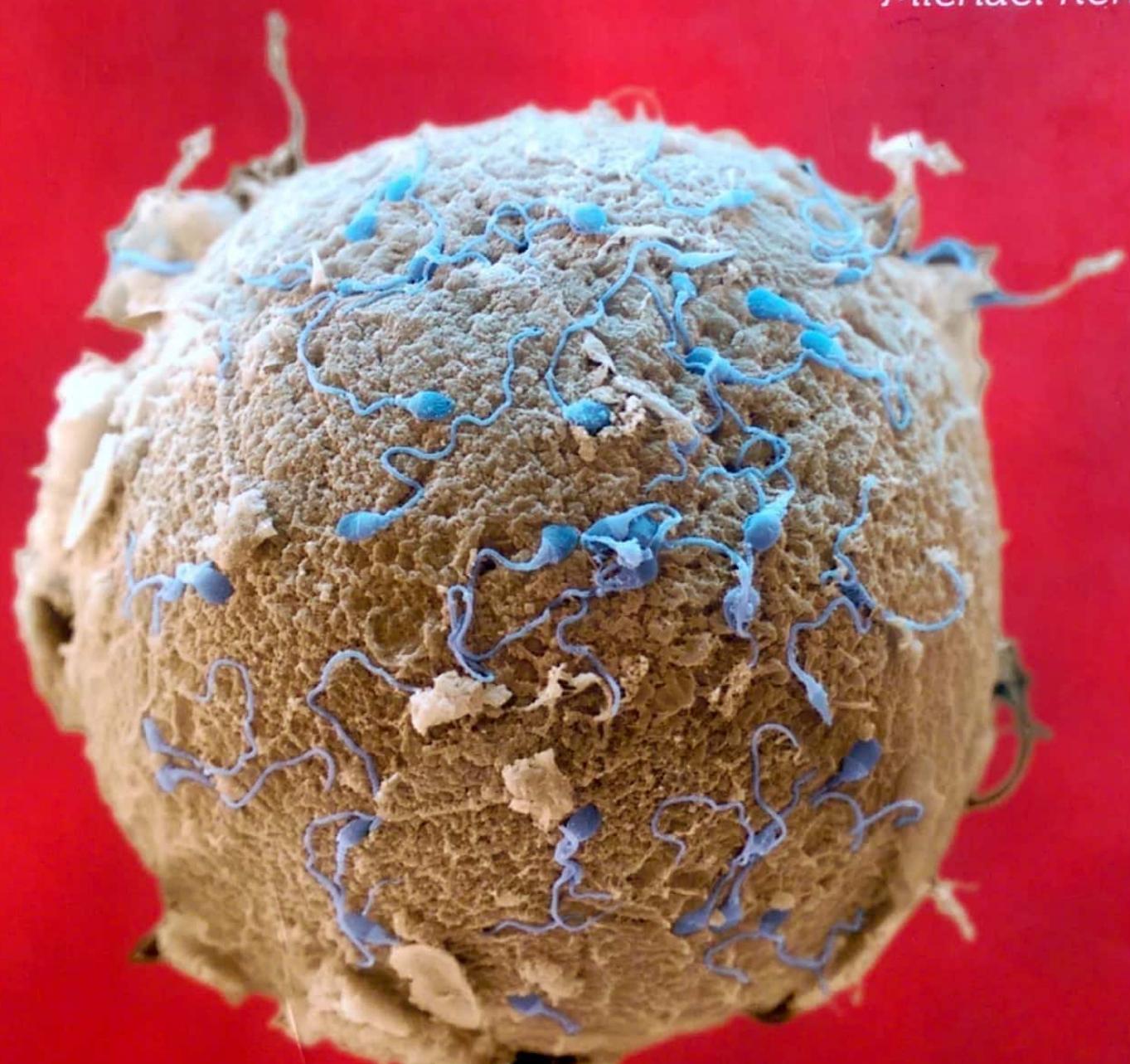


# Advanced **BIOLOGY**

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Compiled by Dante

OXFORD

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The basis of **LIFE**



*We are content to find that living things are made of elements identical with those outside the body. Indeed, we should be astounded and dismayed if anyone claimed that it were not so. Nevertheless, we shall not be satisfied that living things are therefore 'just the same' as non-living ones. We shall not be surprised to find that there is something different and even 'special' about them and particularly about their 'behaviour'.*

J. Z. Young

In this part of the book, we examine the structure of life and some of the fundamental processes that characterise living things. We see that life is organised on many structural levels. Atoms combine to form complex biological molecules. These are arranged into minute structures called organelles which in turn are the components of cells. In multicellular organisms, cells are grouped into tissues and tissues into organs. Each level builds on those below it in such a way that when small components join together to form larger structures, new properties emerge that were not present in the simpler levels of organisation.

*A photomicrograph of a mitochondrion, one of the organelles found in plant and animal cells.*



Biology is the scientific study of life. But what is life? When we see a bird on a rock it may seem obvious that the bird is alive and the rock is not, but what precisely makes the bird alive and the rock not? Throughout history, thinkers in many fields have tried to define life. Although they have failed to provide a universally accepted definition, most scientists agree that all living things share certain basic characteristics:

- Living things are made of organised structures.
- Living things reproduce.
- Living things grow and develop.
- Living things feed.
- Living things respire.
- Living things excrete their waste.
- Living things respond to their surroundings.
- Living things move.
- Living things control their internal conditions.
- Living things are able to evolve.

Non-living systems may show some of the characteristics of living things, but life is the combination of all these characteristics.

### **Organisation**

All things are made of chemicals, but in living things the chemicals are packaged into highly organised structures. The basic structure of life is the cell. Cells themselves contain small organelles that carry out specific functions. A cell may exist on its own or in association with other cells to form tissues and organs. Because of their highly organised structure, living things are known as **organisms**.

### **Reproduction**

**Reproduction** (figure 1) is the ability to produce other individuals of the same species. It may be sexual or asexual. Reproduction involves the replication of DNA. This chemical contains genetic information which determines the characteristics of an organism, including how it will grow and develop.

### **Growth and development**

All organisms must grow and develop to reach the size and level of complexity required to complete their life cycle. **Growth** is a relatively permanent increase in size of an organism. It is brought about by taking in substances from the environment and incorporating them into the internal structure of the organism. Growth may be measured by increases in linear dimensions (length, height, etc.), but is best measured in terms of dry weight as this eliminates temporary changes due to intake of water which are not regarded as growth. **Development** involves a change in the shape and form of an organism as it matures. It is usually accompanied by an increase in complexity.

### **Feeding**

Living things are continually transforming one form of energy into another to stay alive. Although energy is not destroyed during these transformations, heat is always formed. Heat is a form of energy which cannot be used to drive biological processes, so it is sometimes regarded as 'wasted energy'. Living things have to renew their energy

stores periodically from their environment, to continue transforming energy and to replace the 'wasted energy'. They also have to obtain nutrients – chemicals that make up their bodies or help them carry out their biological processes. Living things acquire energy and nutrients by **feeding**, either by eating other organisms, or by making their own food out of simple inorganic chemicals using energy from sunlight or from chemical reactions.

## Respiration

Living things need energy to stay alive and to do work. Although food contains energy, this is not in a directly usable form. It has to be broken down. The energy released during the breakdown is used to make ATP (adenosine triphosphate) in a process called **respiration**. ATP is an energy-rich molecule and is the only fuel that can be used directly to drive metabolic reactions in living organisms.

## Excretion

The energy transformations that take place in an organism involve chemical reactions. Chemical reactions that occur in organisms are called **metabolic reactions**. Waste products are formed in these reactions, some of which are poisonous, so they must be disposed of in some way. The disposal of metabolic waste products is called **excretion**.

## Responsiveness

All living things are sensitive to certain changes in their environments (stimuli) and **respond** in ways that tend to improve their chances of survival. The degree of responsiveness depends on an organism's complexity: a bacterium may be limited to simple responses, such as moving towards favourable stimuli or away from harmful ones; people can make highly sophisticated responses to a wide variety of stimuli which they may perceive either directly or with the aid of technological devices.

## Movement

Responses usually involve some form of **movement**. Movement of whole organisms from one place to another is called **locomotion**. Plants and other organisms that are fixed in one place do not display locomotion, but they can move parts of their bodies. Movements of living things differ from those of non-living things by being active, energy-requiring processes arising from within cells.

## Homeostasis

All living things are, to some extent, able to control their internal conditions so that their cells have a constant chemical and physical environment in which they can function effectively. The regulation and maintenance of a relatively constant set of conditions within an organism is called **homeostasis**. Homeostasis is a feature of all living systems, from a single cell to the whole biosphere (the part of Earth containing life).

## Evolution

Living things are able to change into new forms of life. This **evolution** usually takes place gradually over successive generations in response to changes in the environment.



**Figure 2** Scientists at the Honda Motor Company have been developing a human-shaped robot equipped with artificial intelligence since the early 1990s. In 2008, one model helped conduct the Detroit Symphony Orchestra. In 2011, a new improved model was able to take a drinks order and serve it from a thermos flask.

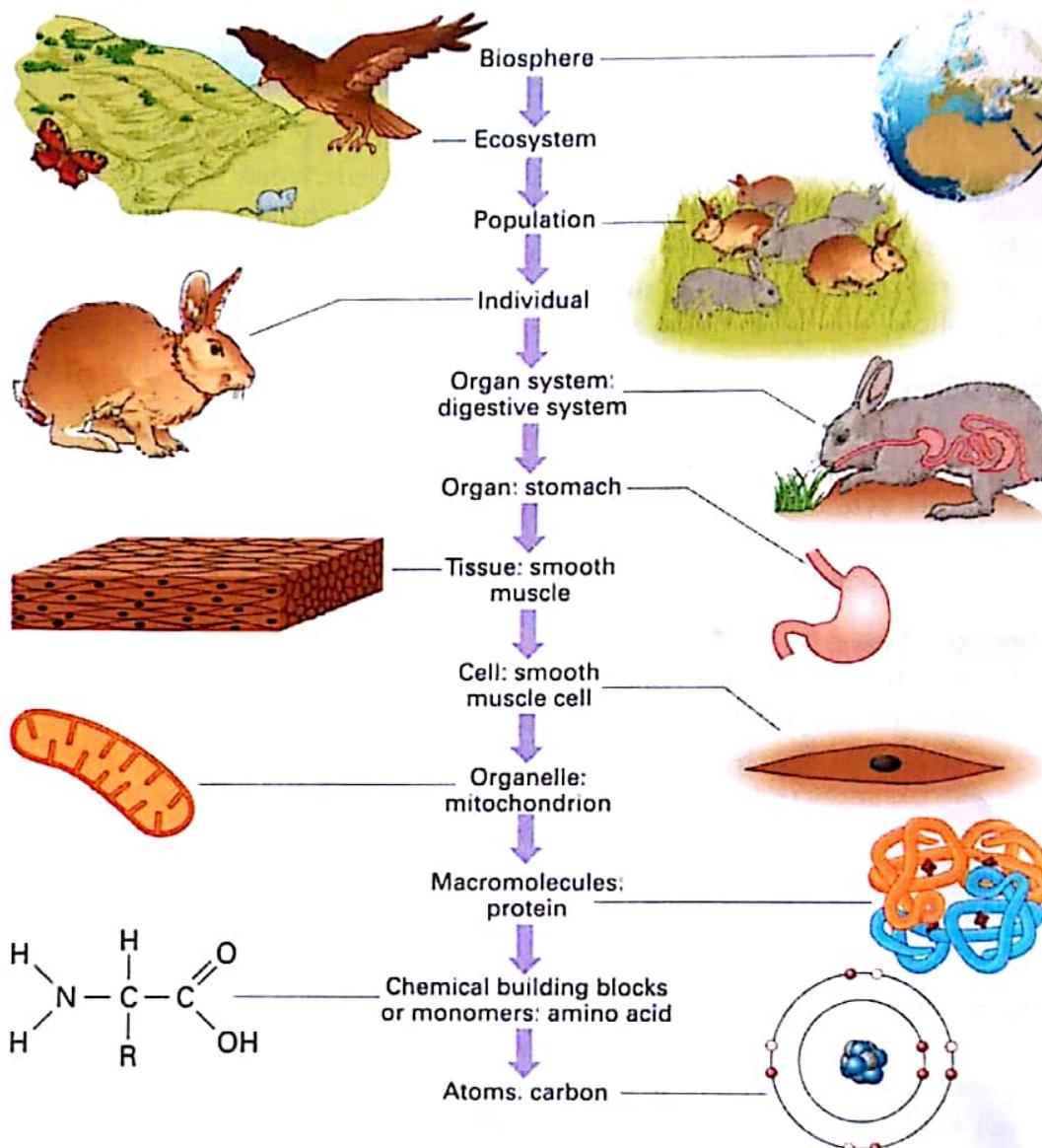
## QUICK CHECK

- 1 What is the difference between:
  - a the growth of a crystal and the growth of a plant
  - b the movement of a cloud and the movement of an animal?

## Food for thought

- 2 You might be familiar with the mnemonic (memory aid) 'Richard Of York Gave Battle In Vain' for remembering the colours of the spectrum (red, orange, yellow, green, blue, indigo, violet). Suggest a mnemonic for the ten characteristic features of living things described in this spread. You can change the order of the features.
- 3 The robot shown in figure 2 can move and respond, and requires energy to maintain its organisation and a constant internal environment. How would you argue that the robot is a non-living object? A robot could be made that has all the characteristic features of living things. Would it still be non-living?

Biologists study every aspect of life at every level of its organisation, from the atoms that make up biological molecules to the ecosystems that form the biosphere (figure 1).



**Figure 1** Levels of biological organisation from atoms, the smallest components of living things, to the biosphere, the entire living planet.

### Aspects of biology

Modern biology is an enormous subject that has many branches.

Specialists in some branches include:

- molecular biologists and biochemists who work at the chemical level, with the aim of revealing how DNA, proteins, and other molecules are involved in biological processes
- geneticists who study genes and their involvement in inheritance and development
- cell biologists who study individual cells or groups of cells, often by culturing them outside organisms; they investigate how cells interact with each other and their environment
- physiologists who find out how organ systems work in a healthy body
- pathologists who study diseased and dysfunctional organs
- ecologists who study interactions between organisms and their environment. Some focus their attention on whole organisms; others study populations, individuals of the same species living together at one location.

There are also biologists who specialise in particular groups of organisms; for example, bacteriologists study bacteria, botanists study

Biologists are employed in many fields including conservation and wildlife management, industry, medicine, health care, horticulture, agriculture, zoos, museums, information science, and marine and freshwater biology. In addition, many biologists are employed as teachers, lecturers, or research workers.

### The scientific method

The definition of biology given in spread 1.1 states that it is a 'scientific study'. This distinguishes biology from other ways of studying life. However, there is no single rigid scientific method that biologists use: there are numerous ways of studying life scientifically. Nevertheless, biological investigations usually include one or more of the following key elements:

- observing: making observations and taking measurements
- questioning: asking questions about observations and posing a problem
- hypothesising: formulating a hypothesis, a statement that explains a problem and can be tested
- predicting: stating what would happen if the hypothesis were true
- testing: testing the hypothesis, usually by carrying out a controlled experiment aimed at producing data that will either support or contradict the hypothesis
- interpreting: interpreting the test results objectively and drawing conclusions that accept, modify, or reject the hypothesis.

A biologist may start an investigation by making observations or by using observations described by other biologists. Such observations may be obtained directly by the senses, such as listening to a bird song, or indirectly through instruments such as recording the song on a computer system. On the other hand, an investigation may start simply by a biologist having an idea (perhaps no more than a hunch) that something happens in a particular way, and then the idea will be tested by making observations or carrying out experiments to see if it is valid. A hypothesis is suggested and then tested in all investigations. One essential aspect of a scientific experiment is that it can be repeated by other scientists working independently.

A typical hypothesis makes a clear link between an **independent** or **manipulated variable** and a **dependent variable**. Variables are conditions or factors (such as light, temperature, or time) that can vary or may be varied. In an experiment, the independent or manipulated variable is the one that is systematically changed; the dependent variable is the effect or outcome that is measured. For example, when investigating the activity of an enzyme at different temperatures, temperature is the independent variable that is manipulated by the scientist; rate of reaction is the dependent variable that is measured at each temperature. Other variables called **controlled variables** are kept constant or controlled at set levels.

At the end of an experiment, the results must be interpreted as objectively as possible. Sometimes they are so clear that it is obvious whether they support or contradict a hypothesis. Often, however, results are variable and need statistical analysis before conclusions can be made. The conclusions may lead to the hypothesis being accepted, modified, or rejected. Even if results support a hypothesis, it is accepted only tentatively because it can never be proved completely. However, it only needs a single contrary observation to **refute** a hypothesis (prove it wrong or incomplete). A hypothesis is therefore only the best available explanation at any time. This makes biology a highly dynamic subject and not merely a collection of facts.

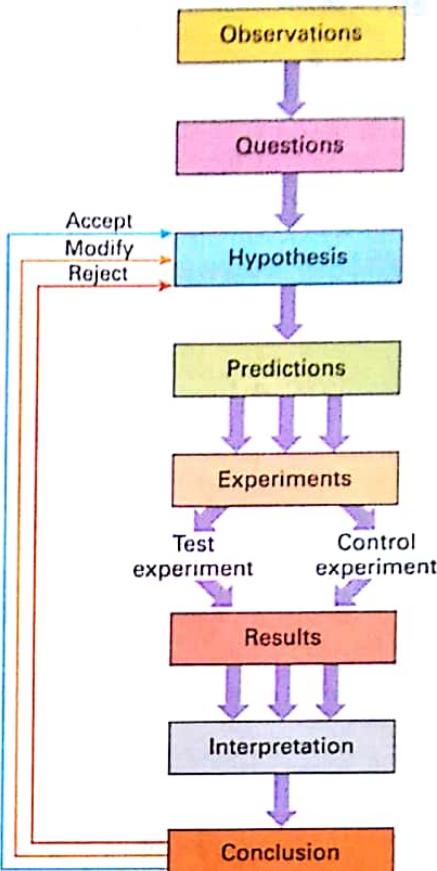


Figure 2 A typical sequence of events in a scientific investigation.

### QUICK CHECK

- 1 What is the difference between a physiologist and a pathologist?
- 2 Which is the highest level of biological organisation on Earth?
- 3 In an experiment in which the rate of photosynthesis of a plant is measured at different light intensities, which is the independent (manipulated) variable and which is the dependent variable?

### Food for thought

The life sciences have made an enormous contribution to human welfare, especially through their applied branches of medicine, agriculture, and biotechnology. However, an important part of understanding biology and the other sciences is realising their limitations. Science does not, for example, deal with hypotheses that are not testable. Suggest questions that might not be possible to answer using a scientific method.

# 2

# The chemicals of life

## ATOMS AND ELEMENTS

### 2.1

#### OBJECTIVES

- By the end of this spread you should be able to:
- understand that all matter is made of elements
  - list the main elements found in living things
  - describe the main components of an atom
  - describe how isotopes can be used in biological investigations.

#### Fact of life

Ninety-nine per cent of all living things is made up of only four elements: hydrogen, oxygen, nitrogen, and carbon

Even organisms as different as a giraffe and the acacia bush that it eats (figure 1) are made up of these elements.

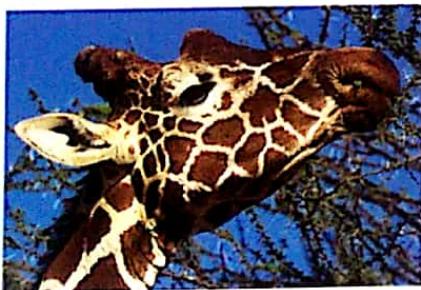


Figure 1 A giraffe and the acacia bush that it eats are made of the same chemical elements.

### The elements in living things

All parts of the Earth are made of 92 naturally occurring substances called **elements**. An element is a substance that cannot be split into simpler substances by chemical means. Living, dead, and non-living things are all made up of elements.

Only about 21 of the 92 naturally occurring elements are important to living organisms. The most common elements in organisms are:

- carbon (C)
- oxygen (O)
- hydrogen (H)
- nitrogen (N).

Other elements occur in living organisms in smaller quantities. These include phosphorus (P), potassium (K), sulphur (S), calcium (Ca), iron (Fe), magnesium (Mg), sodium (Na), and chlorine (Cl).

### Atoms

Each element is made up of **atoms**. A single atom is the smallest amount of any element that can exist. Atoms are far too small to be seen with an ordinary microscope. Many millions of atoms make this comma, and countless billions make each of us. The structures and functions of whole organisms depend on the structures of the atoms they contain.

Atoms can join together with other atoms of the same element, or with atoms of different elements, to form **molecules** (see spread 2.2). Atoms can combine in many different ways to form thousands of different materials. Combinations of different elements are called **compounds**.

### Structure of the atom

The main mass of each atom is in a central **nucleus**. The nucleus is made of even smaller particles called **protons** and **neutrons**. (Hydrogen atoms are the exception. They have one proton in the nucleus but no neutrons.)

**Electrons** are minute particles. They move around the nucleus at very high speed within **electron shells** or **energy levels** (figure 2). Their mass is negligible compared with that of the neutron or proton.

The components of an atom have different electrical properties.

- Neutrons have no electrical charge.
- Electrons have a negative charge (-).
- Protons have a positive charge (+).

An atom with an equal number of electrons and protons has no overall electrical charge.

### Ions

**Ions** are atoms that have gained an electrical charge.

- Some atoms can gain one or more electrons and become negatively charged.
- Others can lose one or more electrons and become positively charged.

### Grouping the elements – the periodic table

The number of protons in the nucleus is different for every element. This number is called the **atomic number**. In a neutral atom the number of protons equals the number of electrons. The periodic table (figure 3)

shows the elements arranged in order of atomic number. Elements with similar properties occur in the same column or **group**.

## Isotopes

The atoms of any particular element always have the same number of protons but the number of neutrons can vary. The atoms may therefore have different masses. These are different versions of the same element, and are called **isotopes**.

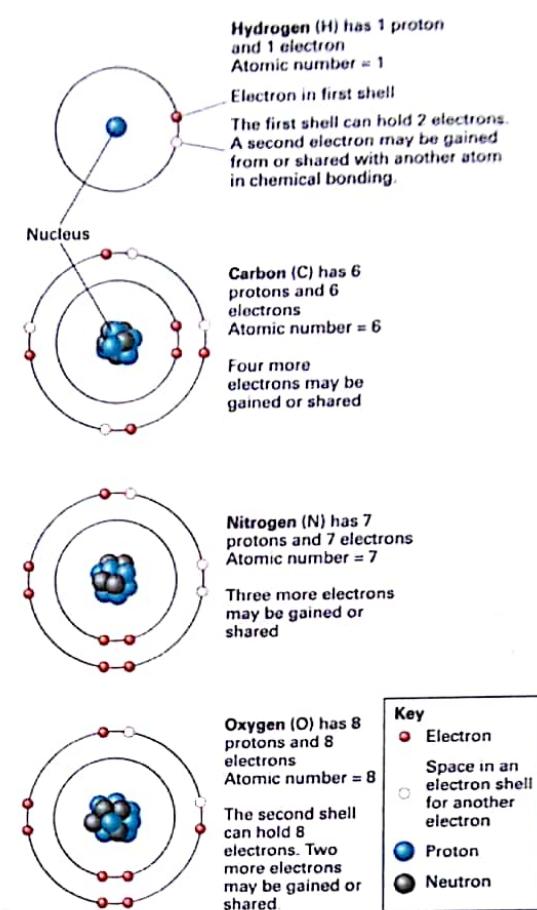
Isotopes are identified by the **mass number** of the atom, which is the number of protons plus the number of neutrons. Different isotopes of the same element have different mass numbers, but they all have the same atomic number. For example, three isotopes of the carbon atom have mass numbers of 12, 13, and 14, but they all have an atomic number of 6. These isotopes are represented as  $^{12}\text{C}$ ,  $^{13}\text{C}$ , and  $^{14}\text{C}$ .

