use will depend upon the data gathered or given to you. They could include:

- calculations (to the appropriate number of significant figures),
- use of simple statistical techniques like chi-squared,
- drawing graphs (using lines of best fit or curves, and recognising anomalous results),
- using these graphs to spot trends and patterns,
- drawing conclusions that are ***consistent*** with your results,
- explaining these conclusions with detailed and appropriate biological knowledge.

Here are some examples to put these points into context.

1 The effect of pH on the activity of catalase.
This graph shows data from the investigation that you have already considered in the planning and implementing sections.



From this data you can see that:

- the rate of reaction of catalase is fastest at pH 7.2,
- the rate of reaction of catalase is slower at pHs more acidic ***and*** more alkaline than pH 7.2.

These are trends and patterns and from them you can conclude that:

- pH does affect the activity of catalase and that it is less active at extremes of pH.

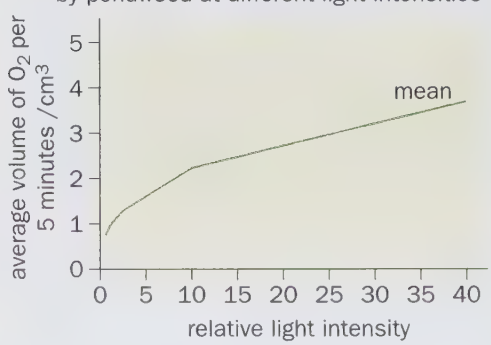
What you ***cannot*** conclude (for reasons that will become clear when you consider the skill of evaluation) is that:

- catalase has an optimum pH of 7.2.

In trying to explain your conclusion you would need to refer to the fact that enzymes are stable over a limited range of pH, and that at extremes outside this range they can be denatured. In your write-up you would, of course, discuss this in more detail.

2 The effect of light intensity on photosynthesis.
This graph shows the results of varying light intensity on photosynthesis in Canadian pondweed (*Elodea*). Here the student measured oxygen production over 5-minute periods.

graph showing the volume of oxygen (O_2) produced by pondweed at different light intensities



From this data you can see that:

- oxygen production increases with light intensity,
- this increase is most pronounced at lower light intensities.

From these patterns you can conclude that:

- the rate of photosynthesis increases with light intensity,
- but at high intensities light begins to have less effect.

For relevant biological knowledge you would need to consider the effect of 'limiting factors' upon the rate of photosynthesis.

Evaluating

The final skill area involves reflecting on your working methods, the quality of evidence produced and therefore the strength of any conclusions drawn.
You should consider:

- the suitability and limitations of the methods used to generate the data,
- any errors in your procedures, including measuring equipment used,
- suggestions for reducing the main sources of error,
- the repeatability and reproducibility of the data (that is, are your results repeatable?),
- suggesting how to improve the quality of data, if appropriate,
- any anomalous results and explaining why they are out of line with the rest of the data,
- the strength of the conclusions you can draw from a set of data,
- what you could do to gather further evidence to support your conclusions.

(You might consider the number of measurements made, or the range over which they were taken. Will any generalisations made be true for readings taken outside the range within which your data lies?)

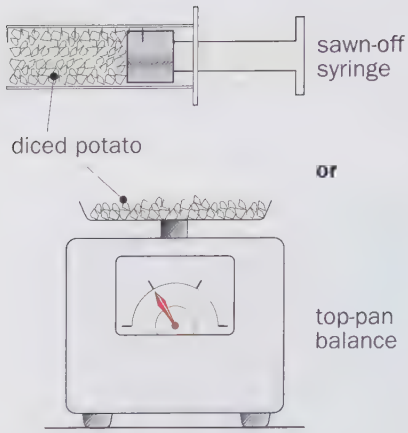
If you consider the examples of biological investigations already discussed, what limitations can you identify?

- The source of enzyme is diced potato, so there is no guarantee that the enzyme concentration is constant between different pHs.
- Even if each potato sample is accurately measured there is no guarantee that each sample has the same surface area exposed to the substrate.
- Each sample may not have come from the same potato (particularly likely if the investigation was carried out over more than one lesson).
- Have you read the meniscus on the burette accurately?
- Have you accounted for air expelled from the flask when the substrate is added?
- Are the pHs chosen adequate in terms of number and range?

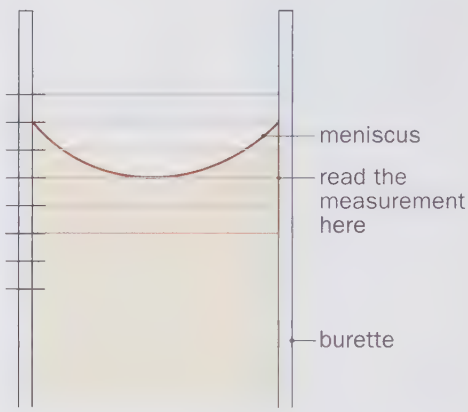
This latter point explains why a conclusion that states 'the optimum pH for catalase activity is 7.2', would not be valid.

A better statement would be 'within the range chosen the optimum pH for catalase activity is 7.2, but further tests using a narrower range of values around this figure would be necessary to confirm the actual optimum'.

There is no set list or number of limitations. What the awarding bodies are looking for is an awareness of the tentative nature of results and the need for subsequent further investigation.



Which apparatus would give the consistency when preparing different potato samples?



A common source of error – misreading a burette

Maths skills

All good biologists need a basic understanding of mathematical skills.
In your examination you can expect 10% of the marks to be awarded for your application of mathematical skills.
The following section gives you a summary of the specific skills, together with examples and references to where you can find their use in this book.

Arithmetic and numerical computation

These skills are the basic requirements of 'doing sums' at this level. As well as adding, subtracting, multiplying and dividing, you need a working knowledge of:

Using appropriate units

When comparing measurements, such as in magnification calculations, it is important to have the same units for each measurement (see page 65).

Example

You can measure the size of the image using your ruler in mm, for example 2.5 mm.
The actual size of the specimen is given in μm.
To calculate the magnification you must have both measurements in mm.
To convert mm to μm you multiply by 1000. For example

$$2.5 \times 1000 = 2500\,\text{mm}$$

Using powers and standard form

A number raised to a power is that number multiplied by itself by the same number as the power. So

10^3 is $10 \times 10 \times 10 = 1000$

In the example above we used powers of 10 in the conversion of units.

We could write 2500 μm as 2.5×10^3 μm. This is known as **standard form**.

In biology we often use ordinary numbers from 1 to 10 raised to powers, which are often negative. 10^{-3} is the same as $\frac{1}{10^3}$, so multiplying an ordinary number by 10^{-3} is the same as dividing that number by 10^3.
Negative powers help us to write very small numbers in a convenient way.
To multiply by 10^y you simply move the decimal point in the number y times to the right. For example

$8.06 \times 10^2 = 806.0$

To multiply by 10^{-y} you simply move the decimal point in the number y times to the left. For example

$8.06 \times 10^{-2} = 0.0806$

We can use powers to calculate outcomes in biology, examples include the number of triplets of DNA possible from four different bases 2^4, and the number of different gametes it is possible to produce in meiosis (2^{23} in humans where there are 23 pairs of chromosomes).
We can use powers to calculate the possible number of DNA molecules produced in PCR.

Example

Starting with a single molecule of DNA, the polymerase chain reaction doubles the number of DNA molecules with every cycle of the reaction. If there are 30 cycles there will be $2^{30} = 1\,073\,741\,824$ molecules of DNA produced.

Ratios, fractions and percentages

These are all ways of expressing the relative proportions of the different parts of a whole.
Ratios and percentages are often used to compare outcomes of investigations where the starting values are different.
In genetic crosses we use phenotypic ratios to represent the possible outcomes (see page 375).

Logarithms

Biologists can use **logarithms** to express quantities with a very large range of values that need to be plotted on a graph.
This is because the logarithm of an ordinary number is the power to which 10 is raised to equal that number.
So the logarithm (or log to base 10) of 1000 is 3 because $1000 = 10^3$.
The main logarithmic scale you will need to know about is the pH scale (see page 8).
On this scale we can express the concentration of hydrogen ions in most solutions as values between 1 and 14.