## Radioactive isotopes

A few elements have isotopes that are radioactive, which means that they are unstable. They change spontaneously to form a different isotope of the same element, or a completely different element. Radiation is given out during this change.

## Tracers

Isotopes of an element have the same chemical properties and so will behave in the same way inside an organism. This is useful for investigating the reactions that go on in an organism. A compound that contains a 'labelled' isotope (e.g. one that is radioactive) is used. It takes part in reactions in the organism in the same way as the unlabelled compound. The isotope and products made from it in the organism can be detected by physical measurements. For example, the radioactive isotope  $^{14}\text{C}$  can be used to trace the path by which carbon in carbon dioxide ( $^{14}\text{CO}_2$ ) is converted into glucose ( $^{14}\text{C}_6\text{H}_{12}\text{O}_6$ ) during photosynthesis.



**Figure 2** Highly simplified representations of hydrogen, carbon, nitrogen, and oxygen atoms. In reality, the electron shells are not spherical. Electrons occupy charged clouds of various shapes, and there is no way of telling exactly where the electrons are at any one instant.

| IA                  |                       | IIA           |  |
|---------------------|-----------------------|---------------|--|
| 1<br>H<br>Hydrogen  |                       | 2<br>Li<br>Be |  |
| 3<br>Na<br>Sodium   | 12<br>Mg<br>Magnesium |               |  |
| 4<br>K<br>Potassium | 20<br>Ca<br>Calcium   |               |  |
| 5<br>Rb             | Sr                    |               |  |
| 6<br>Cs             | Ba                    |               |  |
| 7<br>Fr             | Ra                    |               |  |

| Transition metals |          |                     |                        |                       |                  |                    |                    |                    |                  |
|-------------------|----------|---------------------|------------------------|-----------------------|------------------|--------------------|--------------------|--------------------|------------------|
| 21<br>Sc          | 22<br>Ti | 23<br>V<br>Vanadium | 24<br>Cr               | 25<br>Mn<br>Manganese | 26<br>Fe<br>Iron | 27<br>Co<br>Cobalt | 28<br>Ni<br>Nickel | 29<br>Cu<br>Copper | 30<br>Zn<br>Zinc |
| Y                 | Zr       | Nb                  | 42<br>Mo<br>Molybdenum | Tc                    | Ru               | Rh                 | Pd                 | Ag                 | Cd               |
| La                | Hf       | Ta                  | W                      | Re                    | Os               | Ir                 | Pt                 | Au                 | Hg               |
| Ac                | Ku       | Ha                  |                        |                       |                  |                    |                    |                    |                  |

| IIIB             | IVB                    | VB               | VIB                  | VIIIB              | O<br>He        |
|------------------|------------------------|------------------|----------------------|--------------------|----------------|
| 5<br>B<br>Carbon | 6<br>C<br>Nitrogen     | 7<br>N<br>Oxygen | 8<br>O<br>Sulphur    | 9<br>F<br>Chlorine | 10<br>Ne<br>Ar |
| 13<br>Al         | 14<br>Si<br>Phosphorus | 15<br>P<br>S     | 16<br>S<br>Se        | 17<br>Cl<br>Br     | 18<br>Ar<br>Kr |
| 31<br>Ga         | 32<br>Ge               | 33<br>As         | 34<br>Se<br>Selenium | 35<br>Br<br>Iodine | 36<br>Xe       |
| In               | Sn                     | Sb               | Te                   | 53<br>I<br>Xe      |                |
| Tl               | Pb                     | Bi               | Po                   | At                 | Rn             |

Dividing line between metals (left) and non-metals (right)

**Figure 3** In the periodic table, elements are arranged in order of atomic number. Elements in a column or groups have similar chemical properties. The main elements occurring in living organisms are shown in green.

## QUICK CHECK

- What is the smallest possible amount of any element called?
- Name the four most common elements found in living organisms.
- Give the name of the relatively dense, central part of an atom.
- Which component of an atom varies in number to form different isotopes of the same element?

## Food for thought

Suggest how a biologist could test whether or not the radioactive isotope  $^{14}\text{C}$  behaves in the same way as  $^{12}\text{C}$  when inside a plant.

## OBJECTIVES

By the end of this spread you should be able to:

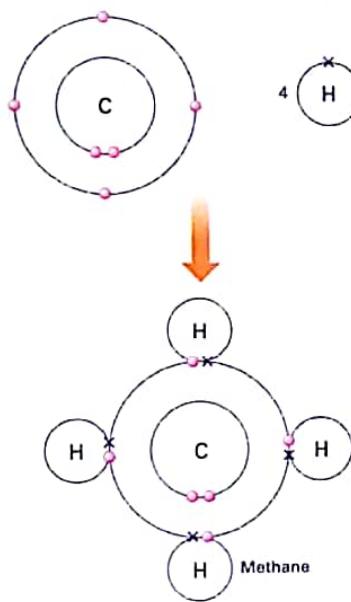
- describe how molecules and compounds are formed
- explain why carbon is uniquely suited to its role as the main element in living organisms.

### Fact of life

In 1828, Friedrich Wöhler was the first person to synthesise a biological material. He synthesised urea from non-biological substances – cyanic acid and ammonia.

This showed that the substances in living organisms are made of the same chemicals as those in non-living things, and that they differ only in the way they are organised.

In methane, the carbon atom shares each of its 4 outer electrons with a hydrogen atom



The carbon atom has now achieved stability: it has a share in 8 electrons in its outer shell. Each hydrogen atom has a share in 2 electrons: it also has a full outer shell.

Figure 1 Covalent bonding in methane.

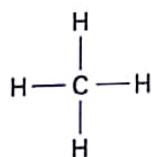


Figure 2 Methane: each single bond between a carbon atom and a hydrogen atom is represented by a single line.

Atoms contain electrons, which orbit the nucleus in shells or energy levels. Isolated atoms are usually unstable because their outer electron shell is not full. They therefore form chemical bonds with other atoms so that their outer shells are filled and they reach a stable state. This can be achieved by two main types of bonding.

- In **covalent bonding** electrons are *shared* between atoms.
- In **ionic bonding** electrons are *transferred* between atoms.

**Compounds** are formed during chemical bonding.

The only elements in which atoms exist separately, without being bonded to other atoms, are the noble gases such as neon. The atoms of these gases have a full outer electron shell.

### Chemical formula

Scientists use a chemical formula to show the composition of a compound. For example,  $\text{H}_2\text{O}$  indicates that a water molecule consists of two hydrogen atoms and one oxygen atom.

### Covalent bonding: making molecules

In a covalent bond, a pair of electrons is *shared* between atoms to form a **molecule**. The atoms may be of the same element, as in the hydrogen molecule ( $\text{H}_2$ ), or of different elements, forming a covalent compound. A molecule is the smallest particle into which a covalent compound can be divided without breaking up the compound.

### Valency

The **valency** of an element tells you how many bonds the atoms need to form to gain a full outer shell of electrons:

- A carbon atom (C) forms 4 covalent bonds.
- An oxygen atom (O) forms 2 covalent bonds.
- A hydrogen atom (H) forms 1 covalent bond.
- A nitrogen atom (N) forms 3 covalent bonds.

For example, in a molecule of methane ( $\text{CH}_4$ ) the carbon atom is linked to four hydrogen atoms by single covalent bonds (figures 1 and 2).

Bonds also act *between* molecules, pulling them together within a solid or a liquid. These bonds are much weaker than the covalent bonds holding the atoms in the molecule together. (See hydrogen bonding, spread 2.3.)

### Ionic bonding: making ions

In an ionic bond, electrons are *transferred* between atoms to form **ions**. Ions have full outer electron shells and are therefore very stable. The valency of an element is the number of electrons that are donated or accepted during ionic bonding.

For example, in sodium chloride ( $\text{NaCl}$ ; table salt):

- the outer electron of a sodium atom is transferred to the outer electron shell of a chlorine atom (figure 3).
- The sodium ion has a positive electric charge (+) because it has lost an electron.
- The chloride ion has a negative electrical charge (-) because it has gained an electron.
- Sodium and chlorine both have a valency of 1.

The opposite charges produce an electrostatic attraction – the ionic bond – which holds the sodium and chloride ions together in solid sodium chloride. However, when dissolved in water, the ions can move about freely, quite independently of each other. In an aqueous solution of sodium chloride, the ions are represented as  $\text{Na}^+(\text{aq})$  and  $\text{Cl}^-(\text{aq})$ .

## Biological molecules

The simplest compounds may be made up of molecules that are quite small and contain just two or three atoms. However, many **biological molecules** are very large: DNA, for example, contains millions of atoms.

Biological molecules contain many carbon atoms combined with atoms of other elements. The study of these carbon-containing compounds is called **organic chemistry**. Carbon-containing compounds are not only obtained from living (organic) sources. Scientists have made many thousands of organic compounds, including pesticides and medicines.

## The special property of carbon

A carbon atom is **tetravalent**, which means that it can form four covalent bonds with other atoms. These can be atoms of other elements, or other carbon atoms. The bonds around a carbon atom point towards the corners of a tetrahedron, which is a very stable arrangement.

Carbon–carbon bonds are very strong. Long chains of carbon atoms can exist in a single molecule, combined with other atoms and branched in many different ways. Hence there is an enormous variety of carbon-containing molecules, some of great complexity (figure 4).

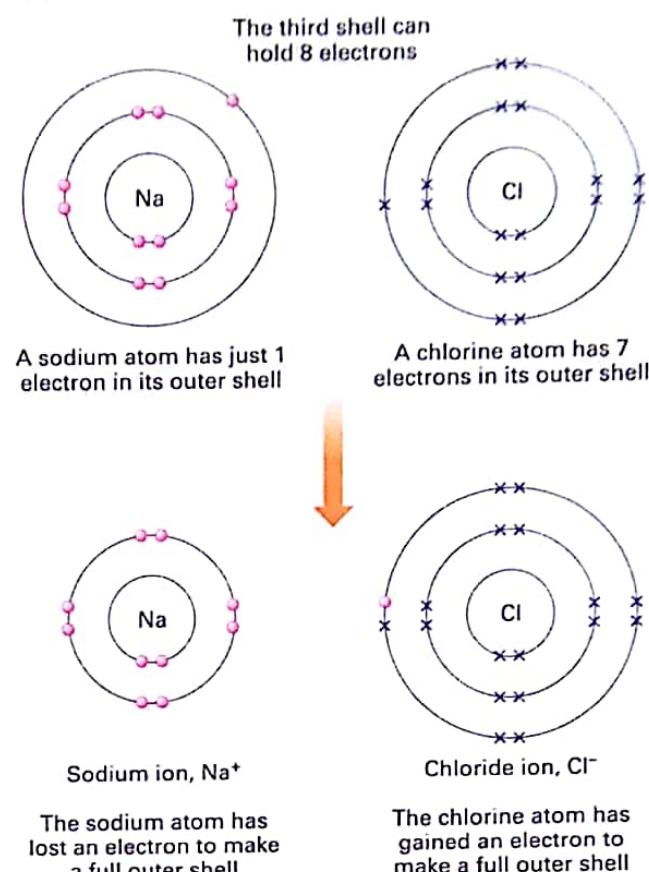


Figure 3 Formation of sodium and chloride ions.

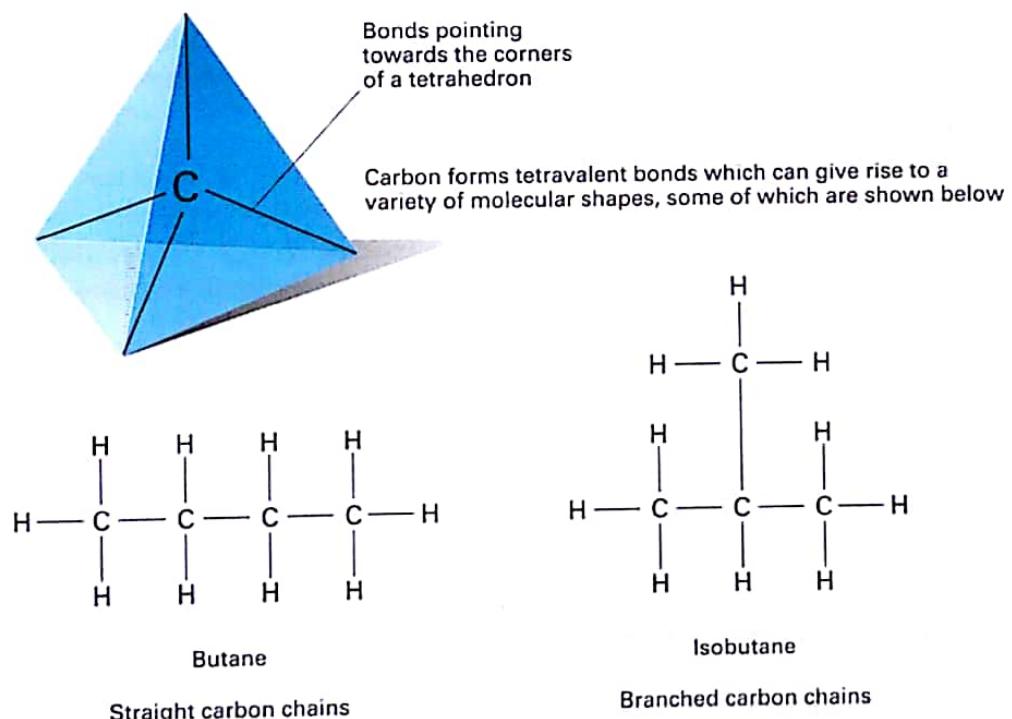


Figure 4 Just a few examples of the variety of molecular shapes based on tetravalent bonding in carbon compounds.

### QUICK CHECK

- 1 Name the type of bond formed:
  - when one atom donates an electron to another atom *ionic bond*
  - when two atoms share electrons. *covalent bond*
- 2 Why is carbon referred to as tetravalent? *it forms four covalent bonds*
- 3 Give three reasons why carbon is uniquely suited to its role as the main element in living organisms.

### Food for thought

Some science fiction writers have suggested that life on other planets could be based on an element other than carbon. Suggest why life on Earth is carbon based.

## OBJECTIVES

By the end of this spread you should be able to:

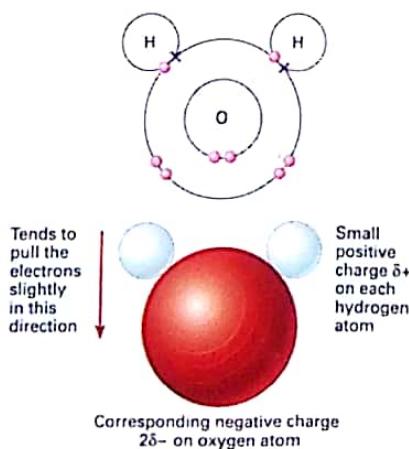
- describe the physical and chemical properties of water.

## Fact of life

In October 2009, NASA scientists smashed a rocket on to the dark side of the Moon creating a debris cloud 2 km high. Spectrometers on a probe that followed the rocket detected significant amounts of water vapour in the cloud. In May 2011, analysis of lunar soil brought back by the Apollo 17 mission revealed 100 times more water than expected, supporting the idea that the Moon once had a sea the size of the Caribbean. Before the 2011 discovery it was thought that all lunar water was brought in by impacts of icy comets or watery meteorites into the Moon. If future explorations find sufficiently large quantities of water, permanent settlements on the Moon might be feasible using the water not only for drinking but also for fuel, using solar energy to convert the water into hydrogen and oxygen.



**Figure 1** An artist's impression of a manned lunar base. Such a base could support a manned mission to Mars.



**Figure 2** The arrangement of atoms in a water molecule. The oxygen atom has a tendency to draw electrons closer to it. This gives the oxygen end of a water molecule a slightly negative charge and the hydrogen end a slightly positive charge. ( $\delta+$  and  $\delta-$  are the conventional ways of representing very small charges.)

## THE UNIQUE PROPERTIES OF WATER

Water is essential for life as we know it. Its unique chemical and physical properties allow it to sustain life. Organisms themselves are mainly water; the human body is about 60 per cent water.

## Water molecules...

A water molecule consists of two hydrogen atoms and one oxygen atom. Each hydrogen atom shares a pair of electrons with the oxygen atom to form two covalent bonds (H—O—H). However, the electrons are not shared equally. Oxygen has a greater pull on the electrons than hydrogen: it has a greater **electronegativity**. This draws electrons away from the hydrogens slightly so that each water molecule has slightly negative and slightly positive regions (figure 2).

Molecules with charged regions are called **polar molecules**; those with two separate charged areas are called **dipolar molecules**. Water is a dipolar molecule.

## ...are 'sticky'

The negative and positive ends of water molecules attract each other so that the molecules tend to stick together (figure 3). These forces of attraction between water molecules are called **hydrogen bonds**. Hydrogen bonding gives water many of its unique properties.

## Water is a liquid at room temperature

Compounds with molecules as small as those of water, for example hydrogen sulphide, are usually gases at room temperature. Water is a liquid at room temperature because of hydrogen bonding. One water molecule can form hydrogen bonds with up to four other water molecules.

## Water is the universal solvent

The dipolar molecules make water an excellent solvent. In fact water is often called the **universal solvent** because so many things dissolve in it.

- Polar and ionic substances have an electrostatic charge, so they are attracted to the charges on water molecules. They dissolve readily in water and are called **hydrophilic** ('water-loving').
- Non-polar substances, such as oil, have no charge on their molecules. They do not dissolve readily in water and are called **hydrophobic** ('water-hating').

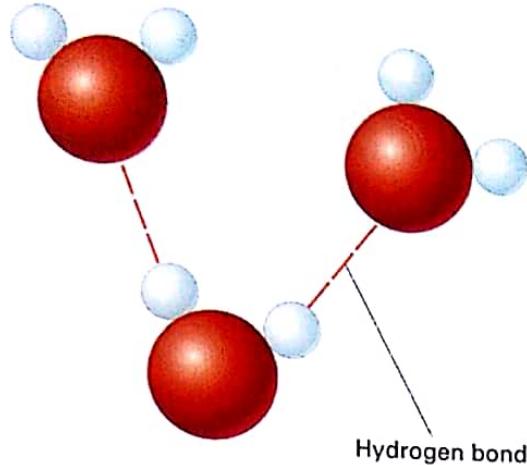
When a salt (an ionic compound) dissolves in water, the ions separate and layers of water molecules form around the ions (figure 4). These layers of water molecules prevent ions or polar molecules from clumping together, and keep the particles in solution.

## Water forms a skin at its surface

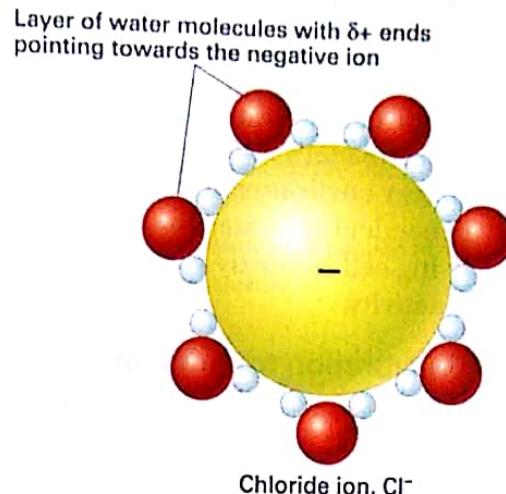
Water molecules have a much stronger attraction to other water molecules than to molecules in the air. At an air–water interface (for example, on the surface of a pond), a water molecule on the surface forms hydrogen bonds with other water molecules around and below it, but not with air molecules above it. The unequal distribution of hydrogen bonds produces a force called **surface tension**. This causes the water surface to contract and form a surprisingly tough film or 'skin'. Surface tension is a measure of how difficult it is to stretch or break a liquid surface; water has a greater surface tension than most other liquids.

## Ice floats on water

Water behaves peculiarly when cooled and is most dense at 4 °C. Consequently, the temperature at the bottom of a large body of water such as an ocean remains relatively constant at 4 °C, despite variations in the temperature of the water at the surface.

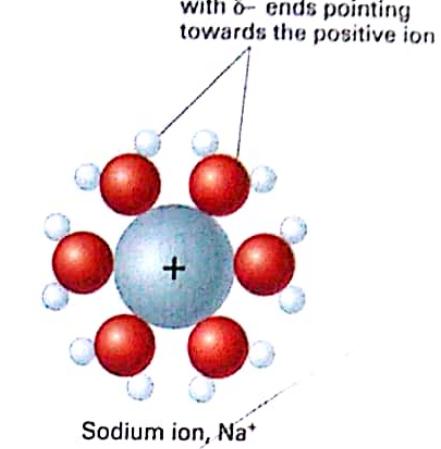


**Figure 3** Water molecules have negative and positive ends so they tend to be pulled together by hydrogen bonding.



**Figure 4** Water is a good solvent because its molecules form a layer around ions or molecules. In a solution of sodium chloride, the positive (hydrogen) ends of water molecules point towards the negative chloride ion, and the negative (oxygen) ends point towards the positive sodium ion.

Layers of water molecules form around many non-ionic organic substances, such as sugars, because they contain polar side-groups.



In liquid water hydrogen bonds are constantly forming and reforming. When water freezes, each molecule forms hydrogen bonds with four other molecules. This makes a rigid lattice, which holds the water molecules further apart than in liquid water. This is why water expands when it freezes. Ice is less dense than liquid water; ice floats on water. Although individual hydrogen bonds are weak, combined in their thousands they make ice as solid as rock.

### Water is wet

Water tends to wet most things (that is, it sticks to them) because its molecules form hydrogen bonds with other polar substances. This attraction between molecules of different substances is called **adhesion**. The attraction between molecules of similar substances is called **cohesion**. Hydrogen bonding gives water considerable cohesive properties. Thus water molecules stick together and also stick to other surfaces, such as the sides of its container. This attraction between molecules enables water to enter and move along very narrow spaces, a process called **capillarity**.

### Water and temperature

An important thermal property of water is its ability to resist temperature changes.