Microbiologists often use logarithmic scales to represent the number of bacteria grown over a length of time, since the numbers are so great.

▶ Handling data

Mean, mode and median

Taking the **mean** (averages) of a set of data by adding them up and dividing by the number of measurements taken is easy enough.
But in biological data we often want to look at the **mode** (most commonly-occurring value) and the **median** (middle value) as well (see page 455).
If the mean, mode and median are similar values for a large set of data then the data has a normal distribution.

Range and standard deviation

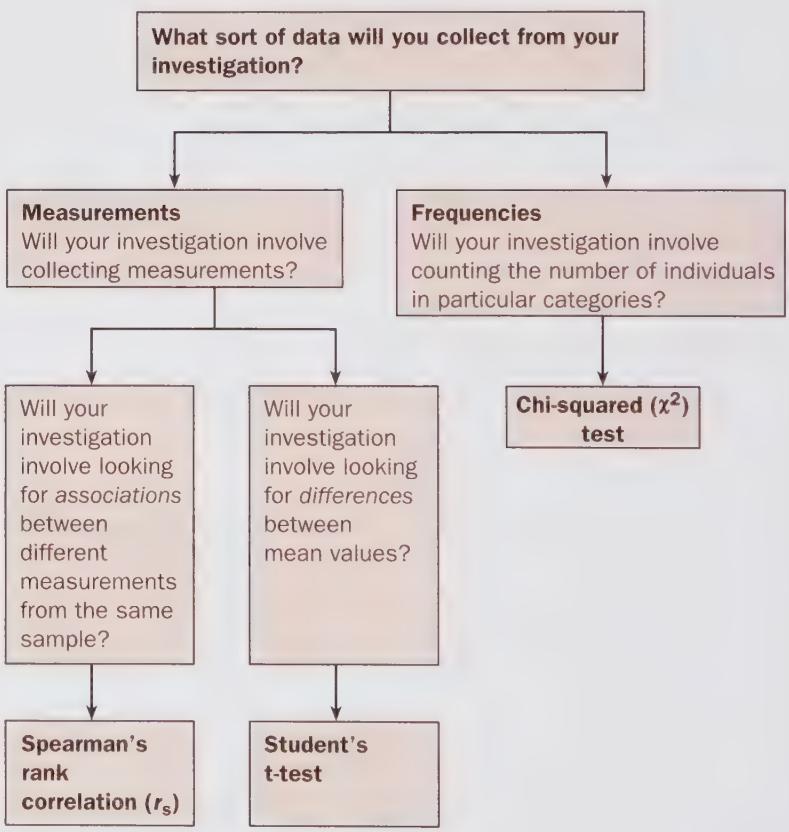
When interpreting data from investigations we can look at the mean, mode and median, but these give little indication of how varied the data may be.
The range of data gives the difference between the highest and lowest values but can be affected by outliers (odd extreme values).
Standard deviation (**SD**) uses all the data and gives a measure of variation around the mean.
It can be shown as the mean + or − the SD in tables or graphs (see page 456).

Sampling

When studying a population of organisms it is impossible to examine every one so we take samples. How we take samples will depend on the organism. However, it is important that samples are taken randomly so no bias is introduced (see pages 450–51).

Using statistical tests

These tests are used to look in more detail at data collected from an investigation. You will be expected to know the most suitable test to use, how to calculate the test statistic, and how to interpret the result in terms of **probability** and **chance**.
The chart will help you decide which test to use.



- **Chi-squared test** (see page 380 for worked example)
- **Spearman's rank correlation (r_s)**
  Use this test when:
  - You wish to find out if there is a significant association between two sets of paired data.
    Positive if as one set of data increases, the other also increases.
    Negative if as one set of data increases, the other decreases.
  - You have between 7 and 30 pairs of measurements.