- Water has a high **specific heat capacity**: it needs to gain a lot of heat energy to raise its temperature. Conversely, it needs to lose a lot of heat energy to lower its temperature.
- Water has a high **latent heat of vaporisation**: a lot of heat energy is needed to evaporate it. When water evaporates from a surface, it draws heat energy out of the material underneath, creating a cooling effect.
- Water has a high **latent heat of fusion**: water at  $0^\circ\text{C}$  must lose a lot of heat energy before it forms ice crystals.

### Other properties of water

Other important physical properties of water include:

- It is transparent to sunlight (figure 5).
- It has a relatively high density compared with air.
- It is difficult to compress.
- It conducts electricity. (Pure water has a low conductivity, but dissolved ions make it a good conductor.)



**Figure 5** The transparency of water allows plants to photosynthesise deep in the oceans.

### QUICK CHECK

- What are hydrogen bonds?
- Explain why sugar and salt dissolve readily in water, but oil does not.
- Why is a plant leaf damaged by frost?

### Food for thought

When water freezes, ice forms on the surface. Suggest what would happen if ice were denser than liquid water. What implications would this have for life on Earth?

## OBJECTIVES

By the end of this spread you should be able to:

- explain the biological significance of the chemical and physical properties of water
- give examples of organisms or biological processes to illustrate the significance of these properties.

## Fact of life

Dehydration (loss of water from the body) is a major hazard to most terrestrial organisms. In humans, dehydration can result in overheating, a potentially dangerous lowering of blood pressure, and a slower circulation of blood around the body. These effects can lead to heat stroke and death.

Mild dehydration commonly occurs during exercise if the amount of water lost through sweating is greater than the water taken in by drinking. This mild dehydration can cause poor performances in sport even in temperate climates. A 2 per cent loss of body weight due to water loss can lead to a 20 per cent drop in the working capacity of muscles. Dehydration reduces both the speed and strength of muscle contractions. To minimise the risk, water should be drunk at intervals during exercise and competitive sports.

## THE BIOLOGICAL IMPORTANCE OF WATER

Water covers more than three-quarters of the Earth's surface, and most of the planet's organisms live in water. Living organisms originated in a watery environment, and in the course of evolution they have remained dependent on water for most of their activities. The content of each living cell is mostly water. Without it, cells die or go into a state of deep inactivity.

## Dehydration for survival

Water is essential to life for many reasons (see table 1). Sometimes, however, it is an advantage for living tissue to become dehydrated. Dehydration results in a state of suspended animation, so that small organisms and reproductive structures such as seeds, pollen, and eggs can survive periods of water scarcity. A very low water content also enables these structures to be small and more easily dispersed.

Some organisms dehydrate to avoid being killed during cold conditions. Examples are the tardigrades, short plump animals better known as 'water bears' which inhabit the films of water that surround terrestrial lichens and mosses (figure 1). When the temperature falls below a certain level, their body composition changes from 85 per cent water to about 3 per cent. Waterless tissues cannot freeze, so there is no danger of dehydrated tardigrades being ripped apart by ice.

## Life on the surface

The surface film of a pond, resulting from water's high surface tension, is a firm elastic platform on which a wide range of surface-dwelling organisms can live. These include whirligig beetles, pond skaters, and raft spiders (figure 2) which use the surface of the water to detect vibrations caused by passing prey.

In addition to those functions mentioned in table 1, water has many other roles. For example, it provides the medium through which sperm swim during fertilisation, and it is necessary for the germination of seeds. Water also plays a major role in the regulation of pH (see spread 2.5).



Figure 1 An electron micrograph of a tardigrade ( $\times 100$ ).



Figure 2 Surface dwellers.

**Table 1** The biological significance of the properties of water.

| Property of water   | Significance for living organisms   |
|---|---|
| Water is a liquid at room temperature   | Provides a liquid environment inside cells and aquatic environments for organisms to live in  |
| Water is the universal solvent: it dissolves more substances than any other common solvent    | The chemical reactions inside cells happen in aqueous solution; water is also the main transport medium in organisms  |
| Water forms a skin at its surface: it has a high surface tension                              | Water forms a surface film at an air–water interface; this allows some aquatic organisms such as pond skaters to land on the surface of a pond and move over it |
| Ice floats on water: ice is less dense than liquid water                                      | Ice forms on the surface of a body of water and insulates the water below, allowing aquatic life to survive   |
| Water is wet: adhesion makes it stick to polar surfaces                                       | Along with low viscosity, this property allows capillarity so that, for example, water can move upwards through narrow channels in soil, against gravity        |
| Water has a very high specific heat capacity  | The environment inside organisms resists temperature changes; aquatic environments have relatively stable temperatures  |
| Water has a high latent heat of vaporisation  | Heat is lost from a surface when water evaporates from it; this is used as a cooling mechanism, for example sweating in mammals and transpiration in plants     |
| Water has a high latent heat of fusion  | Cell contents and aquatic habitats are slow to freeze in cold weather   |
| Water is colourless and transparent   | Transmission of sunlight enables aquatic plants to photosynthesise  |
| Water is denser than air  | Water supports organisms as large as whales; it also supports and disperses reproductive structures such as larvae and large fruits such as coconuts            |
| Water is difficult to compress  | Water is an important structural agent, acting as a skeleton (called a hydrostatic skeleton) in worms and turgid plants   |
| Water takes part in many chemical reactions   | Water is a major raw material for photosynthesis; it also takes part in many digestive reactions, breaking down food molecules by hydrolysis                    |
| Water combines with many organic molecules to form hydrated molecules                         | Most organic molecules occur in a hydrated form in cells; if the water is removed, their physical and chemical properties are affected                          |
| Water has a low viscosity (it flows freely: water molecules can slide easily over each other) | Water can flow freely through narrow vessels; watery solutions can act as a lubricant, for example mucus allows food to move easily down the oesophagus         |
| Water has a high tensile strength: water columns do not break or pull apart easily            | Continuous columns of water can be pulled all the way up to the top of a tree in xylem vessels during transpiration   |

### QUICK CHECK

1 a Which property of water makes it suitable for use as a hydrostatic skeleton? *It is difficult to compress*

b Why is water's transparency essential for most of life on Earth? *Because it allows photosynthesis to take place in aquatic plants*

2 Which biological process uses the latent heat of vaporisation of water?

*Sweating and transpiration in plants*

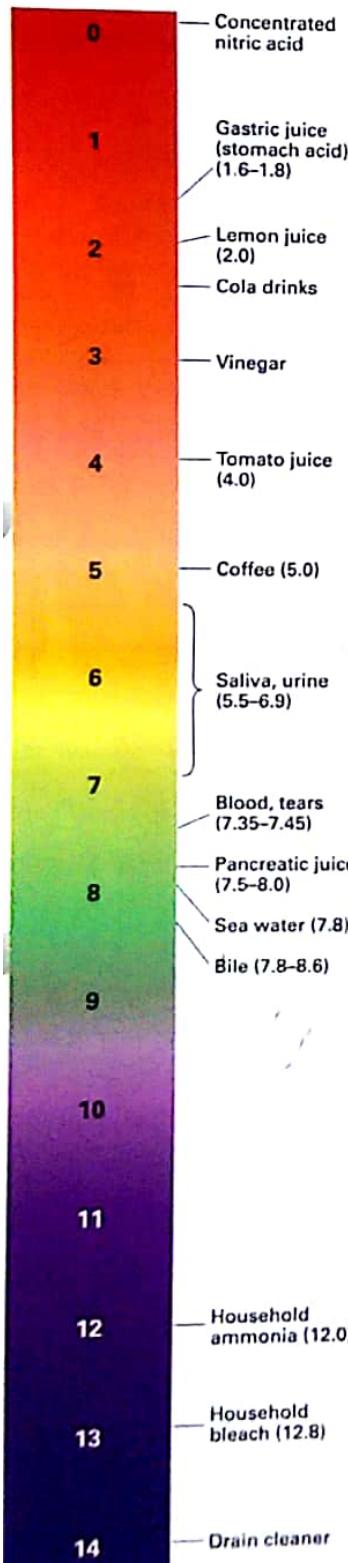
### Food for thought

Suggest what would happen to pond skaters if their pond became polluted with detergent.

of this spread you should be  
he different components of a  
hat the pH scale is  
he causes and effects of acid  
on.

**Fact of life**

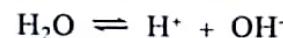
In 1974, a rainstorm in Scotland had a pH of 2.4; it was more acidic than vinegar.

**In solution**

Almost all the processes that are essential to life take place in solution. A **solution** is a homogeneous mixture (that is, a mixture that is uniform throughout) of two or more substances in which the particles are completely dispersed. The substance of which there is more, forming the greater part of the solution, is called the **solvent**. The substance dissolved in the solvent is called the **solute**.

**Acids, bases, and alkalis**

In living organisms, water is the most important solvent. In spread 2.3 we discussed how water molecules are made of hydrogen and oxygen atoms. However, we did not mention that these molecules can **dissociate** or split to form protons and hydroxide ions:



Pure water dissociates only a little, producing an equal number of protons ( $\text{H}^+$ ) and hydroxide ions ( $\text{OH}^-$ ), both at a concentration of  $10^{-7} \text{ mol dm}^{-3}$ . The concentration of protons in a solution is more conveniently expressed in terms of **pH**, the negative logarithm (to the base 10) of the  $\text{H}^+$  concentration in  $\text{mol dm}^{-3}$ . Water has a pH of 7; it is neutral, neither acidic or basic. **Acids** are substances that release free protons in solution. Therefore, acidic solutions have a higher concentration of free protons than pure water and a pH less than 7. The stronger the acidity, the more protons are released and the lower the pH. **Bases** are substances that react with acids to form salts. Bases that dissolve in water are called **alkalis**. These molecules are proton acceptors, removing protons from aqueous solutions and so increasing the pH. Alkaline (or basic) solutions therefore have a higher concentration of hydroxide ions than pure water, and a pH greater than 7.

Because pH is measured on a logarithmic scale, each pH number below 7 is ten times more acidic than the next number (for example, a solution at pH 5 has a concentration of protons ten times higher than a solution at pH 6). The same is true for pH numbers above 7 (for example, a solution at pH 9 has a concentration of hydroxide ions ten times higher than a solution at pH 8). The pH scale (figure 1) extends from 0 (most acidic) to 14 (most alkaline).

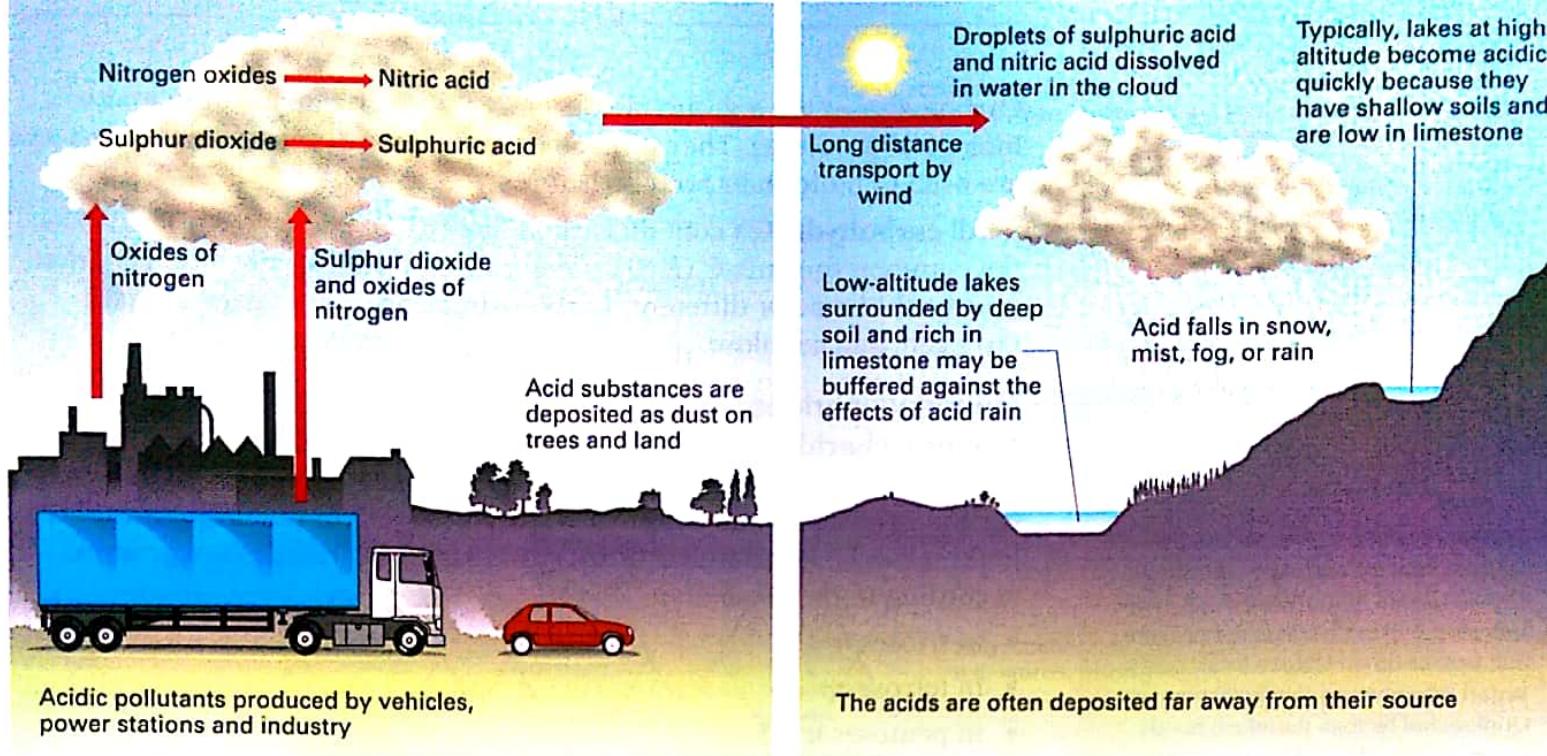
**Acid precipitation**

The bodies of living organisms can tolerate only a limited pH range. Even a slight change in pH can be harmful because molecules in cells are very sensitive to concentrations of protons and hydroxide ions.

Rain and other forms of precipitation contain dissolved gases from the atmosphere, principally carbon dioxide and sulphur dioxide, and so naturally have a pH less than 7. **Acid precipitation** has a pH less than 5.0. The increased acidity is caused mainly by fumes from burning fossil fuels reacting with water in the atmosphere to form acids of sulphur and nitrogen (figure 2).

**Effects on fish, trees, and soil**

In the 1970s, rain with a pH as low as 3 was first noticed in Scandinavia, then in the north-eastern United States and south-eastern Canada, and later in northern Europe and Japan. This mixture of acids was responsible for the depletion of fish stocks in lakes and the destruction of large areas of forest. The acids react with soil minerals, releasing aluminium ions into lakes which interfere with oxygen uptake in the gills and kill the fish. The effect is so dramatic that about 700 of Norway's lakes are now completely devoid of fish. Acid precipitation has its greatest



**Figure 2** Acidic gas emissions cause acid precipitation many miles from the source of the pollution.

effect in spring when ice melts, releasing into the lakes all the pollutants accumulated during the winter. This inflow of acidic water coincides with the breeding period of many fish.

The effect of acid precipitation on trees is complex. Acid rain may affect plants directly, though only if the pH is exceptionally low. Acid mist, which clings to leaf surfaces, and airborne acid particles deposited directly on leaves probably cause more direct harm to trees than acid rain. Heavy deposits of acid cause yew and birch trees to lose their young branches, and conifers to lose their needles and become discoloured.

Acid precipitation has a dramatic effect on soils, which might explain some of the devastation in forests. As the acid precipitation trickles down through the soil, the protons interact with soil minerals. For example, they displace aluminium ions and magnesium ions, which are then dissolved in the soil water. This liberation of toxic aluminium ions can kill plants directly or make them susceptible to pests and diseases, while the dissolved magnesium ions, essential for plant growth, are leached away and lost from the soil.

International efforts are being made to reduce emissions of sulphur dioxide and other gases that contribute to acid precipitation. Unfortunately, the response of many governments has been slow because the financial cost of reducing emissions is high.

### QUICK CHECK

1 Copy and complete the following sentence, using the terms **solvent**, **solute**, and **solution**:

A solute dissolves in a solvent to form a solution.

2 How many more protons are there in a solution at pH 6 compared with an equal volume of a solution at pH 8?

3 Suggest why the term 'acid precipitation' is used in the text rather than 'acid rain'.

### Food for thought

Norway is not a densely populated country, nor is it heavily industrialised. However, the acidification of its rivers and lakes has been rapid. In 1940, rivers and lakes had a pH range of 5.0–6.5; by 1976, they had a pH of 4.6–5.0, with a pH as low as 3.0 after snow melt or during dry spells. Suggest why Norway's lakes became so quickly acidified even though they are not in heavily industrialised regions.

Snow melt occurs in spring. Why does this have such a devastating effect on fish such as trout and salmon?

### Buffers

Common aquatic plants and animals generally flourish in a fairly narrow range of pH. However, most of these organisms possess buffers which allow them to tolerate, at least for a short time, water at a pH of 6–9. A **buffer** is a substance that keeps the pH constant when small amounts of acid or alkali are added. They work to maintain a constant pH within an organism (see spread 2.9 for a fuller account of how buffers work).

## 2.6

### OBJECTIVES

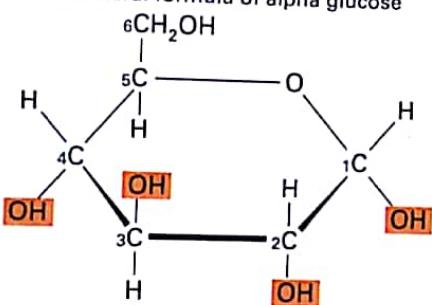
By the end of this spread you should be able to:

- describe the main physical and chemical properties of sugars
- describe the polymerisation of monosaccharides to form polysaccharides
- discuss the functions of sugars.

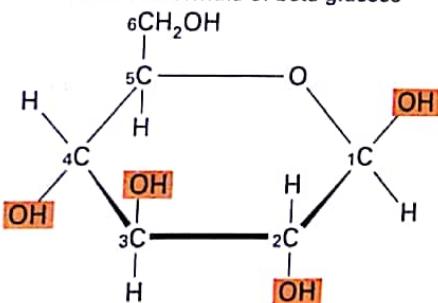
#### Fact of life

Untreated cow's milk is not the perfect food for cats – it can cause diarrhoea and flatulence (wind)! As adults, many cats stop making lactase, the enzyme that breaks down lactose (milk sugar) into glucose and galactose. Undigested lactose therefore builds up in the gut. This encourages the growth of bacteria, which produce the unpleasant gut problems.

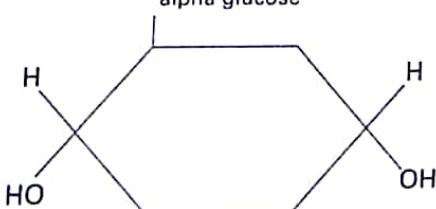
Structural formula of alpha glucose



Structural formula of beta glucose



Simplified structural formula of alpha glucose



**Figure 1** The structure of glucose, C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>. The ring lies at right angles to the plane of the paper. The groups connected by thick lines lie in front of the groups connected with thin lines. The various side groups stick up and down at angles.

## CARBOHYDRATES: SIMPLE SUGARS

Carbohydrates are a group of substances that are important in many biological processes. They provide energy-rich nutrients to organisms and are used to build their body structures.

All carbohydrates contain carbon, hydrogen, and oxygen. They have the common formula C<sub>x</sub>(H<sub>2</sub>O)<sub>y</sub>, where x and y are variable numbers that may be the same or different. Carbohydrates include sugars, starch, glycogen, and cellulose.

### Monosaccharides

Monosaccharides are simple sugars. (The word means 'single sugar'.) They all have the general formula (CH<sub>2</sub>O)<sub>n</sub>, showing that the elements carbon, hydrogen, and oxygen are always present in the same ratio. The letter n can be any number from 3 to 7. Monosaccharides are grouped according to the value of n:

- in trioses n = 3
- in tetroses n = 4
- in pentoses n = 5
- in hexoses n = 6
- in heptoses n = 7.

**Glucose** is the best known and most abundant hexose. Its chemical formula is C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, showing that each molecule contains 6 atoms of carbon, 12 atoms of hydrogen, and 6 atoms of oxygen.

The formula does not show how these atoms are structurally arranged. In fact, glucose can take up a number of different shapes. This phenomenon is called **isomerism**. Each **isomer** has the same chemical formula but a different structural formula.

### Glucose has different isomers

Figure 1 shows two common isomers of glucose: alpha glucose and beta glucose. Notice that the ring structures are very similar – only the positions of the hydrogen and hydroxyl groups on carbon atom 1 are different. Imagine the glucose molecule as a three-dimensional structure. The CH<sub>2</sub>OH group is above the ring in both alpha and beta glucose. The OH group is *below* carbon atom 1 in alpha glucose but *above* carbon atom 1 in beta glucose.

The different arrangements of glucose have important biological consequences: for example, alpha glucose molecules combine to form starch whereas beta glucose molecules combine to form cellulose (see spread 2.7). This illustrates a key concept in biology: the detailed positions of atoms in a biological molecule determine both the shape of the molecule and its function.

Other important hexoses include fructose (fruit sugar) and galactose. Important pentoses are ribose and deoxyribose (constituents of the genetic material, RNA and DNA).

### Disaccharides

Figure 2 shows how two monosaccharides can combine to form a 'double sugar' or **disaccharide**. This is called a **condensation** reaction because it involves the removal of a water molecule.

- **Maltose** (malt sugar) is a disaccharide made up of two glucose molecules.
  - **Sucrose** (table sugar) is made up of a glucose and a fructose.
  - **Lactose** (milk sugar) is made up of a glucose and a galactose.
- Disaccharides can be broken down to their constituent monosaccharides by **hydrolysis**. This is a chemical reaction involving the addition of water.

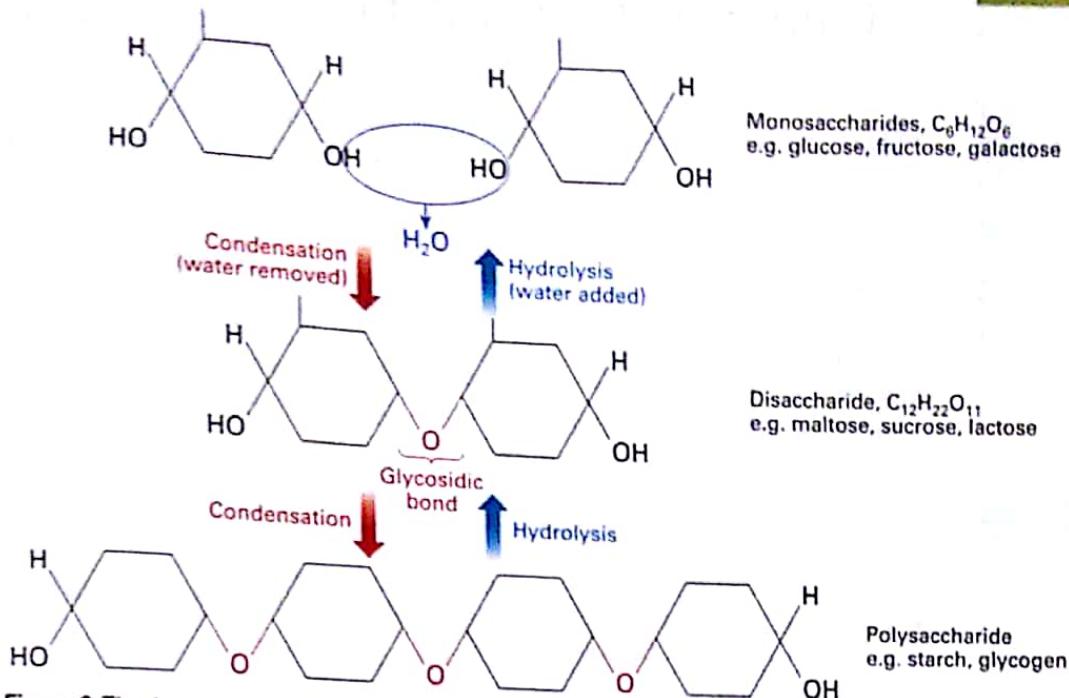


Figure 2 The formation of disaccharides and polysaccharides by condensation (removal of water), and their breakdown to monosaccharides by hydrolysis (addition of water).

## Polysaccharides

Many monosaccharide units can be added to a disaccharide by a series of condensation reactions. A molecule with between three and ten monosaccharide units is called an **oligosaccharide**. More and more units can be added to form a very large molecule called a **polysaccharide**. This process is called **polymerisation**. Large molecules that are made up of repeating units are called **polymers**; each unit is called a **monomer**. Polysaccharides are polymers made up of monosaccharide monomers.

Monosaccharide monomers may also combine with other types of molecule to form **conjugated molecules**. Chains of monosaccharide units can combine with lipids to form glycolipids, or with proteins to form glycoproteins. These molecules are important in cell membranes.

## Functions of simple sugars in living organisms

- Glucose is the major source of energy for most animals. Each gram of glucose yields approximately 16 kJ of energy when fully broken down during respiration. Glucose is also the main form in which carbohydrate is transported around the body in animals.
- Lactose is the main sugar in milk.
- Maltose is produced by the breakdown of amylose in many germinating seeds.
- In plants, carbohydrate is moved from one region to another as sucrose.

### QUICK CHECK

- What is the general formula for monosaccharides?
- Name the bond that links the glucose monomers in a disaccharide.
- In what form is carbohydrate transported in:
  - mammals
  - flowering plants?

### Food for thought

Fructose is the sweetest of natural sugars, one and a half times sweeter than sucrose. Fructose is used as a sugar substitute for diabetics. Suppose a cake recipe specifies 100 g of sugar; how much fructose would you need?

### Sugar in the diet

Monosaccharides, disaccharides, and oligosaccharides all have fewer than ten monosaccharide units (monomers). They are therefore classified as **sugars** – sweet-tasting crystalline substances that are soluble in water.

In the UK, the average person eats more than 1 kg of sugar each week, mostly sucrose. Sugar is a source of energy but has no other nutritional value. (It is sometimes described as 'empty calories'.) **Moderate** consumption of sugar is not harmful. However table sugar has been referred to as 'pure, white, and deadly' because eating very large amounts of it may be associated with obesity, diabetes, and heart disease. These diseases are likely to be linked to general overeating and lack of exercise, as well as eating too much sugar. Nevertheless, people who are obese or who respond adversely to high levels of sugar (including diabetics) should not eat too much sugar.

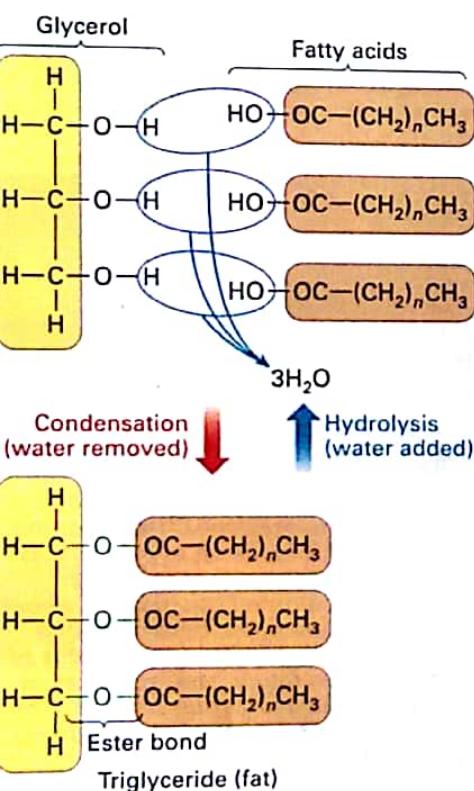
High sugar intake can also lead to tooth decay and to certain fungal infections. For example, candidosis (commonly called thrush) is a disease caused by yeast-like fungi that thrive in sugary environments.

## OBJECTIVES

- By the end of this spread you should be able to:
- describe the structure of fats and oils
  - list the functions of lipids
  - describe the structure of a phospholipid.

### Fact of life

Worldwide, humans use 80 million tonnes of fats and oils every year, most of which are derived from plants. In addition to being used in margarines, soaps, candles, putties, printing inks, and varnishes, plant oils are also used as a fuel for vehicles. Biodiesel, oil derived from plants such as oil palms and sunflowers, is commonly used in tropical areas that do not have their own 'fossil' oils.



**Figure 1** Formation of a triglyceride molecule from glycerol and three fatty acids. Three molecules of water are removed in the condensation reaction.

**Lipids** are a diverse group of compounds which are insoluble in water but dissolve readily in other lipids and in organic solvents such as ethanol, chloromethane, and diethyl ether. Lipids all contain carbon, hydrogen, and oxygen, though the proportion of oxygen is lower than in carbohydrates.

### Fats and oils

**Triglycerides** (called **triacylglycerols** by chemists) are lipids made from glycerol and fatty acids. Triglycerides are also called true fats or neutral fats.

**Glycerol** is an alcohol that contains three carbon atoms each linked to a hydroxyl group. A **fatty acid** has a long chain of hydrogen and carbon atoms (a **hydrocarbon chain**) ending in an acidic carboxyl (—COOH) group. The carboxyl group ionises in water to release a hydrogen ion (proton), which gives fatty acids their acidic properties:



A triglyceride is formed when each hydroxyl group on the glycerol molecule combines with a carboxyl group on a fatty acid molecule, in a reaction called a **condensation** (figure 1). Water is removed and an **ester bond** is formed.

Triglycerides are sometimes referred to under the umbrella term 'fats'. Sometimes they are grouped according to their physical state: those that are liquid at room temperature (20 °C) are called oils; those that are solid are called fats. Each molecule of a fat or oil is made up of glycerol combined with three fatty acids which may all be the same, or may be different.

Fatty acids may be **saturated** or **unsaturated**. In a **saturated fatty acid**, each carbon atom in the hydrocarbon chain is linked to a carbon atom on either side and also to two hydrogen atoms. The carbon atoms are bonded to the maximum number of other atoms. Saturated fatty acids have only single bonds in the hydrocarbon chain (table 1). This makes the chain relatively straight so that triglycerides that contain only saturated fatty acids are able to pack closely together. Thus, triglycerides of saturated fatty acids tend to be solid ('fats') at room temperature.

In an **unsaturated fatty acid**, such as oleic acid (a major constituent of olive oil), the carbon atoms in the hydrocarbon chain are not bonded to the maximum number of other atoms. Two or more carbon atoms have double bonds between them (table 1). Fatty acids with one double bond are called **monounsaturated fatty acids**; those with two or more double bonds are called **polyunsaturated fatty acids**.

The atoms around a double bond may be arranged in either the *cis*-form or the *trans*-form (figure 2). Triglycerides with a high proportion of unsaturated *cis*-fatty acids tend to be oils because the *cis*-double bonds kink the hydrocarbon chain, which prevents the molecules from packing closely together. This means the attractions between the molecules are very weak, so the substance is a liquid rather than a solid. Triglycerides

**Table 1** Structures of some common fatty acids. The skeletal formula shows only the carbon-to-carbon bonds in the hydrocarbon chain and the terminal carboxyl (COOH) group.

| Trivial name | Systematic name                   | Structural formula  | Skeletal formula |
|--------------|-----------------------------------|---|------------------|
| Stearic acid | Octadecanoic acid                 | CH <sub>3</sub> (CH <sub>2</sub> ) <sub>16</sub> COOH                                     |                  |
| Oleic acid   | Octadec- <i>cis</i> -9-enoic acid | CH <sub>3</sub> (CH <sub>2</sub> ) <sub>7</sub> CH=CH(CH <sub>2</sub> ) <sub>9</sub> COOH |                  |

with more unsaturated *trans*-fatty acids tend to be solids because the hydrocarbon chains are straighter, leading to properties similar to saturated fatty acids.

### Functions of fats and oils in living organisms

Triglycerides have a higher proportion of hydrogen than either carbohydrates or protein, making them a more concentrated source of energy: each gram of fat or oil yields about 38 kJ, more than twice the energy yield of a gram of carbohydrate. In mammals, excess fat is laid down for storage in special connective tissue (called **adipose tissue**) under the skin.

As well as being an energy store, fats and oils have other functions, including:

- heat insulation – fat is a bad conductor of heat; mammals tend to increase their adipose tissue in winter to reduce heat loss
- shock absorption – delicate mammalian organs such as the kidneys which are vulnerable to knocks and bumps have relatively thick layers of fat around them
- buoyancy – many single-celled aquatic organisms produce an oil droplet to aid buoyancy.

Dietary fat is also a source of fatty acids (see spread 9.7) and of phospholipids, which have a number of essential functions.

### Phospholipids

**Phospholipids** form a major part of cell membranes (see spread 4.6), including the myelin sheath around nerve fibres that allows the rapid conduction of nerve impulses. Phospholipids consist of glycerol attached to two (not three) fatty acid chains. The third hydroxyl group of glycerol combines with phosphoric acid to form a polar phosphate group.

Schematic diagrams such as figure 3 show a phospholipid as a tadpole-like structure with the phosphate group forming the head and the fatty acid chains a double tail. The polar head is strongly attracted to water: it is hydrophilic ('water-loving'). The non-polar tail, being made up of hydrocarbon chains, is oily and therefore repelled by water: it is hydrophobic ('water-hating'). The phospholipid molecule is said to be **amphipathic**: that is, one end is hydrophilic and the other end is hydrophobic.

In water, phospholipid molecules collect together in a single (monomolecular) layer with the hydrophilic heads poking into the water. In cells, both the intracellular environment and immediate external environment are watery. This causes phospholipids to form a double layer, with the hydrophobic tails pointing inwards, away from the watery environment. The phospholipid bilayer gives cell membranes their fluid properties and allows lipid-soluble substances to pass easily through them (see spread 4.6).

### Waxes, cholesterol, and steroids

Apart from triglycerides and phospholipids, other lipids include waxes, cholesterol, and steroids. **Waxes** are similar to triglycerides, but contain fatty acids bonded to long-chain alcohols rather than to glycerol. Waxes are usually relatively hard solids at 20 °C, providing protection and waterproofing on the surfaces of insects and leaves.

**Steroids** contain four rings of carbon and hydrogen atoms with various side chains. Many animal hormones are steroids, including oestrogen and testosterone (see spread 12.4), which are made from cholesterol, a lipid which shares the same four-ring structure.

**Cholesterol** is also a raw material for the manufacture of vitamin D. It is a vital component of mammalian cell membranes, strengthening the membranes at high body temperatures.

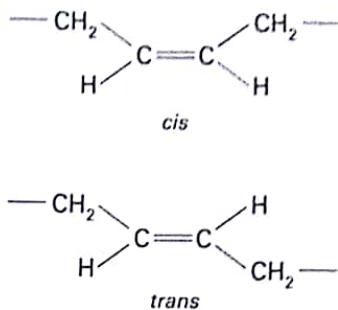


Figure 2 The arrangement around the double bond in a cis-fatty acid and a trans-fatty acid.

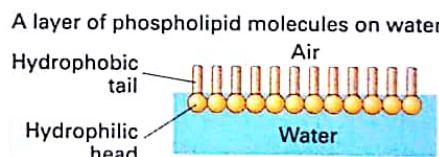
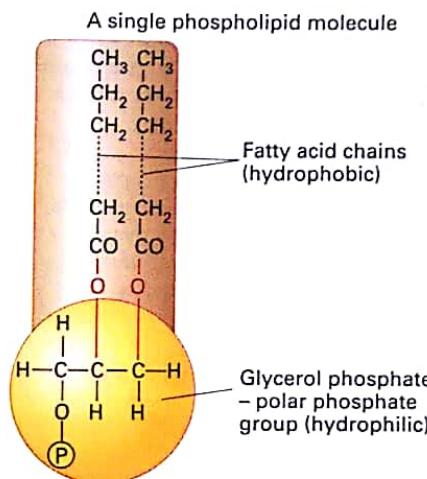


Figure 3 Structure of a phospholipid.

### QUICK CHECK

- Name the products of digestion of true fats.
- Why is fat such a rich store of energy?
- State two ways in which a phospholipid differs from a triglyceride.

### Food for thought

Solid margarines are produced by treating plant oils with a stream of hydrogen in the presence of a metal catalyst such as platinum. Suggest how this process changes the plant oils.

What are the health implications of eating solid margarines rather than plant oils?

## OBJECTIVES

- By the end of this spread you should be able to:
- list the main functions of proteins
  - describe the structure of an amino acid
  - show how amino acids polymerise
  - explain how amino acids can act as buffers.

### Fact of life

Simple organisms such as bacteria have about 1000 different types of protein molecule in their cells; humans probably have between 50 000 and 100 000. The largest known human protein is dystrophin. It has a relative molecular mass of 350 000 and contains about 3500 amino acids. Some people cannot make dystrophin in their cells, and this leads to muscular dystrophy, an inherited disorder characterised by muscle weakness and wasting.

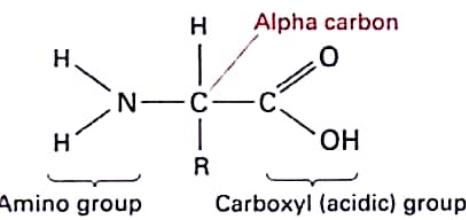


Figure 1 General structure of an amino acid.

Table 1 The amino acids that make up proteins.

Proteins are large complex biological molecules which play many diverse roles in all organisms. Proteins make up a high percentage of the structure of living things (for example, about 18 per cent of the human body is protein) and also take part in the fundamental processes that make up life. Every organism contains thousands of different kinds of proteins, each with its own unique three-dimensional structure which enables it to carry out a specific function.

### Types of protein

Proteins can be grouped into seven major classes based on their functions:

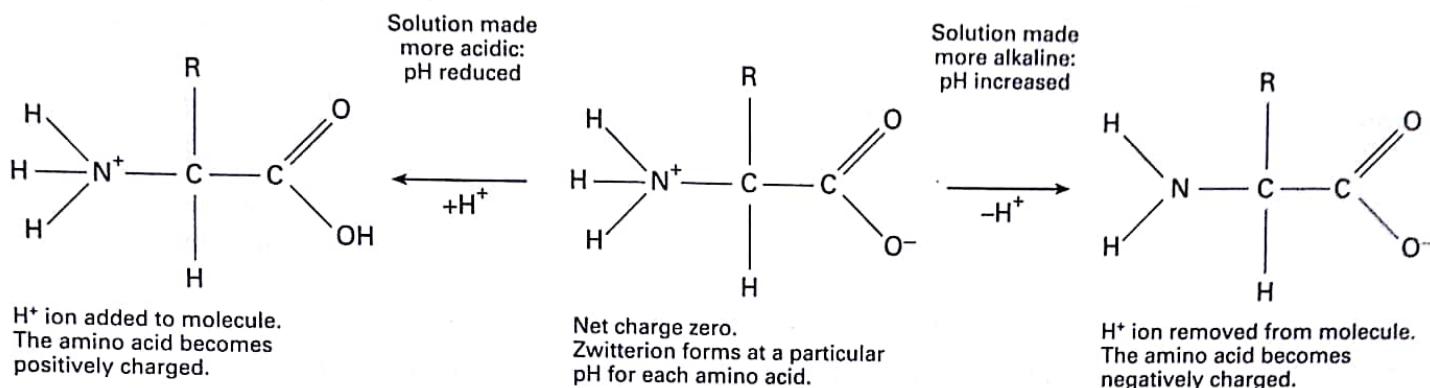
- enzymes**, biological catalysts that control biochemical reactions, for example amylase, which catalyses the digestion of starch, and ATPase, an enzyme found in all cells which catalyses the breakdown and formation of ATP
- structural proteins** that form part of the body of an organism, for example the silk of spiders, collagen that makes up tendons and ligaments, and keratin, the major component of hair
- signal proteins** that carry messages around the body, for example insulin, a hormone involved in controlling glucose levels in the blood
- contractile proteins** involved in movement, for example actin and myosin, the proteins that enable muscles to contract
- storage proteins**, for example albumen, the protein store that forms the white of eggs
- defensive proteins**, for example blood antibodies that fight infections
- transport proteins**, for example haemoglobin, the carrier of oxygen in the blood.

Although there are many millions of proteins, all are made from the same basic building blocks, namely approximately 20 kinds of **amino acid**.

### Amino acids

All amino acids have an **amino group** ( $-\text{NH}_2$ ) and a **carboxyl group** ( $-\text{COOH}$ ), as shown in their general structure (figure 1).

| Amino acid | Abbreviations | R group (side chain) | Amino acid   | Abbreviations | R group (side chain) |   |   |
|------------|---------------|----------------------|--|---------------|----------------------|---|---|
| Glycine    | Gly           | G                    | H  | Aspartic acid | Asp                  | D | CH <sub>2</sub> COOH                              |
| Alanine    | Ala           | A                    | CH <sub>3</sub>                                      | Asparagine    | Asn                  | N | CH <sub>2</sub> CONH <sub>2</sub>                 |
| Valine     | Val           | V                    | CH(CH <sub>3</sub> ) <sub>2</sub>                    | Glutamic acid | Glu                  | E | (CH <sub>2</sub> ) <sub>2</sub> COOH              |
| Leucine    | Leu           | L                    | CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>    | Glutamine     | Gln                  | Q | (CH <sub>2</sub> ) <sub>2</sub> CONH <sub>2</sub> |
| Isoleucine | Ile           | I                    | CH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>  | Proline       | Pro                  | P |   |
| Serine     | Ser           | S                    | CH <sub>2</sub> OH                                   | Tryptophan    | Trp                  | W |   |
| Threonine  | Thr           | T                    | CH(OH)CH <sub>3</sub>                                | Phenylalanine | Phe                  | F |   |
| Lysine     | Lys           | K                    | (CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>      | Tyrosine      | Tyr                  | Y |   |
| Arginine   | Arg           | R                    | (CH <sub>2</sub> ) <sub>3</sub> NHCNHNH <sub>2</sub> | Methionine    | Met                  | M | (CH <sub>2</sub> ) <sub>2</sub> SCH <sub>3</sub>  |
| Histidine  | His           | H                    |  | Cysteine      | Cys                  | C | CH <sub>2</sub> SH                                |



**Figure 2** The amphoteric nature of an amino acid.

The amino group is attached by a covalent bond to a central carbon atom called the alpha carbon. A hydrogen atom, another carbon atom, and a side chain represented by the letter R are also linked to the alpha carbon. The R group is different for each of the 20 amino acids (table 1) and it determines the specific properties of a given amino acid.

In the simplest amino acid, **glycine**, the R group is a single hydrogen atom. Several amino acids contain R groups with relatively complex ring structures. Some R groups are non-polar and hydrophobic (such as that of leucine), while others are polar and hydrophilic (such as those of cysteine and serine). Proteins containing amino acids with hydrophilic R groups can dissolve more readily in solutions within cells.

### Amino acids as buffers

Amino acids are **amphoteric**; that is, they have both acidic and basic properties when they dissociate in water (figure 2). The acidic properties are derived from the carboxyl group which can donate a proton so the molecule becomes negatively charged when in an alkaline solution. The basic properties are derived from the amino group which can take up a proton so the molecule becomes positively charged in an acidic solution. At a pH that is quite specific for each amino acid, the molecule ionises so that different parts of the molecule have positively and negatively charged groups simultaneously. Ions like this with both positive and negative charges are called **zwitterions**.

The ability to donate or receive protons causes amino acid solutions to behave as **buffers**. A buffer solution tends to resist changes in pH when an acid or base is added to it. Buffer systems play an essential role in the human body, keeping the pH of the blood and of other fluids within tolerable levels.

### Peptide bonds: joining amino acids together

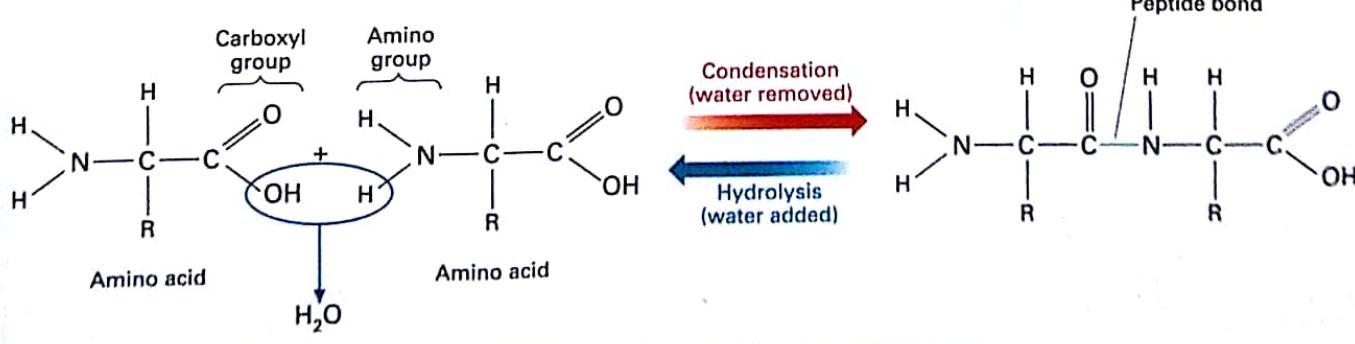
Two amino acids can combine to form a **dipeptide** by a condensation reaction between the carboxyl group of one and the amino group of the other (figure 3). The resulting bond linking the two amino acids is called a **peptide bond**. Further amino acids can be added to either end of the dipeptide to form a **polypeptide chain**. Proteins consist of one or more polypeptide chains: they are polymers made up of amino acid monomers.

### QUICK CHECK

- What is the function of:
  - haemoglobin
  - amylase
  - insulin?
- Name the four elements found in all amino acids.
- Draw a peptide bond.
- What is a zwitterion?

### Food for thought

Over 170 amino acids are known to occur in cells and tissues. Of these, only 20 are commonly found in proteins. Suggest one function that all amino acids and proteins share.



Condensation reaction resulting in a peptide bond.