Example

Great tits are small birds. In a study of growth in great tits, the relationship between the mass of the eggs and the mass of the young bird on hatching was investigated. Some of the data collected are given below.

Mass of eggs (g)	1.37	1.49	1.56	1.70	1.72	1.79	1.93
Mass of chick on hatching (g)	0.99	0.99	1.18	1.16	1.17	1.27	1.75

State **null hypothesis**: *there is no association between the mass of the eggs and the mass of the chicks which hatch from them.*
Calculate the **correlation coefficient (r_s)**.

1 Insert paired data into table (columns 2 and 4).

2 Rank the data: for both sets of data, give each of the values a number in order of their size, with the smallest value 1, and the largest 7 (for the example given).
If two or more values are the same, an average ranking is calculated and allocated to them all.

3 Calculate the difference between the **rank values** (***D***).

4 Square the difference (D^2).

Pair number	Egg mass (g)	Rank (egg mass)	Chick mass (g)	Rank (chick mass)	Difference in rank (D)	D^2
1	1.37	1	0.99	1.5	−0.5	0.25
2	1.49	2	0.99	1.5	0.5	0.25
3	1.56	3	1.18	5.0	−2.0	4.0
4	1.70	4	1.16	3.0	1.0	1.0
5	1.72	5	1.17	4.0	1.0	1.0
6	1.79	6	1.27	6.0	0.0	0.0
7	1.93	7	1.75	7.0	0.0	0.0

5 Add up all of the squares of the differences: $\Sigma D^2 = 6.5$.

6 Calculate the value of the r_s.

$$r_s = 1 - \left(\frac{6 \times \Sigma D^2}{n^3 - n}\right)$$

where n = number of pairs of data in the sample

$$r_s = 1 - \left(\frac{6 \times 6.5}{7^3 - 7}\right)$$
$$= 1 - \left(\frac{39}{336}\right)$$
$$= 1 - 0.116$$
$$= 0.884$$

Interpret the r_S value

The r_s value will always be between +1 and −1.

A ***positive value*** indicates a ***positive association*** between the paired data.
A ***negative value*** indicates a ***negative association*** between the paired data.
A number ***near to 0*** shows ***little/no association***.

Use the table to obtain the **critical value** (**CV**) for the number of paired values in the sample, for example for seven paired values the CV (p = 0.05) is 0.79.

Number of pairs of measurements	CV (p = 0.05)
5	1.00
6	0.89
7	0.79
8	0.74
9	0.68
10	0.65
12	0.59
14	0.54
16	0.51
18	0.48

Compare the r_s with the CV.

If the r_s value is *greater than* the CV then:

- There is a *significant association* between the paired data. There is a less than 5% probability that the association occurred by chance.
- Therefore, the *null hypothesis is rejected*.

If the r_s value is *less than* the CV then:

- There is *not* a *significant association* between the paired data. There is a greater than 5% probability that the association occurred by chance.
- Therefore, the *null hypothesis is accepted*.

In the example shown:
r_s = 0.884
CV = 0.79

Since the r_s is greater than the CV, it can be said that:

- There is a significant association between the mass of egg and the mass of chick which hatch from them. There is a less than 5% probability that the association could have occurred by chance.
- Therefore, the ***null hypothesis is rejected***.

- **Student's t-test**
  This test is used to find out if there is a significant difference between two means, and the data is normally distributed.

Example

Gammarus pulex is a small freshwater shrimp which is sometimes infected with the parasite *Polymorphus minutus*. Infected individuals of *Gammarus* are easy to identify because of the bright orange colour of the parasite.
In an experiment, 30 uninfected specimens and 30 infected specimens were collected using hand nets, and the length of each specimen was measured accurately.