## OBJECTIVES

By the end of this spread you should be able to:

- describe the four levels of protein structure
- explain the significance of a protein's shape
- classify proteins according to their structure.

### Fact of life

Every organism has its own particular complement of proteins. This is called its **proteome**. Different proteomes have evolved to function in different environments. The proteomes of thermophilic bacteria living in hot volcanic springs, for example, have evolved to function at temperatures in excess of 80°C, while the proteome of ice fish living in Antarctic waters have evolved to function at sub-zero temperatures. The ability of a protein to carry out its specific function in a particular environment depends on its structure. The study of the structure and function of the complete complement of proteins of an organism is called **proteomics**.

Proteins consist of one or more chains of amino acids (polypeptide chains) folded into a unique three-dimensional shape. The shape is determined by up to four levels of structure: the primary structure, the secondary structure, the tertiary structure, and the quaternary structure. Each level of structure determines the next. **Simple proteins** consist of amino acids only; **conjugated proteins** also contain a non-amino acid part called a **prosthetic group**.

### Primary structure: the sequence of amino acids

A protein's **primary structure** is the sequence of amino acids that make up its polypeptide chain or chains. Figure 1 shows the primary structure of myoglobin, an oxygen-carrying conjugated protein consisting of one polypeptide chain of 153 amino acids, and an iron-containing prosthetic group attached to the polypeptide chain.

1 GLSDGEWQLVLNVWGKVEADIPHGOEVILRLFKGHPETLEKFDRFKHLK  
51 SEDEMKASEDLKKHGATVLTALGGILKKKGHEAEIKPLAQSHATKHKIP  
101 VKYLEFISEAIIQVLQSKHPGDFGADAQGAMNKALELFRKDMASNYKELG  
151 FQG

**Figure 1** The primary structure of myoglobin: the sequence of 153 amino acids in the myoglobin polypeptide chain. The letters refer to the single-letter abbreviations in table 1 on spread 2.9.

In order for myoglobin or any other protein to carry out its specific function, it must contain the correct amino acids arranged in a precise order. Its ability to function may be severely disrupted if only one amino acid is out of place. For example, a particular change in a single amino acid in haemoglobin, the main oxygen-carrying blood protein, causes sickle-cell anaemia, a serious blood disorder.

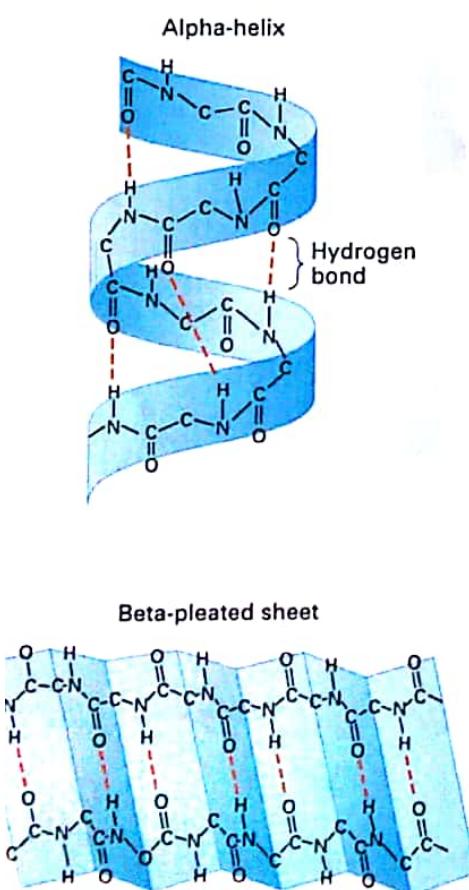
### Secondary structure: folding and coiling

When amino acids join up in the polypeptide chain, a variety of forces between different parts of the molecule and hydrogen bonding (see spread 2.3) cause the chain or regions of the chain either to coil into an **alpha-helix** or to fold into a **beta-pleated sheet** (figure 2). This coiling or folding is the protein's **secondary structure**. The shape of the helix or sheet is maintained by regularly spaced hydrogen bonds each formed between the —N—H group of one amino acid and the —C=O group of another amino acid in a different part of the polypeptide chain. A single polypeptide chain may have some regions coiled into an alpha-helix and others folded into beta-pleated sheets.

### Tertiary structure: the 3D shape

The **tertiary structure** refers to the overall three-dimensional shape of a polypeptide chain (figure 3). Proteins are classified into two main groups on the basis of their tertiary structure:

- **Fibrous proteins** consist of parallel polypeptide chains cross-linked at intervals to form long fibres or sheets. Fibrous proteins are usually insoluble in water and physically tough, which suits them for their mainly structural functions. Fibrous proteins include collagen (a major constituent of tendons and bone), silk (which forms the threads of a spider's web), and keratin (the main component of hair).
- In **globular proteins**, the polypeptide chains are tightly folded to form a spherical shape. Many globular proteins are folded so that their hydrophobic groups are on the inside of the molecule and the hydrophilic groups face outwards, making these proteins soluble in water. Globular proteins include enzymes, antibodies, and many hormones.



**Figure 2** An alpha-helix and a beta-pleated sheet. Both secondary structures depend on hydrogen bonding of the peptide bond parts of the molecule at different areas along the polypeptide chain.

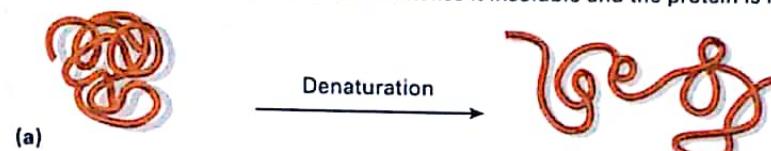
The precise three-dimensional shape of a globular protein molecule determines its function: every coil and twist, bump and indentation is important. The shape is maintained by various bonds including ionic bonds (spread 2.2), hydrogen bonds (spread 2.3), disulphide bonds (figure 4), and **hydrophobic interactions** (interactions between non-polar, water-repellent groups in the protein). The tertiary structure of a protein depends on its primary structure, as the bonds holding the tertiary structure together can only form if the correct amino acids are at specific points along a polypeptide chain.

### Breaking down the tertiary structure: denaturation

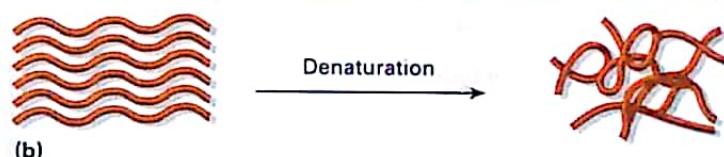
If the bonds holding the protein in shape are broken, a process called **denaturation** occurs. The primary structure is retained but the polypeptide chains unravel and lose their specific shape (figure 5). As a result, denatured globular proteins lose their specific function.

Denaturation is nearly always irreversible. It can be caused by changes in pH, salt concentration, or temperature. The effects of denaturation are dramatically demonstrated every time an egg is boiled or fried: the heat quickly causes the transparent protein-containing area around the yolk to irreversibly solidify and become white and opaque.

If the protein is soluble, denaturation makes it insoluble and the protein is inactivated



If the protein is an insoluble fibrous protein, it loses its structural strength



**Figure 5** Denaturation of: (a) a globular protein and (b) a fibrous protein.

### Quaternary structure: the association of polypeptide chains

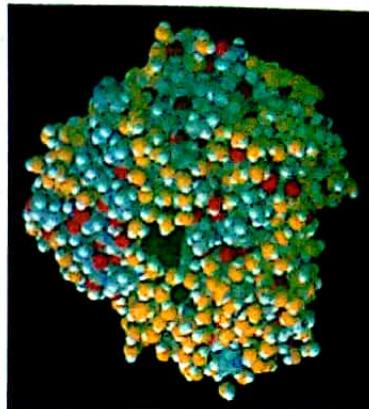
Many proteins consist of more than one polypeptide chain chemically bonded to each other. The **quaternary structure** refers to the way these polypeptide chains are arranged in the protein. Haemoglobin, for example, has four polypeptide chains of two distinct types and four non-protein haem groups (figure 6), whereas **collagen**, a fibrous protein, consists of three helical polypeptides that are supercoiled to form a ropelike structure of great strength.

#### QUICK CHECK

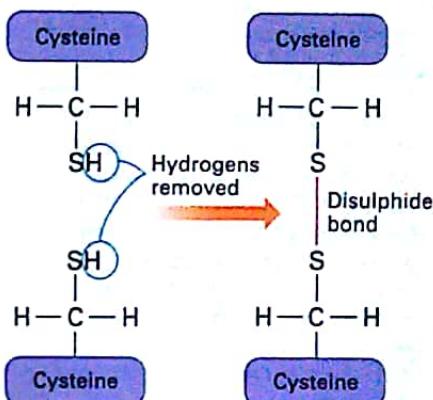
- With reference to haemoglobin, distinguish between tertiary and quaternary structure.
- What happens when a protein is exposed to excessive heat or extremes of pH?
- Which class of protein, fibrous or globular, has mainly structural functions?

#### Food for thought

In humans, fats and carbohydrates are the main sources of energy. One gram of protein yields about the same amount of energy as one gram of carbohydrate. Suggest why protein normally contributes no more than 10 per cent of energy requirements, and why it has been called the 'fuel of last resort'.

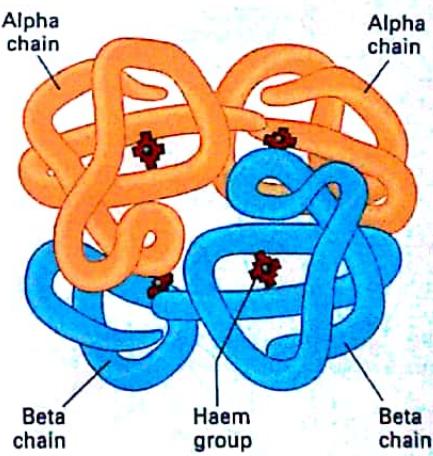


**Figure 3** The tertiary structure of a myoglobin molecule, represented as a space-filling model which shows the molecule's surface shape.

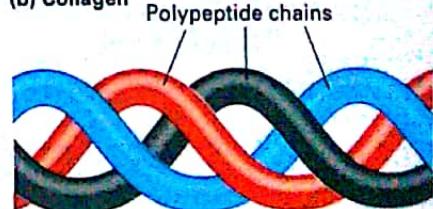


**Figure 4** A disulphide bond forms between two cysteine amino acids. It is one of the strongest and most important bonds that maintain the shape of protein molecules.

#### (a) Haemoglobin



#### (b) Collagen



**Figure 6** The quaternary structure of (a) haemoglobin and (b) collagen. Haemoglobin has two alpha and two beta polypeptide chains, each about 140 amino acids long. The quaternary structure of collagen consists of a triple helix of three polypeptide chains wrapped around each other in a rope-like manner.

## NUCLEOTIDES AND NUCLEIC ACIDS

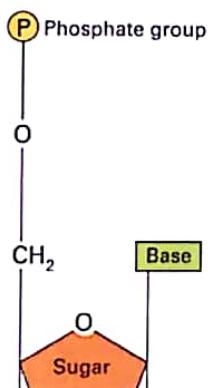
### OBJECTIVES

By the end of this spread you should be able to:

- describe the main components of a nucleotide
- explain how nucleotides can combine to form a polynucleotide chain
- discuss the structure and function of ATP
- outline the structure of DNA.

### Fact of life

The breakdown of ATP releases energy used to do work. A contracting muscle cell requires about two million ATP molecules per second to drive its biochemical machinery. A runner uses the equivalent of about 75 kg of ATP during a marathon race. However, the human body has only a small store of ATP (approximately 100 g in an average person). This store would be exhausted after only one second of the race if the runner could not continuously regenerate ATP from ADP and inorganic phosphate by respiration of metabolic fuels, mainly carbohydrate and fat.



**Figure 1** The basic structure of a nucleotide.

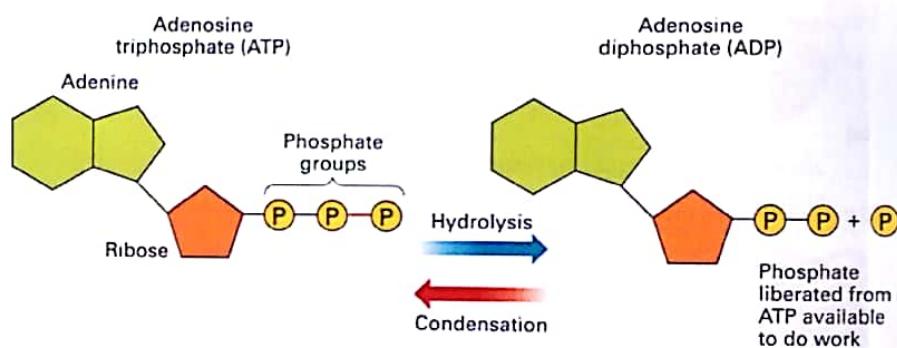
**Nucleotides** are nitrogen-containing organic substances which play a vital role in every aspect of an organism's life. Nucleotide molecules occur singly (**mononucleotides**) or combined in numbers from two to many thousands (**polynucleotides**). Each nucleotide is made of three parts (figure 1):

- a nitrogen-containing organic **base**
- a five-carbon sugar (a **pentose**)
- one or more **phosphate groups**.

### ATP: energy to drive reactions

**Adenosine triphosphate** or ATP is a mononucleotide. The base is **adenine** and the sugar is **ribose**. Attached to the ribose are three phosphate groups (figure 2). The covalent bond linking the second and third phosphate groups is unstable, and is easily broken by hydrolysis.

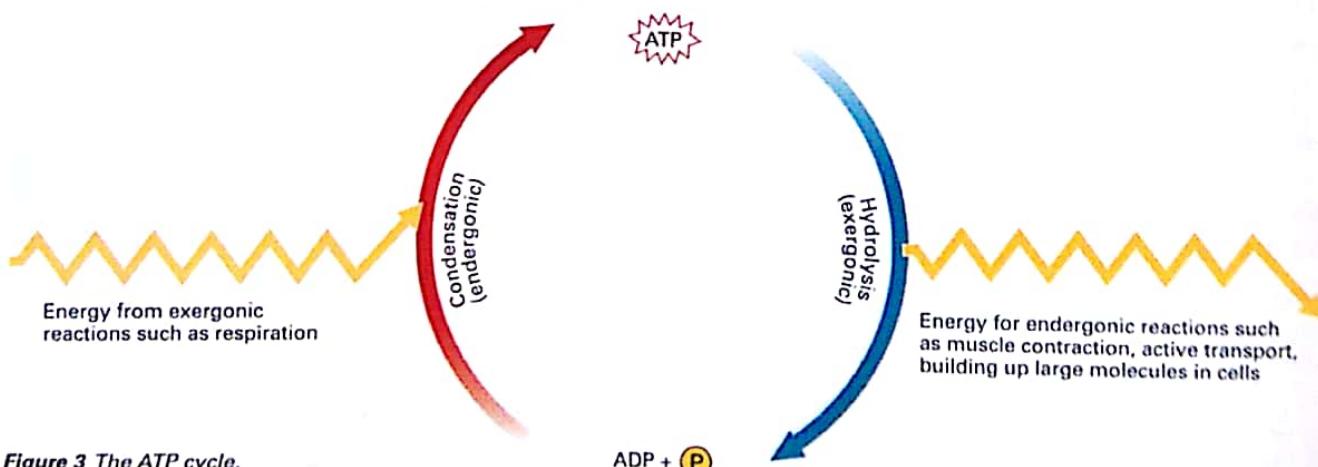
When this bond is broken a phosphate group ( $P_i$ ) is removed, and ATP becomes **ADP** (**adenosine diphosphate**). At least 30 kJ of energy is released during this reaction. It is called an **exergonic** reaction because energy is released.



**Figure 2** The breakdown and formation of adenosine triphosphate.

ATP can be resynthesised from ADP and inorganic phosphates by a condensation reaction. The energy needed to drive this reaction comes from respiration. The **ATP cycle**, which shows the relationship between ATP, ADP, and respiration, is outlined in figure 3.

ATP is vital because it is the main source of chemical energy for energy-consuming reactions. In cells, the exergonic breakdown of ATP is coupled to energy-consuming (**endergonic**) reactions. Phosphate groups liberated from ATP attach to other molecules (a process called **phosphorylation**). This energises the molecules, enabling them to do work.



**Figure 3** The ATP cycle.

## Nucleic acids: DNA

Nucleotides can combine by condensation reactions to form long chains (figure 4). These chains are called **nucleic acids** or polynucleotides. The best known nucleic acid is **deoxyribonucleic acid** or **DNA**. DNA has been called 'the molecule of life' because it plays a key role in inheritance and protein synthesis. It influences every physical and behavioural characteristic of an organism.

The basic structure of DNA seems simple because it is made of only four types of nucleotide. However, these nucleotides can link together in many ways to form chains of infinite variety. This infinite variability enables DNA to store the genetic information for all the millions of organisms on Earth, as we shall see later.

Each DNA nucleotide has a phosphate group, a pentose sugar (deoxyribose), and one of four types of base (all of which contain nitrogen): **adenine** (A), **cytosine** (C), **guanine** (G), or **thymine** (T).

### DNA forms a double helix

- The DNA molecule consists of two strands, each of which is a polynucleotide chain.
- Each strand has a helical (spiral) shape, so that DNA has become known as the '**double helix**'.
- The polynucleotide chains run in opposite directions and are joined by pairs of bases (figure 5).
- The bases are held together by hydrogen bonds between the hydrogen atoms of a base in one chain and the oxygen and nitrogen atoms of a base in the other chain.
- The shapes and sizes of the bases mean that nucleotides can pair up only in certain ways. Guanine always pairs with cytosine, and adenine always pairs with thymine.

The sequence of bases along the polynucleotide chain forms the genetic code, which determines the characteristics of an organism that are inherited from its parents (see spread 18.6). The sugar-phosphate backbone of DNA is the same for all organisms, from bacteria to whales, but the base sequence is extremely variable. See spread 18.1 for a more detailed account of DNA structure.

## Nucleic acids: RNA

The other main nucleic acid is **ribonucleic acid** or **RNA**.

- RNA usually consists of a single polynucleotide chain that forms an alpha-helix.
- The pentose sugar in the RNA nucleotides is ribose rather than the deoxyribose that occurs in DNA nucleotides.
- RNA nucleotides contain one of four types of base: adenine, guanine, cytosine, or **uracil** (which replaces the thymine of DNA).

There are several types including messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). Each differs in length and shape, but all share the same basic structure. The different types of RNA are involved in different aspects of protein synthesis. The exact function is related to the precise shape, length, and nucleotide composition of the RNA (see spread 18.7).

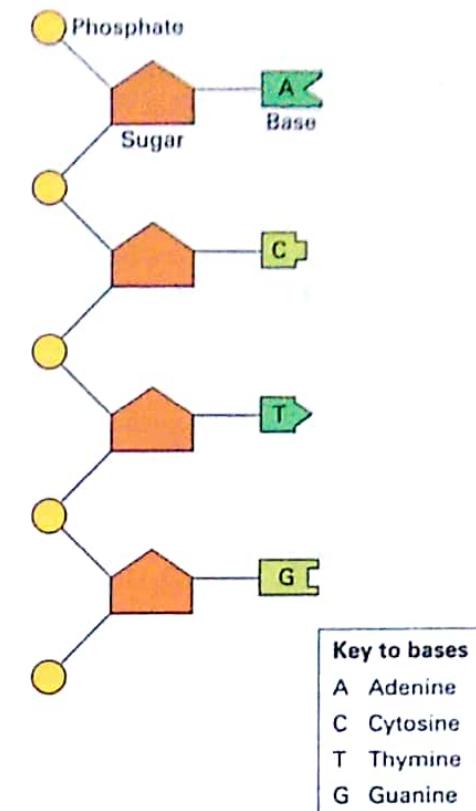


Figure 4 Part of a polynucleotide chain.

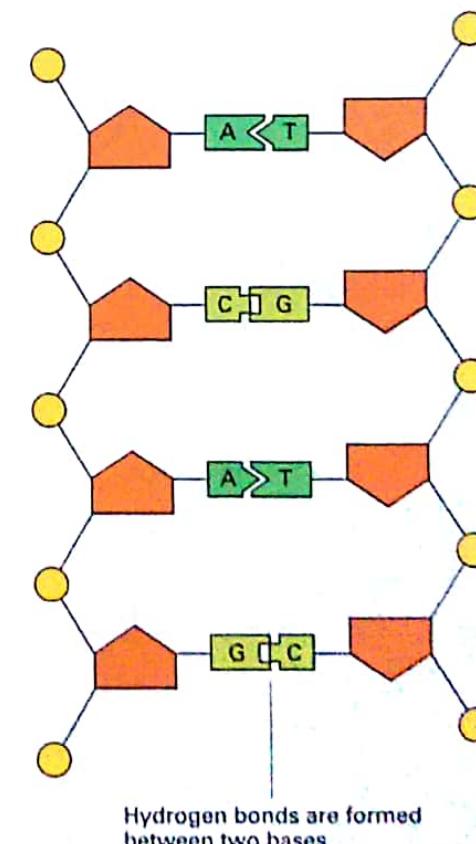


Figure 5 Part of a DNA molecule.

### QUICK CHECK

- 1 List the three components found in all nucleotide molecules.
- 2 How are the bases in a double strand of DNA held together?
- 3 Name three cellular activities that use ATP as an energy source.

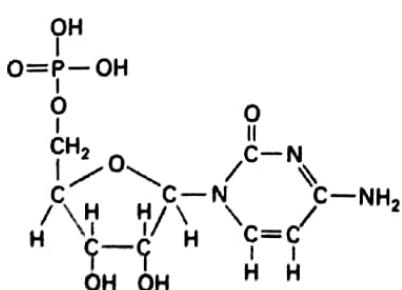
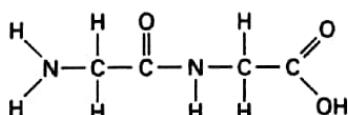
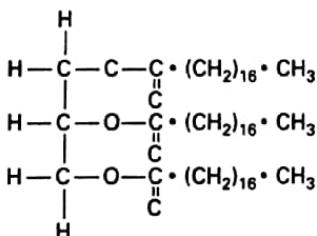
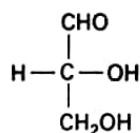
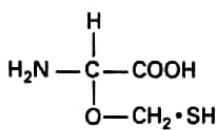
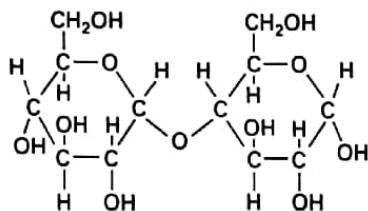
### Food for thought

Biochemists often refer to ATP as the 'energy currency of cells' because all cells use ATP as the source of energy to drive their metabolic machinery. Cells may have many different fuel molecules, but these cannot supply metabolic energy until they are converted to ATP. Suggest why cells do not use fuel molecules, such as carbohydrates and fats, directly.

## PRACTICE EXAM QUESTIONS

- 1 The diagrams below represent organic molecules. Using only the letters adjacent to the diagrams, indicate
- which structure contains a peptide bond [1]
  - which structure contains a glycosidic bond [2]
  - which structure is an amino acid [1]
  - which structure is a nucleotide [1]
  - which structure is characterized by its solubility in organic solvents [1]
  - which structure is a monosaccharide [1]
  - which structures have been made as a result of a condensation reaction [2]
  - which structure would require 3 water molecules for complete hydrolysis. [1]

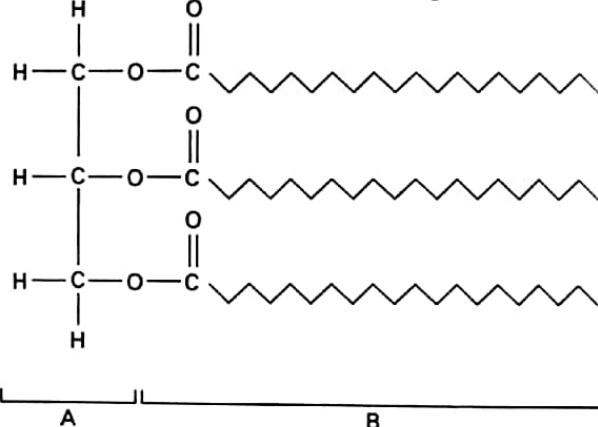
[Total 10 marks]



- 2 The diagram below shows the structure of a lipid molecule.

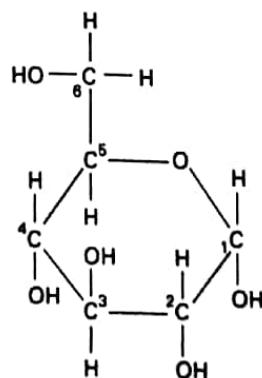
- i Name the parts labelled A and B. [2]
- ii Name this type of lipid. [1]
- iii Name the chemical reaction used to form the bonds between A and B. [1]
- i State one function of this type of lipid in living organisms. [1]
- ii State one feature of the molecules of this type of lipid which makes them suitable for the function you have given. [1]

[Total 6 marks]



- 3 Starch is a polymer of glucose. It has two components, amylose and amylopectin. Amylose is a straight chain molecule of several thousand alpha glucose molecules linked by 1-4 glycosidic bonds. Amylopectin is a branched molecule formed from thousands of alpha glucose molecules linked by 1-4 glycosidic bonds with branches formed from 1-6 glycosidic bonds every 25 units (the numbers associated with the bonds refer to which carbon atoms are involved in the bond).

- The diagram below represents a molecule of alpha glucose. Using a simplified structure construct a diagram to show a short length of amylopectin consisting of three glucose units in one chain linked to a branch of two units. [2]

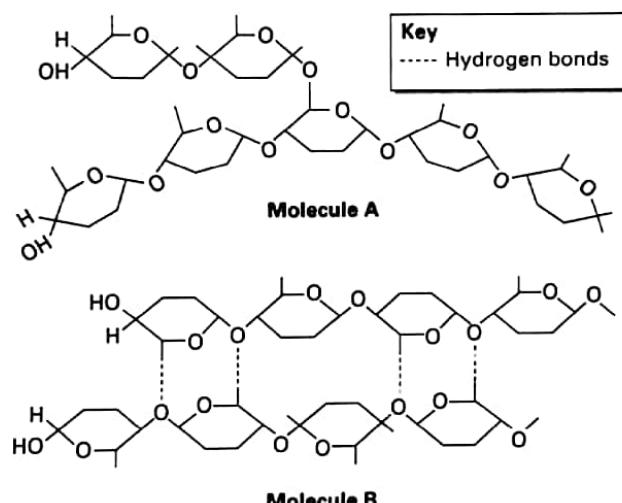


- b** The hydrolysis of the molecule which you have drawn would result in the production of monosaccharides.
- What is meant by the term *hydrolysis*? [2]
  - Give two different ways by which this hydrolysis could be brought about. [2]
  - Suggest why the hydrolysis of a branched molecule will be faster than a straight chain molecule. [1]
- [Total 7 marks]

- 4** Copy and complete the table by giving four differences between cellulose and glycogen. [4]

|   | Cellulose | Glycogen |
|---|-----------|----------|
| 1 |           |          |
| 2 |           |          |
| 3 |           |          |
| 4 |           |          |

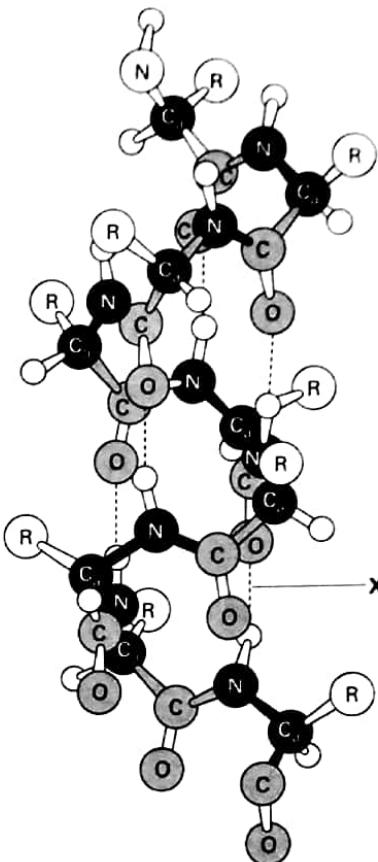
- 5** The diagrams show part of the molecular structures of two polysaccharides. The hexagonal shapes represent hexose sugars.



- Give the name of molecule A. [1]
  - Give one difference between hexose sugars in molecules A and B. [1]
  - Both polysaccharides contain hexose sugars joined by 1-4 glycosidic bonds.
    - Explain, using an annotated diagram, how these bonds in molecule A are hydrolysed in the process of human digestion. [2]
    - Using information in the diagram of molecule B, suggest one reason why it cannot be digested by humans. [1]
- [Total 5 marks]

- 6** The figure above right shows a diagram of part of a polypeptide chain. This type of twisted structure is commonly found in proteins of many different types.

- Name the repeating unit of a polypeptide chain. [1]
- State the name given to the twisted structure shown in the figure. [1]
- Identify the type of bond in the structure labelled by the letter X. [1]
- Explain briefly what would happen to the polypeptide chain if it were heated to about 70 °C. [2]

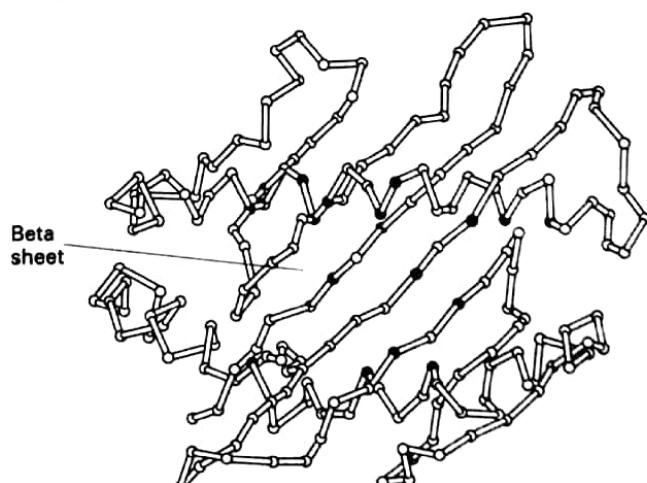


**v** The twisted arrangement seen in the figure above is referred to as a secondary structure. Explain what is meant by the term *secondary structure*. [2]

- b** Another common secondary structure is known as the beta sheet. State *one* difference between the beta sheet and the structure shown in the figure above. [1]

- c** **i** Proteins can be classified as fibrous or globular. Name **one** example of each type of protein. [2]

Globular proteins such as that shown in the figure below are often described as tertiary structures. However, as indicated in the diagram, many globular proteins may also have sections of secondary structure.



- ii** Explain what is meant by the term *tertiary structure*. [2]

- d** Monosaccharides can also be linked together to form long chain molecules called polysaccharides. Give **three** differences (other than the presence of monosaccharides or amino acids) between a polypeptide and a polysaccharide chain. [3]

[Total 15 marks]

## 3.1

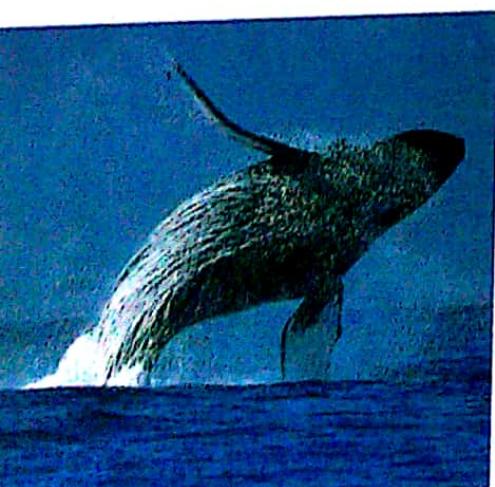
## OBJECTIVES

By the end of this spread you should be able to:

- understand that living organisms obey the laws of thermodynamics
- distinguish between catabolic and anabolic reactions
- explain what is meant by the activation energy of a metabolic reaction.

## Fact of life

It used to be thought that all organisms on Earth obtained their energy from the Sun. However, in 1977 scientists made a stunning discovery that changed forever our understanding of life on Earth. They found a vast assemblage of weird and wonderful organisms living on the bottom of the Pacific Ocean close to hydrothermal vents (areas of underwater volcanic activity) and so deep that no light can reach them. The organisms ranged in size from microscopic bacteria to giant clams and tube worms. The bacteria, which acquire their energy from sulphur compounds released from the hot vents, are the primary food suppliers.



## Types of energy

Every living organism on Earth requires energy to stay alive. Energy is the capacity to do work: the ability to move matter in a direction in which it would not move without an input of energy. The amount of matter may be small or large: an ion passing through a cell membrane, or a whale swimming in an ocean.

Energy exists in many forms, including chemical, electrical, nuclear, heat, light, and mechanical energy. There are two types of mechanical energy: kinetic energy and potential energy. Matter that is moving and performing work is said to have **kinetic energy** (for example, a whale rising out of the sea; figure 1). Matter that is not actually performing work, but that has the ability to do so because of its position, is said to have **potential energy** (for example, the whale at the top of its jump, when it is momentarily static). The atoms within a sugar molecule also have potential energy, because of the arrangement of their atoms. Sugar possesses **chemical energy**, the most important type of potential energy for life.

## Laws of thermodynamics

Organisms survive by transforming one form of energy into another. Plants use energy from light to make glucose and starch, and animals use energy from other organisms so that they can move, grow, and carry out all their many activities. Energy that can be used to do useful work is called **free energy**.

Under most circumstances, two laws govern the energy transformations that occur in all matter, be it living, dead, or non-living. The laws are called the first and second laws of thermodynamics. (**Thermodynamics** is the study of energy transformations.)

The **first law of thermodynamics** (the **law of conservation of energy**) states that energy can be neither created nor destroyed; it can only be changed from one form into another. Therefore, when energy changes take place within an organism, the energy input always equals the energy output.

The **second law of thermodynamics** is based on the assumption that the universe is becoming increasingly disordered. This is related to the fact that when energy is transformed from one type to another, some of the energy is converted into heat. Because we are warm blooded, we tend to think that heat is useful. However, whenever heat is generated, less energy is available to do useful work. Therefore, heat energy is regarded as a low-order form of energy. The second law of thermodynamics is sometimes called the **law of entropy** because entropy is another word for the disorder or randomness in a system. (Entropy is also a measure of the unavailability of energy for useful work, a consequence of disorder.)

Organisms appear to disobey the second law because they become more ordered and complex during their lives. However, they can do this only at the expense of the environment. Organisms are 'energy parasites'; they become increasingly complex by using energy taken from their surroundings. The sum effect of life is to increase disorder in the universe.

*Figure 1 Energy is required by all organisms to carry out their functions. This humpback whale expends a great deal of energy in projecting itself out of the sea. It appears to breach the water to stun or panic shoals of fish, and to communicate to other members of its group.*

## Chemical reactions and metabolism

A chemical reaction leads to a chemical change in matter. A reaction can be represented by a chemical equation. For example, the following chemical reaction takes place when oxygen and hydrogen combine to form water:



Two molecules of hydrogen ( $\text{H}_2$ ) and one molecule of oxygen ( $\text{O}_2$ ) are the starting materials, called the **reactants** or **substrates**. Two molecules of water ( $2\text{H}_2\text{O}$ ) are the resultant substances, called the **products**. The arrow indicates the direction of the reaction, that is from hydrogen and oxygen to water. Notice that the numbers of hydrogen atoms and of oxygen atoms on the left-hand side of the equation equal the numbers on the right-hand side. All equations are **balanced** in this way, reflecting the fact that chemical reactions do not destroy or create matter.

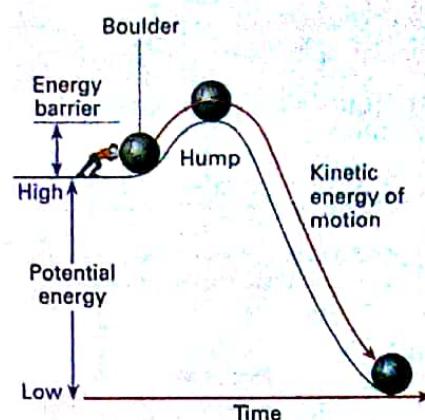
Cells carry out thousands of chemical reactions. The sum of all these reactions is called **cellular metabolism**. There are two main types of metabolic reaction: catabolism and anabolism. During **catabolism**, substances break down and release energy. During **anabolism**, chemical reactions take in energy to synthesise complex molecules from simple molecules. Reactions that liberate more energy than they take in are called **exergonic**; those that take in more energy than they liberate are called **endergonic**.

### Activation energy

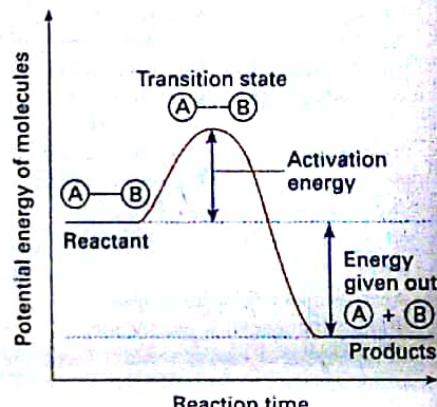
Most molecules are in a relatively stable state and require an input of energy in order to react with each other. Chemicals in a match head, for example, do not react until the match is struck against a suitable surface. There is an **energy barrier** to the reaction. The amount of energy required to overcome this barrier and start a reaction is called the **activation energy**. Friction supplies the activation energy required to ignite the match. Once lit, the match generates much more energy, mainly as light and heat.

The energy changes of a chemical reaction can be likened to those of a boulder rolling down a hill (figure 2). The hump over which the boulder must be pushed before it can roll freely represents the energy barrier, and the energy needed to push the boulder up over this barrier is equivalent to the activation energy.

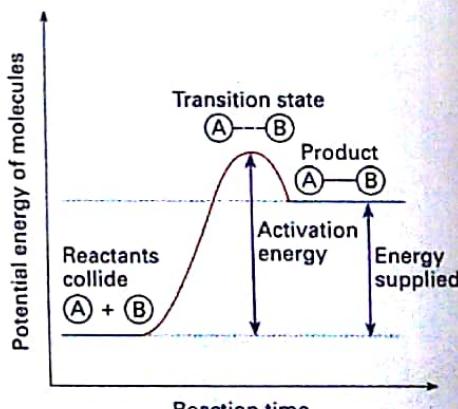
Catabolic reactions are exergonic (give out energy) because they have a small activation energy compared with the energy released during the reaction; the reaction products contain less energy than the substrates (figure 3). Anabolic reactions are endergonic because their activation energy is greater than the energy released during the reaction. The products of the reaction contain more energy than the reactants, therefore extra energy must be supplied for the reaction to proceed (figure 4).



**Figure 2** The boulder analogy: the potential energy of the boulder must be increased before it can roll down the hill.



**Figure 3** Potential energy of molecules during a catabolic reaction.



**Figure 4** Potential energy of molecules during an anabolic reaction.

### QUICK CHECK

- 1 Organisms obey the law of conservation of energy. Why then do they appear to lose energy when they transform one form of energy into another (for example, when an animal converts chemical energy into kinetic energy during locomotion)?
- 2 Is photosynthesis an anabolic or catabolic process?
- 3 What is activation energy?

### Food for thought

The energy change that occurs during a chemical reaction is referred to by the measure  $G^\ominus$ , the standard free energy change. In any reaction, if  $G^\ominus$  is

negative, the reaction is exergonic, that is, it releases energy which can do work. If  $G^\ominus$  is positive, the reaction is endergonic, that is, it requires an energy input for the reaction to take place. Suggest whether  $G^\ominus$  is positive or negative in the following reactions:

- a glucose + oxygen  $\longrightarrow$  carbon dioxide + water
- b ADP + P<sub>i</sub>  $\longrightarrow$  ATP
- c water + carbon dioxide  $\longrightarrow$  glucose + oxygen
- d C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> (glucose)  $\longrightarrow$  2CH<sub>3</sub>CH(OH)COOH (lactate)

Most of the chemical reactions in cells are endergonic. What is their source of energy?

## OBJECTIVES

By the end of this spread you should be able to:

- list the main differences between enzymes and inorganic catalysts
- describe the lock-and-key theory and induced-fit theory of enzyme action.

## Fact of life

A very small amount of enzyme can bring about a change in a large amount of substrate. For example, at 0°C, the enzyme catalase has a turnover number of 50 000. This means that at 0°C, one molecule of catalase can catalyse the breakdown of 50 000 molecules per second of hydrogen peroxide to water and oxygen. At human body temperature, one molecule of the enzyme catalyses the breakdown of about 600 000 molecules per second of hydrogen peroxide.

An average enzyme undergoes about 1000 reactions per second. Catalysts can speed up reaction rates by as much as a trillion ( $10^{12}$ ) times. According to Dianne Gull and Bernard Brown, 'This is roughly equivalent to speeding up your life from cradle to grave so that it is completed in a single heartbeat.'

## HOW ENZYMES WORK

## The transition state: the hump in the energy hill

Every cell carries out many thousands of metabolic reactions. Each reaction starts with the **reactants** (the molecules undergoing the reaction) and ends with a **product** or products. Metabolic reactions usually take place in a number of steps, with each intermediate step forming an intermediate molecule on the way to the final product. The intermediate molecules are less stable (hence contain more energy) than either the reactants or the products. The intermediate molecule containing the most energy is called the **transition state**. Before a reaction can get underway, there must be enough energy available to convert the reactants to the transition state. This energy is the activation energy described in spread 3.1.

## Enzymes: biological catalysts

Almost every cellular reaction, whether it involves breaking things down (a catabolic reaction) or building things up (an anabolic reaction), is controlled by a biological catalyst called an **enzyme**. Enzymes can increase the rate of biochemical reactions to more than a billion times their normal rate, without the enzymes being changed themselves by the reaction. Because they can be reused, even small concentrations of enzymes can be very effective. However, like other catalysts, enzymes cannot make reactions occur which otherwise would not happen, and they do not alter the final amount of product formed, just the speed at which it is formed.

Unlike an inorganic catalyst, which might catalyse many different types of reaction, an enzyme will usually catalyse only one type of reversible reaction. Enzymes are affected by temperature and pH much more than inorganic catalysts are (spread 3.3). Each enzyme works effectively only in a limited range of temperature, pressure, and pH; inorganic catalysts often function very effectively in widely varying conditions of temperature and pressure, and extremes of pH.

## Reducing the activation energy

Their ability to accelerate reactions and their high selectivity make enzymes among the most important compounds found in biology. Most enzymes catalyse reactions by combining with the substrate of a reaction to form a series of **enzyme-substrate complexes**. The complex in which the substrate binds most tightly to the enzyme corresponds to the transition state. This transition state is more stable (of lower energy) than the transition state in the uncatalysed reaction. Consequently, an enzyme reduces the activation energy of the reaction it catalyses (figure 1).

There are two main theories explaining how enzyme-substrate complexes form: the lock-and-key theory and the induced-fit theory.

## The lock-and-key theory

Most enzymes are huge globular protein molecules made up of many thousands of atoms along with some metal ions. Their molecules have a very precise shape (tertiary structure) which includes a cleft or pocket called the **active site**. In the **lock-and-key theory** of enzyme action, the substrate fits into a rigid active site like a key into a lock (figure 2). Various types of bond including hydrogen bonds and ionic bonds hold the substrate in the active site to form an **enzyme-substrate complex**. Once the enzyme-substrate complex is formed, the enzyme can help change the substrate, either splitting it apart or linking pieces together.

In the lock-and-key theory, the shape of the substrate must fit the enzyme exactly if a reaction is to be catalysed. This explains why enzymes are specific, and why any change in enzyme shape, no matter

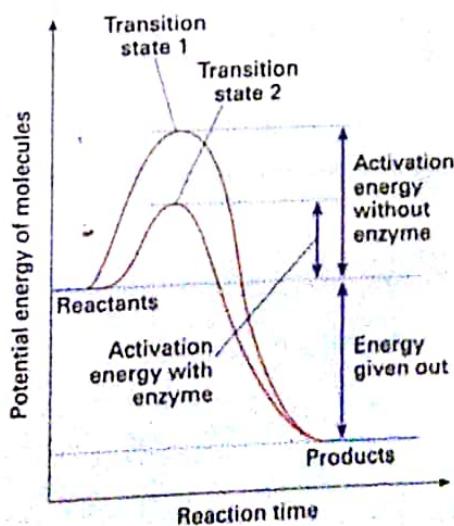


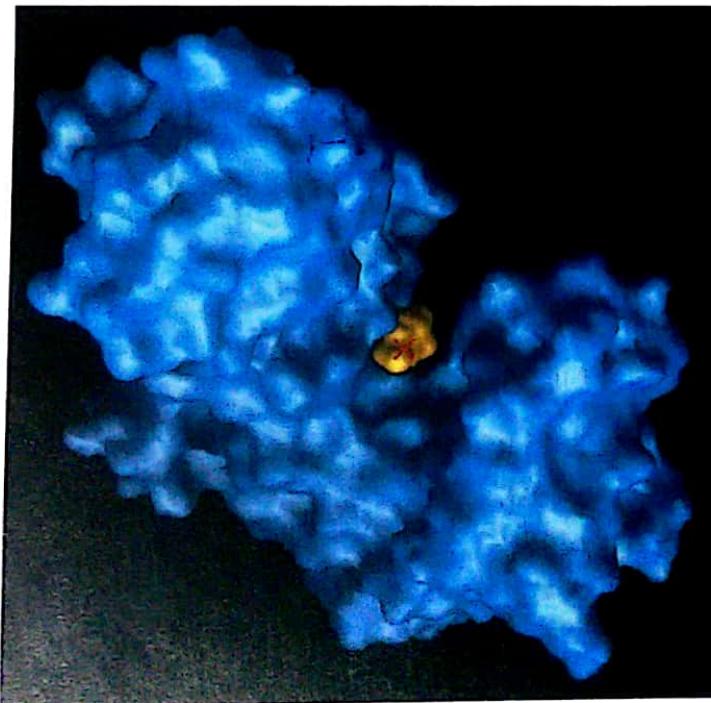
Figure 1 The lowering of the activation energy by an enzyme.

how small, alters its effectiveness. However, it is not a totally satisfactory explanation of enzyme action. If the theory is correct, enzyme action depends on the unlikely event of randomly moving substrate molecules entering the active site in the right orientation. This would be analogous to trying to get a key in a lock by throwing it ... with your eyes shut!