The mean ($\bar{x}$) for each set of data is:
uninfected = 7.733 mm infected = 5.113 mm

The SD for each set of data is:
uninfected = 1.665 mm infected = 1.331 mm

State **null hypothesis**: *there is no difference in the mean length of Gammarus pulex between those infected with Polymorphus minutus and those uninfected.*

1 Calculate t by substituting into the following equation:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{(s_x)^2}{n_x} + \frac{(s_y)^2}{n_y}}}$$

where:
$\bar{x}$ = mean of sample
$\bar{y}$ = mean of sample
s_x = standard deviation of x
s_y = standard deviation of y
n_x = size of sample x
n_y = size of sample y

$$t = \frac{7.733 - 5.113}{\sqrt{\frac{(1.665)^2}{30} + \frac{(1.331)^2}{30}}} = \frac{2.62}{\sqrt{0.0920 + 0.059}}$$

$$t = \frac{2.62}{0.389} \quad t = 6.74$$

2 Degrees of freedom is $n_x + n_y - 2$
So for this example $30 + 30 - 2 = 58$
CV at $p = 0.05$ is 2.00 (you would look this up in a table of t values).

3 Compare the t value with the CV.

If the t value is *greater than* the critical value then:

- There is a *significant difference* between the two means. There is a less than 5% probability that the difference occurred by chance. Therefore the *null hypothesis is rejected*.

If the t value is *less than* the critical value then:

- There is *not a significant difference* between the two means. There is a greater than 5% probability that the difference occurred by chance. Therefore the *null hypothesis is accepted*.

In the example shown:
$t = 6.74$
CV = 2.00

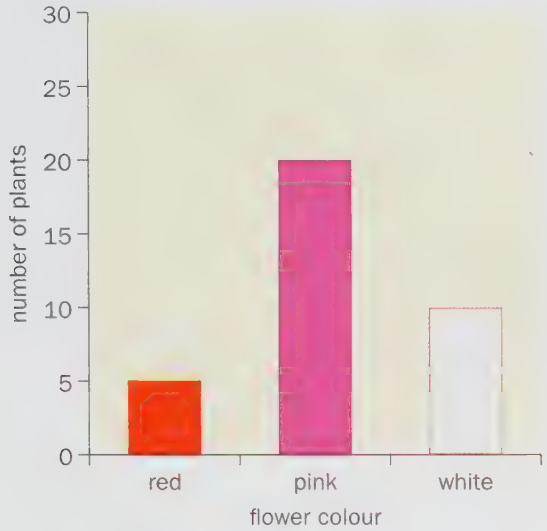
Since t is greater than the CV, it can be said that:

- There is a *significant difference* in the mean length of uninfected and infected *Gammarus*. There is a less than 5% probability that the difference occurred by chance. Therefore, the ***null hypothesis is rejected***.

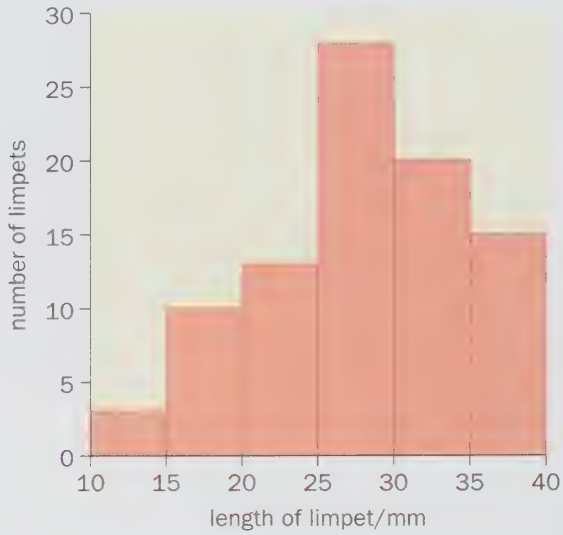
Graphs

It is important to know what type of graph to draw for different types of data.