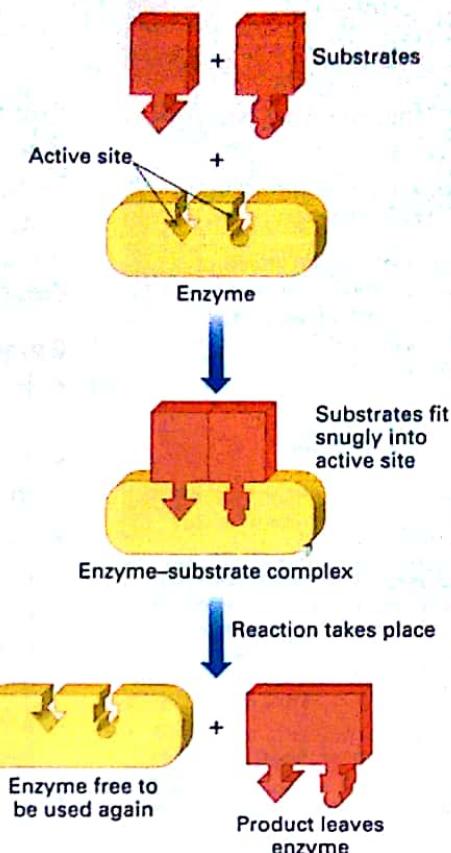
### The induced-fit theory

The **induced-fit theory** of enzyme action is a modified version of the lock-and-key theory. It does not depend on such precise contact being made between the substrate and the active site. In this model, the active site is able to change its shape to enfold a substrate molecule. The enzyme takes up its most effective catalytic shape after binding with the substrate. The shape of the enzyme is affected by the substrate, just as the shape of a glove is affected by the hand wearing it.

The distorted enzyme molecule in turn distorts the substrate molecule, straining or twisting the bonds. This makes the substrate less stable, reduces its potential energy, and thus lowers the activation energy of the reaction. The reaction occurs and products are formed which no longer bind to the active site, and so move away. The flexible enzyme then returns to its original shape, ready to bind the next substrate molecule (figure 3).



**Figure 3** The induced-fit model of enzyme action. Hexokinase is an enzyme which catalyses the phosphorylation of glucose. When the substrate, glucose (x), enters the active site it induces a slight change in the shape of the protein. This enables an enzyme–substrate complex to form.



**Figure 2** Much simplified view of the lock-and-key theory of enzyme action. The reaction can go either way: the enzyme also catalyses the conversion of product to substrate.

### QUICK CHECK

- 1 List three differences between inorganic catalysts and enzymes.
- 2 Give the main difference between the lock-and-key and induced-fit theories of enzyme action.

### Food for thought

The role of enzymes has been compared with matchmakers. Indeed, the Chinese word for catalyst, *tsoo mein*, means 'marriage broker'. Is this comparison appropriate? Suggest another comparison.

### 3.3

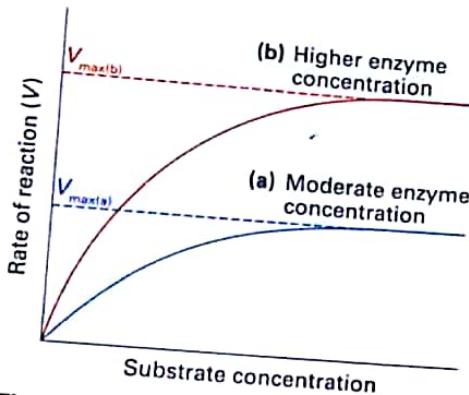
## FACTORS AFFECTING ENZYMES (1)

### OBJECTIVES

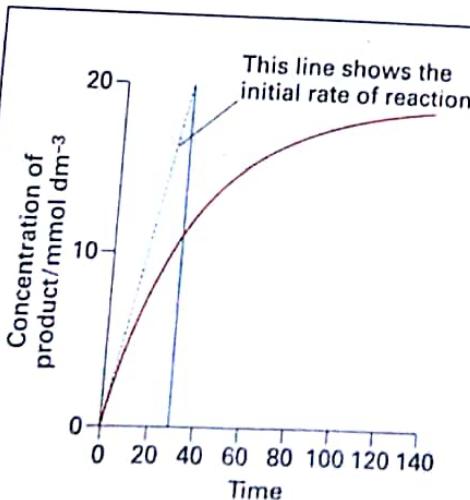
- By the end of this spread you should be able to:
- explain why enzymes affect specific types of reactions
  - describe and explain the effects of enzyme concentration, substrate concentration, incubation time, temperature, and pH on enzyme-catalysed reactions.

### Fact of life

Water spewing out of deep-sea volcanic vents reaches temperatures in excess of 350 °C. Certain bacteria can live in the heated surrounding water because they have evolved special thermostable enzymes that are not denatured by temperatures as high as 115 °C.



**Figure 1** Two saturation curves for an enzyme-catalysed reaction. For any given concentration of enzyme, the rate of reaction increases with increased substrate concentration until a constant rate of product formation (saturation) is reached. At saturation, all enzyme active sites are bound to the substrate and the rate of the reaction is at its maximum ( $V_{max}$ ). At a higher enzyme concentration, the saturation point is raised because more active sites become available, and  $V_{max}$  is increased. The substrate concentration (usually expressed in molar units, M) that gives  $\frac{1}{2}V_{max}$  is referred to as  $K_m$ .



**Figure 2** Measuring the initial rate of reaction.

Enzymes are globular proteins. The three-dimensional shape, particularly that of the active site, determines the specificity of an enzyme and also how it is affected by other factors, such as temperature and pH.

The box at the bottom of the page explains how you can measure the initial rate of a reaction. This initial rate is used to compare the effect of different factors on an enzyme-catalysed reaction.

### Enzyme specificity: which reaction does it catalyse?

- Enzymes catalyse reactions by combining with a substrate to form an enzyme-substrate complex (see spread 3.2).
- The formation of an enzyme-substrate complex can only take place if the substrate fits snugly into the active site of the enzyme.
- An enzyme will only catalyse a reaction in which the substrate and active site have complementary shapes.
- Consequently, an enzyme usually catalyses only one reaction or a closely related group of reactions.
- These reactions are usually reversible; if an enzyme can catalyse a reaction in one direction, then it will also be able to catalyse the reverse reaction.
- The degree of enzyme specificity varies. Some enzymes are so specific that they catalyse only one reaction. For example catalase catalyses only the breakdown of hydrogen peroxide (a by-product of cellular respiration) to water and oxygen. Other enzymes, particularly digestive enzymes, are a little less specific. Some lipases will break down a range of fats into fatty acids and glycerol.

### Substrate concentration

The rate of an enzyme-catalysed reaction increases in direct proportion to the substrate concentration until the reaction reaches a maximum rate. After the maximum all the active sites of the enzyme molecules are filled, so increasing the substrate concentration further has no effect on the rate of reaction (figure 1).

### Enzyme concentration

The rate of an enzyme-catalysed reaction is directly proportional to the concentration of the enzyme present, as long as no other factors are limiting the rate (figure 1). Therefore, this relationship holds only when pH, pressure, and temperature are constant, and the substrates are present in excess concentrations. Under these conditions, the more active sites there are available, the more substrate can be converted to product.

### Measuring the initial rate of reaction

Biologists often need to be able to measure the initial rate of an enzyme-catalysed reaction so that they can compare the rate under different conditions. The rate of reaction is usually expressed as the amount of substrate converted to product per unit time.

Figure 2 shows you how to calculate the initial rate of reaction from a graph of product concentration against time. First draw a line at a tangent to the beginning of the curve, as close as possible to time 0. Continue this line until you reach a convenient point along the x-axis; in this case, 30 seconds. Then read off how much product would be made if the initial reaction rate continued for 30 seconds. In this case, 20 mmol (millimoles, a measure of the number of molecules) of product would have been made in 30 seconds. This gives a rate of  $0.66 \text{ mmol s}^{-1}$  or  $40 \text{ mmol min}^{-1}$ .

## Incubation time

The **incubation time** is the length of time over which a reaction has taken place. Generally, the average (not initial) rate of an enzyme-catalysed reaction decreases as the incubation time increases, even when there is excess substrate present. This decrease is probably because the enzyme gradually becomes denatured with time (spread 2.10). As the protein molecule becomes increasingly deformed, it loses its effectiveness as an enzyme.

## Temperature

The rate of an enzyme-catalysed reaction increases with temperature up to a maximum, called the **optimum temperature**.

- At suboptimal temperatures, increasing temperature increases the kinetic energy of the reactants. As they move faster, they are more likely to collide and interact with each other and with the enzyme.
- The change in rate of a reaction for each  $10^{\circ}\text{C}$  rise in temperature is called the **temperature coefficient**,  $Q_{10}$ :

$$Q_{10} = \frac{\text{rate of reaction at } x + 10^{\circ}\text{C}}{\text{rate of reaction at } x^{\circ}\text{C}}$$

At suboptimal temperatures the  $Q_{10}$  for enzyme-catalysed reactions is approximately 2 (the rate doubles for each  $10^{\circ}\text{C}$  rise in temperature).

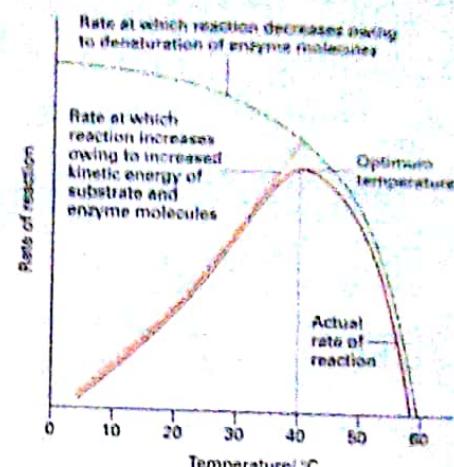
- The rate continues to rise until it reaches a peak at the optimum temperature.
- Above this temperature, the rate usually falls dramatically. This is because the increased energy causes bonds that maintain the enzyme's shape to break, and the enzyme becomes denatured (see spread 2.10). The changed shape means that the substrate can no longer fit into the active site, and enzyme activity is lost.
- The optimum temperature for an enzyme-catalysed reaction is related to the enzyme's usual thermal environment. In humans many enzymes work best at core body temperature – about  $37^{\circ}\text{C}$ .

## Hydrogen ion concentration (pH)

Most enzymes are effective in only a narrow pH range. Within this range there will be one particular pH (the **optimum pH**) at which activity is greatest. The optimum pH for an enzyme usually matches its usual pH environment. Digestive enzymes in the stomach, for example, work best under acidic conditions, whereas those in the small intestine work best in alkaline conditions (figure 4). Deviations from the optimum pH can cause bonds to be broken (especially hydrogen bonds and ionic bonds) so that the enzyme becomes denatured. The substrate no longer fits readily into the active site to form an enzyme–substrate complex, so the enzyme becomes less effective.

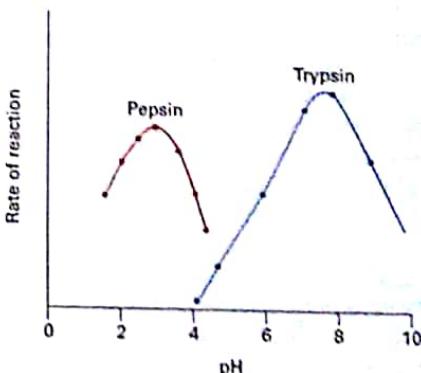
## QUICK CHECK

- Why does an enzyme catalyse only a specific reaction or a closely related group of reactions?
- a State two effects of temperature on enzyme-catalysed reactions.  
b What is the optimum pH of trypsin and of pepsin shown in figure 4?  
c In which parts of the alimentary canal would you expect to find each of these enzymes?



**Figure 3** The effect of temperature on an enzyme-catalysed reaction. Increasing the temperature tends to speed up the reaction because the kinetic energy of the reactants increases. However the reaction also tends to slow down as the enzyme is denatured. The balance of these opposing activities is different at different temperatures:

- Below the optimum temperature, the rate rises because the effect of the increased kinetic energy of reactants exceeds the effect of denaturation.
- At the optimum temperature, the rate levels off because the increase in kinetic energy of reactants is cancelled out by the denaturation of the enzyme.
- Above the optimum temperature, the rate falls because the effects of denaturation exceed the effects of increased kinetic energy of the reactants.



**Figure 4** The effect of pH on the rate of reaction of pepsin and trypsin.

## Food for thought

The factors that affect enzymes can be grouped according to whether they alter the tertiary structure of an enzyme, or whether they alter the chance of an enzyme and substrate colliding (the so-called collision theory of enzyme action). Suggest to which group each of the factors in this spread belong. Are there any factors that belong to both groups?

## OBJECTIVES

By the end of this spread you should be able to:

- explain how cofactors enable enzymes to work
- distinguish between competitive and non-competitive inhibitors
- describe an example of end-product inhibition.

## Fact of life

Enzyme inhibitors can act as poisons, pesticides, antibiotics, and painkillers. Cyanide blocks the active site of cytochrome oxidase. This enzyme catalyses the oxidation of hydrogen to form water, the final product in aerobic respiration. Cyanide is therefore a respiratory poison, preventing aerobic respiration.

The pesticide malathion inhibits the action of acetylcholinesterase. This is how malathion kills insects and other pests, by disrupting the transmission of nerve impulses. Unfortunately, malathion also affects humans and other animals, but at higher doses than those required to kill insects.

The antibiotic penicillin kills bacteria by inhibiting an enzyme that is essential for the synthesis of bacterial cell walls.

Possibly the most widely used painkiller in the world is 2-ethoxyloxybenzenecarboxylic acid, known by pharmacists for many years as acetylsalicylic acid, better known by those in pain as aspirin. Aspirin relieves pain by inhibiting an enzyme called PGHS which is required for the formation of prostaglandins. Prostaglandins are a group of compounds derived from fatty acids. They increase the sensitivity of nerve endings, making a person more aware of pain. Prostaglandins are produced after injury as part of the inflammation response.

## Cofactors: activators and coenzymes

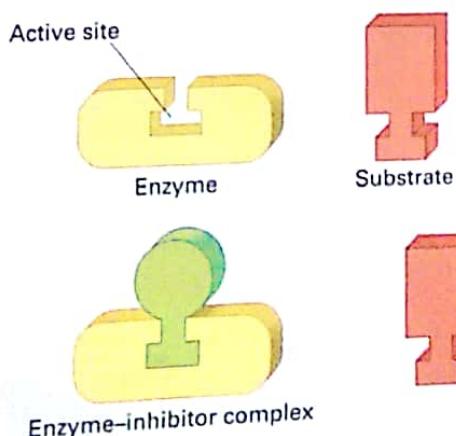
Some enzymes work only in the presence of a non-protein substance called a **cofactor**. A cofactor that is tightly bound to its enzyme is called a **prosthetic group**. Cofactors may be inorganic or organic. Inorganic cofactors are called **activators**, and these include metal atoms such as copper, iron, or zinc. Activators may attach onto the active site of the enzyme to make its shape more efficient. Organic cofactors are called **coenzymes**, and many coenzymes are vitamins or compounds made from vitamins. Some coenzymes transfer chemical groups, atoms, or electrons from one enzyme to another. For example, nicotinamide adenine dinucleotide or NAD, derived from vitamin B<sub>3</sub>, transfers hydrogen during cellular respiration.

## Inhibitors

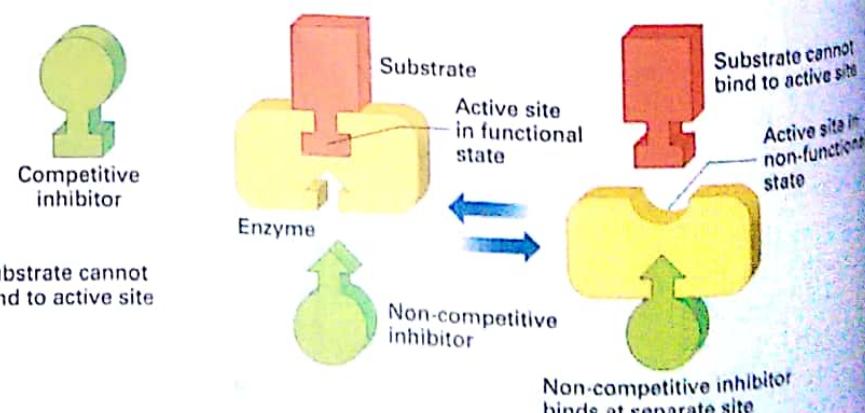
Many substances can interfere with enzymes, reducing or even completely destroying their action. These substances are called **inhibitors**. There are two main groups of inhibitors: competitive and non-competitive. Inhibitors of either type can be either reversible or irreversible. **Reversible inhibitors** generally bind to an enzyme with weak bonds such as hydrogen bonds which are easily broken. Reversible inhibitors affect the enzyme only so long as they are attached to it. As soon as they are detached, the enzyme can function normally again. **Irreversible inhibitors** attach to an enzyme with strong covalent bonds which are difficult to break without damaging the enzyme. Consequently the effect of an irreversible inhibitor is permanent.

A **competitive inhibitor** of a particular enzyme has a shape resembling the enzyme's normal substrate. Consequently, the inhibitor can compete with the normal substrate to occupy the active site. If the inhibitor occupies the site, it prevents the enzyme from combining with its normal substrate (figure 1). Antibiotic drugs called sulphonamides act as competitive inhibitors. Their shape resembles that of a substance called para-aminobenzoate (PAB) which is used by some harmful bacteria in the synthesis of folic acid. An enzyme catalyses the conversion of PAB into folic acid. Sulphonamides compete with PAB for the active site on this enzyme. So if sufficient sulphonamide is present, the enzyme will be inhibited and the bacteria will become deprived of folic acid and die.

The effect of the competitive inhibitor depends on its concentration compared with that of the substrate, and on how tightly the enzyme binds to the inhibitor and to the substrate. An enzyme-catalysed reaction is likely to proceed if there is more substrate than inhibitor, but will tend to become slower as the proportion of inhibitor increases.



**Figure 1** A competitive inhibitor has a shape similar to that of the normal substrate. The substrate and inhibitor compete for the active site. If the inhibitor binds to the active site, it prevents the binding of the substrate.



**Figure 2** A non-competitive inhibitor does not bind to the active site but attaches to an enzyme at some other place. The attachment causes the shape of the active site to change, preventing the formation of an enzyme-substrate complex.

A **non-competitive inhibitor** does not attach to the active site, but binds with the enzyme at another site. Once attached, the non-competitive inhibitor causes the active site to change shape, preventing the normal substrate from binding there (figure 2). In most organisms, heavy metals such as mercury and cadmium act as irreversible non-competitive inhibitors, and are therefore poisonous. However, not all non-competitive inhibitors are harmful. Figure 3 shows how the end-product of a reaction can act as a non-competitive inhibitor, controlling a series of enzyme-catalysed reactions. This phenomenon is called **end-product inhibition**.

### End-product inhibition and metabolic control

You might be under the impression that all inhibitors are harmful substances, and indeed many enzyme inhibitors do act as pesticides and poisons. However, many other inhibitors are beneficial. Some, especially reversible inhibitors, are important regulators of cellular metabolism.

Most metabolic reactions take place in a series of steps called a **metabolic pathway**. In many cases, a metabolic pathway is regulated by the final substance produced by it. When this substance reaches a certain concentration, it acts as a non-competitive inhibitor. It binds onto one of the enzymes in the metabolic pathway (often the first one), causing the enzyme to change shape. This prevents the reaction series from progressing (figure 3). The reactions start up again when the concentration of the end-product falls to a sufficiently low level. This type of inhibition is called **end-product inhibition**. Control of a metabolic pathway by an end-product is an example of **negative feedback**.

The enzyme that is inhibited, which functions as a regulatory enzyme in the metabolic pathway, is often an **allosteric enzyme**. Allosteric enzymes have a site separate from the active site to which another substance can bind. The shape of the binding substance has to fit that of the allosteric site in much the same way as the normal substrate has to fit the active site. The binding substance may be either an inhibitor or an activator, so either slowing down or speeding up the reaction. (The word 'allosteric' means 'at another place'.)

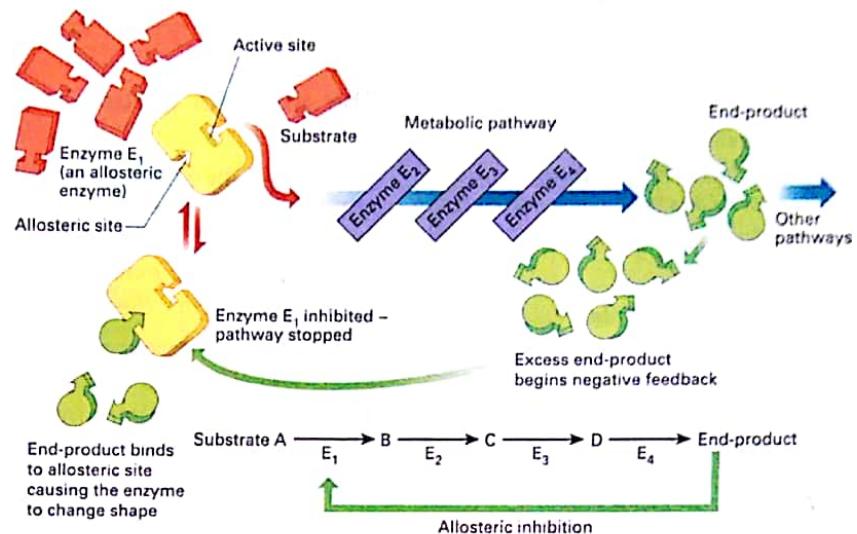
ATP production by glycolysis (the first stage of aerobic and anaerobic respiration) is regulated by end-product inhibition of the enzyme **phosphofructokinase** which functions near the start of the metabolic pathway. If there is a lot of ATP (the end-product) in the cell, this enzyme is inhibited. Respiration slows down and less ATP is produced. As ATP is used up, for example by contracting muscle cells, the inhibition stops and the reaction speeds up again.

### QUICK CHECK

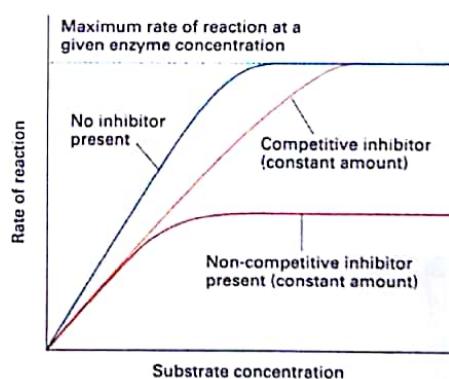
- Distinguish between a cofactor and a coenzyme.
- List the different types of enzyme inhibitor.
- Describe how end-product inhibition works.