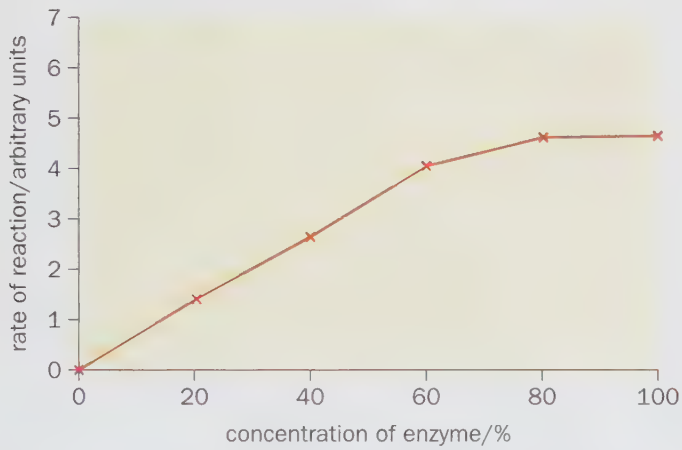
- **Bar chart** – use when independent variable is categoric and dependent variable is continuous, for example number of plant flowers of certain colours.



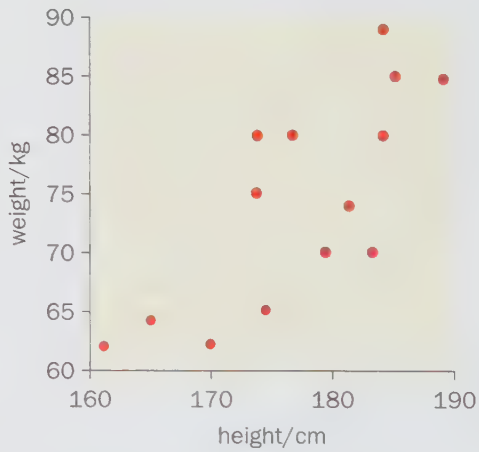
- **Histogram** – use to show frequency distributions, for example number of limpets of different lengths found on a shore.



- **Line graph** – use to show relationship between the independent and dependent variable, for example effect of enzyme concentration on the rate of a reaction.



- **Scattergram** – use when looking for a relationship between two variables, for example height and weight in men.



Remember when drawing graphs, always plot the independent variable (the one you choose to vary) along the horizontal (*x*) axis, and the dependent variable (the one you use to judge the effect of changing the independent variable) up the vertical (*y*) axis.

A sensible scale should be selected (for example 1, 2 or 5 units not 3 units per 20 mm square on the grid).

For line graphs the points should be plotted with a small, neat 'x' using a sharp pencil.

If the points appear to be on a straight line or curve then a line of best fit may be drawn – either with a ruler for a straight line, or a smooth freehand line for a curve.

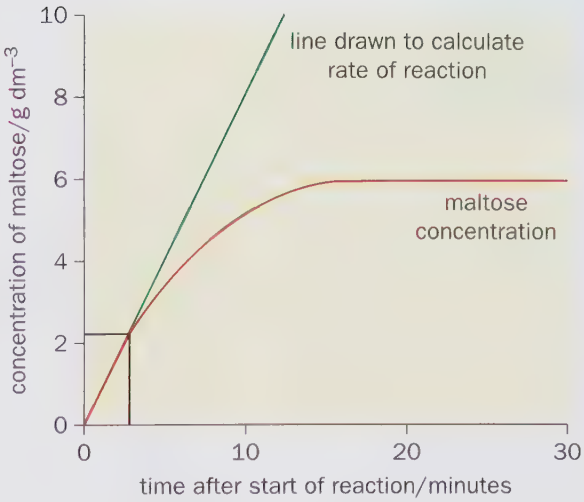
If it is not possible to predict the shape of the curve the points should be joined dot-to-dot.

Many biological investigations do not give predictable curves.

Using the slope of a tangent to a curve as a measure of rate of change

To find the initial rate of reaction we can draw a line at a tangent to the curve at the start as shown in the diagram.

Then draw a line from the curve to the *y* axis and down to the *x* axis. Read off the values. Divide the value from the *y* axis by the time on the *x* axis and this will give you the initial rate.



Algebra

The main skill you need is to be able to change the subject of a mathematical equation.
The 'subject' is the quantity that you want to find the value of.
Calculations involving magnification are an example of this (see page 78).
Biology has many other similar equations to be manipulated.

Example

A person has a tidal volume of 0.8 dm^3, and a pulmonary ventilation rate of 10 dm^3 min^{-1}. What is his breathing rate?

The question has asked you to work out the breathing rate (*BR*) of a person, given their tidal volume (*TV*) and pulmonary ventilation rate (*PV*).
You know that

$$PV = BR \times TV$$

so you need to make BR the subject of the equation.
So if we divide both sides of the equation by *TV*, the *TV* on the right will cancel out leaving just *BR*.

$$\frac{PV}{TV} = BR$$

Substituting in the values we are given in the question into the equation above:

$$\frac{10}{0.8} = 12.5 = 12.5 \text{ breaths per minute}$$

Geometry and trigonometry

Surface area and volumes influence processes such as diffusion in organisms.
Calculating surface area to volume ratio is something you need to be able to do. (See pages 88 and 134.)
In experiments we may want to measure volumes by using a manometer or capillary tubing. An example of this is the potometer (see page 184).
We determine the rate of transpiration by measuring the volume of water taken up in a certain time.
If we know the radius of the capillary tube we can work out its cross-sectional area $A = \pi r^2$.
If we multiply this figure by the distance moved by the bubble (h) in the tube we can work out the volume, $\pi r^2 h$.

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I would like to thank Mike Blackmore for reviewing and improving the manuscript; Linda Turner for her work on Further Questions, Synoptic Questions and Maths Skills; Sarah Morgan for drafting the section on Epigenetics; and Nick Paul for Practical Skills.

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Particular thanks are due to Keith Johnson for his advice and insight into the ways in which we learn and what makes a good textbook. His influence on my writing of both this book and GCSE Biology for You, has been huge.

Thanks also to Annie Hamblin, Julie Thornton and Michelle Sadler for all their helpful advice and suggestions.

Other books by Gareth Williams
Biology for You
Spotlight Science 7, 8, 9 with Keith Johnson and Sue Adamson
Essential Biology for Cambridge IGCSE Top
Biology Grades for You

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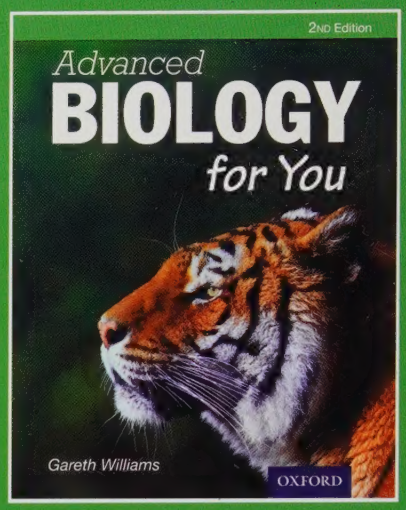

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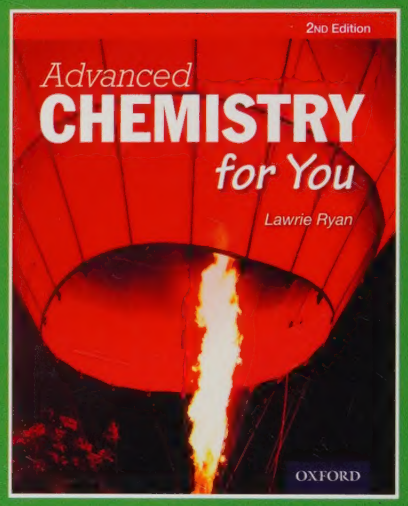

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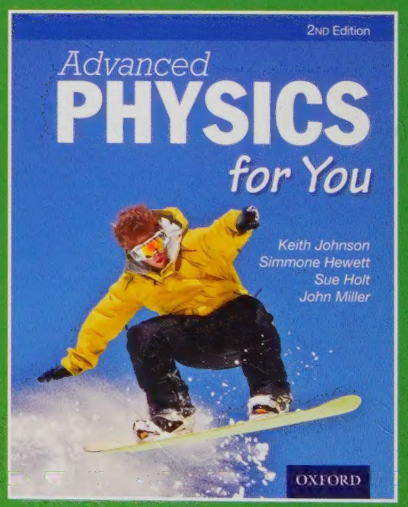

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