### Food for thought

A **Michaelis curve** is a graph that shows the effect of increasing substrate concentration on the rate of reaction in the presence of a fixed amount of enzyme (spread 3.3, figure 1). Figure 4 shows that in the presence of a competitive inhibitor, the maximum



**Figure 3** End-product inhibition in a metabolic pathway. Enzyme E1 acts as a molecular switch that is turned on or off depending on the concentration of the end-product: on when the concentration is low; off when it is higher. Inhibition results from the attachment of the end-product on to the allosteric site of the enzyme, causing the enzyme to change shape so that the substrate can no longer fit onto its active site.



**Figure 4** The effect of a competitive inhibitor and a non-competitive inhibitor on the rate of an enzyme-catalysed reaction.

possible rate can still be achieved, but only when more substrate is available to overcome the effect of the competitive inhibitor. The non-competitive inhibitor has the same effect as lowering the enzyme concentration. Suggest what the graph would look like if:

- a smaller amount of competitive inhibitor was used
- a smaller amount of non-competitive inhibitor was used.

## 3.5

### OBJECTIVES

By the end of this spread you should be able to:

- classify enzymes using the six main classes specified by the International Union of Biochemistry.

#### Fact of life

In 1897 the Buchner brothers ground yeast with sand in a mortar and extracted a lifeless juice from the mixture which was able to ferment alcohol. They called the active ingredient of this juice 'enzyme' (which means 'in yeast'). Since 1897, thousands of new enzymes have been discovered. In 1992, The International Union of Biochemistry and Molecular Biology published the sixth and current edition of *Enzyme Nomenclature* in which they list 3196 enzymes. By 2012 approximately 5000 enzymes had been named.

#### The main enzyme classes

- 1 Oxidoreductases
- 2 Transferases
- 3 Hydrolases
- 4 Lysases
- 5 Isomerases
- 6 Ligases

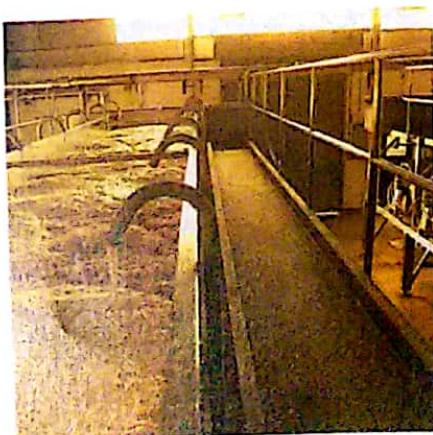


Figure 1 Yeast produces enzymes that ferment sugar to produce alcohol. Carbon dioxide is given off in the reaction, and causes the mixture to bubble and froth.

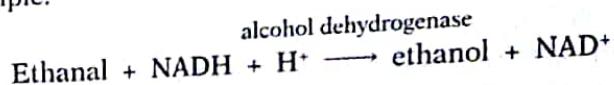
## CLASSIFICATION OF ENZYMES

### The six major groups of enzyme

It is difficult to estimate the total number of enzymes that exist. This is not surprising when you realise that over a thousand different reactions can take place in an individual cell, and each reaction has its own specific enzyme. Classification of these enzymes is therefore a mammoth task. The task is made even more difficult by new enzymes being discovered or synthesised every day.

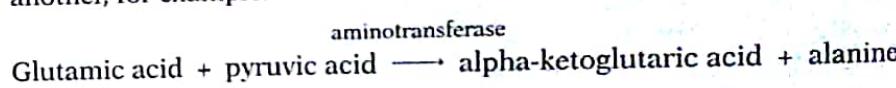
Until 1964 there were several different classification systems of enzymes which led to considerable confusion. In 1964, the International Union of Biochemistry introduced a system aimed at dispelling the confusion. The system is based on the type of reaction the enzyme catalyses. There are six major classes:

- 1 **Oxidoreductases** catalyse redox reactions (biological oxidation and reduction reactions, see box on opposite page) by the transfer of hydrogen, oxygen, or electrons from one molecule to another, for example:



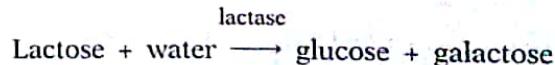
Hydrogen is simultaneously lost from NADH and gained by ethanal. NADH is oxidised to  $\text{NAD}^+$ , and ethanal is reduced to ethanol. This particular process takes place in anaerobic respiration in yeasts and plants.

- 2 **Transferases** catalyse the transfer of a group from one compound to another, for example:



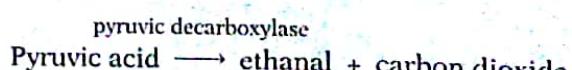
The R group on the amino acid, glutamic acid, is exchanged with the R group on a keto acid, pyruvic acid. A new amino acid, alanine, is formed along with a new keto acid, alpha-ketoglutaric acid. This specific type of process is called transamination and it enables us to make non-essential amino acids from the essential amino acids.

- 3 **Hydrolases** catalyse the splitting of a large substrate molecule into two smaller products. Water is involved in the reaction: it is a hydrolysis, for example:



The disaccharide lactose is broken down into two monosaccharides by the addition of water. Hydrolases enable many condensation reactions (such as the polymerisation of glucose to glycogen) and hydrolytic reactions (such as the digestion of proteins to amino acids) to take place.

- 4 **Lysases** catalyse the addition of a group across a double bond, for example:



Pyruvic acid is converted into ethanal and carbon dioxide by breakage of its double bond and the addition of a new group to the 'freed' bonds. This particular reaction takes place during the fermentation of sugar by yeast. The ethanal is then converted to ethanol (alcohol).

**Redox reactions**

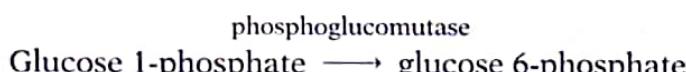
The term **redox** is an abbreviation of reduction–oxidation. Reduction and oxidation are two chemical processes that usually occur simultaneously in the same reaction.

**Oxidation** may involve the addition of oxygen or the removal of hydrogen, but it always involves a loss of electrons. Conversely, **reduction** may involve the addition of hydrogen or the removal of oxygen, but it always involves the gain of electrons.

Redox reactions play a fundamental role in many biological processes, including respiration and photosynthesis, so this is an essential concept for biology students to learn. OIL RIG is a useful mnemonic to help you remember that, in relation to electrons,

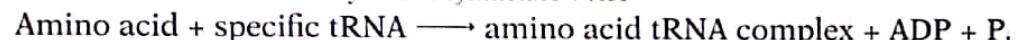
**Oxidation****I**s**L**oss**R**eduction**I**s**G**ain

**Isomerases** catalyse rearrangements within a molecule, converting one isomer to another, for example:



The position of a phosphate group in the glucose 1-phosphate molecule is changed to form the isomer glucose 6-phosphate. This reaction takes place during respiration.

**Ligases** catalyse bond formation between two compounds. The reaction uses energy that comes from the hydrolysis of ATP to ADP and phosphate, for example:



An amino acid is joined to a tRNA molecule. This particular process is called tRNA activation and it is an essential step in protein synthesis.

**Enzyme nomenclature**

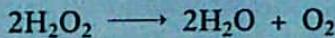
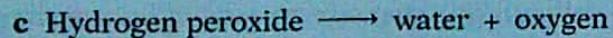
The naming of enzymes has suffered from a confusion similar to that of enzyme classification. Some enzymes are known by three different names: for example, an enzyme found in human saliva is known as ptyalin, salivary amylase, and alpha-1,4-glucan-4-glucanohydrolase. The first two names are referred to as **trivial names**. The first name, ptyalin, dates from before there were any agreed conventions on enzyme nomenclature. The second, salivary amylase, is based on the secretion that contains the enzyme (saliva) and the substrate on which the enzyme acts (amylose). This name also illustrates the convention of using the suffix '-ase' to indicate that it is the name of an enzyme.

The third name is the **systematic name** agreed by the International Union of Biochemistry. The name reflects the nature of the substrate and the type of reaction catalysed, with all the information based on the formal chemical equation describing the reaction.

The systematic name is used for scientific purposes when it is important to be exact in order to avoid confusion. However, it is a rather long name, difficult to remember, and clumsy. Therefore, where confusion is unlikely or exactness is not important, trivial names are still used.

**QUICK CHECK**

1 Using the classification of the International Union of Biochemistry (see box on opposite page), identify the type of enzyme that would catalyse the reactions shown below. You may use each type of enzyme once, more than once, or not at all.

**Food for thought**

Abzymes and ribozymes are two groups of biological catalyst which do not fit neatly into the traditional classification of enzymes. **Abzymes** are large antibodies manufactured by biotechnologists using a technique called monoclonal antibody cloning (see spread 15.6). These antibodies are large protein molecules which fit specific substrates very precisely. They are used in many industrial and medical processes, for example, to break down blood clots and remove scar tissue.

**Ribozymes** were discovered in 1983. They are sections of nucleotides which cut themselves out of nuclear RNA during the formation of messenger RNA (see spread 18.7); they have been described as autosplicing catalysts. Suggest points for and against classifying abzymes and ribozymes as enzymes.

## OBJECTIVES

By the end of this spread you should be able to:

- explain why enzymes are used in industrial processes
- describe how enzymes are extracted for industrial use
- discuss the use of immobilised enzymes in industrial processes.

## Fact of life

The world market for enzymes is worth many millions of pounds and is rising daily as new uses for enzymes are discovered. However, the use of enzymes in commercial and industrial processes is nothing new. They have been used for centuries in cheese-making and the leather industry, and protein-digesting enzymes were patented for use in washing powders in 1913.

## Enzymes from microorganisms

For thousands of years, natural enzymes made by microorganisms (especially bacterial and fungal cells) have been used to make products such as cheeses, bread, wine, and beer. Today enzymes are used for a wide range of industrial processes (see spread 3.7). The study of industrial enzymes and their uses is called **enzyme technology**.

The use of enzymes in industrial processes has a number of advantages over using inorganic catalysts for the same processes. Unlike inorganic catalysts, most enzymes work at room temperature, atmospheric pressure, and within moderate pH ranges. Enzymes are much more specific than inorganic catalysts. Some enzymes, for example, catalyse reactions involving only one type of isomer. Consequently enzymes can be used in the food, drinks, and drugs industries to produce chemically pure products. Non-enzymatic reactions often yield a number of unwanted products with harmful side-effects.

## Producing the enzyme: culturing the microbes

Microbial cells are still the most common source of industrial enzymes. Microorganisms produce enzymes inside their cells (**intracellular enzymes**) and may also secrete enzymes for action outside the cell (**extracellular enzymes**). The chosen microorganisms are usually cultured in large fermentation chambers under controlled conditions designed to maximise enzyme production. The microorganisms may have specific genes introduced into their DNA by genetic engineering so that they produce enzymes naturally made by other organisms (see spreads 18.9 and 18.10).

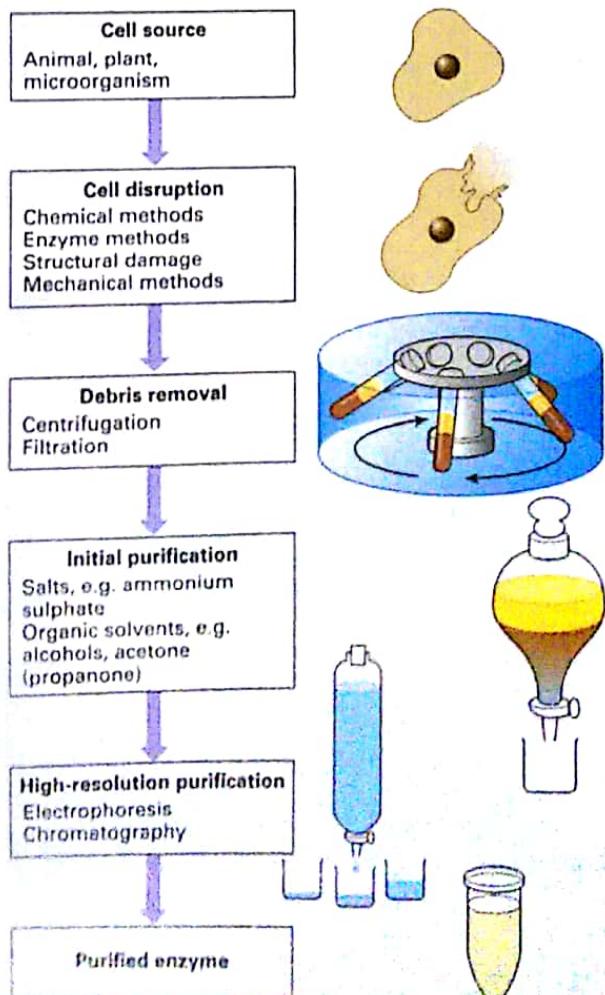


Figure 1 Steps in the purification of an intracellular enzyme produced by cultured microbes.

## Isolating the enzyme

Enzymes used in industry are usually needed in a pure form, so must be isolated from the microbial cells. To obtain an intracellular enzyme, the microbe cells are harvested from the culture and broken open. The resultant mixture is centrifuged to remove large cell fragments, and the enzyme is precipitated from solution by a salt (such as ammonium sulphate) or an alcohol (such as propan-2-ol). The enzyme can be purified (figure 1) by techniques such as electrophoresis or column chromatography (see appendix). Extracellular enzymes are present in the culture outside the microbial cells. They are often soluble in water, so they can be extracted from the culture medium and purified.

## Using whole cells

In some circumstances it is not possible to use isolated enzymes to carry out an industrial process, and whole cells are used instead. This may happen, for example, when it is too difficult or expensive to extract and purify an intracellular enzyme, or when two or more enzymes are required to work together. When using whole cells, the substrate has to diffuse into the cells before the reaction takes place. The product may diffuse out of the cells, or the cells may be disrupted to release it. The product can then be extracted and purified.

## Improving the enzyme: enzyme stability

The **stability** of an enzyme refers to its ability to retain its tertiary structure (its three-dimensional shape) so that it continues to be effective under a wide range of conditions. Enzyme stability is a key factor in the industrial use of enzymes.

Most enzymes are relatively unstable and work only within narrow ranges of temperature and pH. They quickly become denatured when subjected to unnatural environments. Many industrial processes require enzymes to work in the presence of chemicals such as organic solvents, at high temperatures, and extremes of pH, conditions which cause most enzymes to lose their shape and become inactive. It is possible to overcome this problem by taking advantage of microbes that live naturally in harsh environments.

Organisms evolve to produce enzymes that are adapted to their environmental conditions. Thermophilic bacteria living in hot volcanic springs, for example, produce **thermostable** enzymes that do not denature at high temperatures: they work effectively in the temperature range 65–75 °C. These enzymes are also resistant to organic solvents and tolerate a wide range of pH. The gene for the thermostable enzyme has been isolated from thermophilic bacteria and transferred to *Bacillus subtilis*, a microbe that can be used in industrial processes. This enables a thermostable version of the desired enzyme to be produced.

### Improving the enzyme: immobilisation

Unstable enzymes may be **immobilised** by being attached to or located within an insoluble support (figure 2). Once attached, an enzyme's stability is increased, possibly because its ability to change shape is reduced.

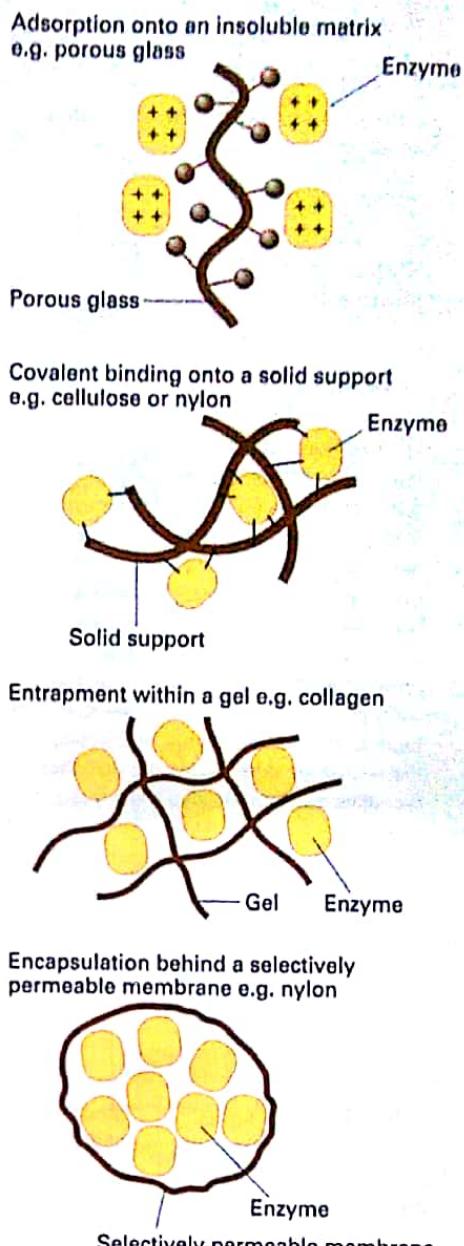
Compared with free enzymes, immobilised enzymes have several other advantages:

- Immobilised enzymes can be recovered easily from a reaction mixture and reused over and over again.
- Because immobilised enzymes are held in an inert (unreactive) matrix, the reaction products are not contaminated by the enzyme.
- Enzymes that have been immobilised by incorporating them into a solid material such as a nylon membrane can be manipulated easily; they can, for example, be placed at a precise position in a reaction system, and they can be added or removed from a reaction system easily, giving greater control over the reaction.
- Immobilising enzymes makes continuous production of a substance easier. For example, reactants could be added to the top of a column containing immobilised enzymes. The reactants trickle down through the enzymes, and the product is collected continuously as it leaves the bottom of the column.

Table 1 shows the four main types of enzyme immobilisation. Similar methods can be used to immobilise whole cells.

**Table 1** Advantages and disadvantages of different types of immobilisation.

| Type of immobilisation   | Advantages   | Disadvantages  |
|--|--|--|
| Adsorption onto an insoluble matrix (e.g. glass)                   | Easy to immobilise; relatively safe  | Becomes detached easily; susceptible to pH and temperature changes   |
| Covalent binding to a solid support (e.g. cellulose or nylon)      | Fairly resistant to pH and temperature changes                               | May require the use of chemicals that can damage the enzyme  |
| Trapping within a gel (e.g. collagen, alginate beads)              | Gentle conditions help preserve the enzyme; no chemical change in the system | Enzyme may leak away; enzyme substrate has to diffuse into the gel   |
| Encapsulation behind a selectively permeable membrane (e.g. nylon) | Little chance of the enzyme leaking away                                     | May be difficult to bring the enzyme substrate in close contact with the enzyme; encapsulation may damage the enzyme |



**Figure 2** Methods of enzyme immobilisation.

### QUICK CHECK

- 1 List three advantages and two disadvantages of using enzymes rather than inorganic catalysts in industrial processes.
- 2 What is the role of alcohol or ammonium sulphate during the extraction of enzymes?
- 3 Why is the thermostability of enzymes so important for many industrial processes?

### Food for thought

Surprisingly, cats have difficulty digesting cow's milk. They lack the enzyme lactase which catalyses the breakdown of lactose into galactose and glucose. Suggest how immobilised enzymes can help.

## OBJECTIVES

By the end of this spread you should be able to:

- detergents
- the food industry
- biosensors.

### Fact of life

The fact that the active sites of enzymes conform to the shape of specific molecules is used to detect illicit drugs. In the 1990s, a gel was developed that changes colour in the presence of heroin. The gel contains two enzymes. The first comes from the bacterium *Rhodococcus* and it breaks heroin down to morphine. The second enzyme comes from another bacterium, *Pseudomonas*, and breaks the morphine down in such a way that it causes a colourless dye to turn red.

## Detergents

Because of their ability to catalyse specific reactions at normal temperatures and pressures, enzymes are of great commercial and industrial importance (table 1). Enzymes have been used for centuries in cheese-making, brewing, baking, and the leather industry. Although they were used in washing powders at the beginning of the twentieth century, it was not until the 1950s and 1960s that Novo industries of Denmark developed an enzyme stable enough for use in a general washing powder. The enzyme, **subtilisin**, was obtained from the bacterium *Bacillus subtilis*. It is a protein-digesting enzyme (protease) which is active up to 60 °C.

Table 1 Examples of industrial applications of enzymes.

| Application           | Enzyme     | Use   |
|-----------------------|------------|---|
| Baking                | Amylase    | Catalyses breakdown of flour starch to sugar    |
| Biofuel               | Cellulases | Break down cellulose to sugars for fermentation |
| Biological detergents | Lipases    | Remove fatty stains                             |
| Brewing               | Proteases  | Remove cloudiness from stored beers             |
| Confectionary         | Invertase  | Helps make soft centres in chocolates           |
| Dairy industry        | Rennin     | Coagulates milk in cheese manufacture           |
| Fruit juices          | Pectinases | Clarify juices                                  |
| Meat industry         | Trypsin    | Tenderises meat and pre-digests baby foods      |
| Paper industry        | Ligninases | Break down lignin to soften paper               |
| Rubber industry       | Catalase   | Helps convert latex into foam rubber            |

## Food and drink

The food and drinks industry depends heavily on enzymes. Enzymes produced by yeast have been used for thousands of years in brewing and baking (see spread 17.10). The word 'enzyme' actually means 'in yeast'. It was first used to describe the chemicals that enable yeast to convert sugar into alcohol during fermentation.

High fructose syrups contain fructose and glucose in roughly equal proportions. The high fructose syrups are in greater demand than pure glucose as food and drink sweeteners, because fructose is sweeter than glucose. Therefore, if glucose can be converted into fructose, its commercial value is increased greatly. The production of high fructose syrups by enzyme technology not only helps satisfy the sweet tooth of millions of consumers, but also helps the food industry solve a waste-disposal problem. The raw material for high fructose syrups is starch (especially corn starch in the USA), a major waste product of the food industry. The conversion of starch to high fructose syrups uses a combination of three main enzymes (figure 1): alpha amylase and glucoamylase (to convert starch to glucose), and glucose isomerase (to change glucose to its isomer, fructose). Alpha amylase and glucose isomerase are obtained from bacteria belonging to the genus *Bacillus*, and glucoamylase is obtained from the fungus *Aspergillus niger*.

**Pectinases** are enzymes used to clarify fruit juices. Plant cell walls contain polysaccharides called pectins, and these make fruit juice viscous and difficult to extract. The juice is also cloudy and the flavour may deteriorate. To overcome this, the pectins are hydrolysed using pectinases.

**Cellulases** are enzymes produced by microbes that live in the guts of ruminants such as cattle. These enzymes are used to break down lignin (see spread 2.7) in pulp so that the lignin can be removed during the manufacture of paper. Lignin discolours paper and makes it rougher. Removing the lignin is a cheaper and less environmentally damaging alternative to bleaching the pulp with chlorine.

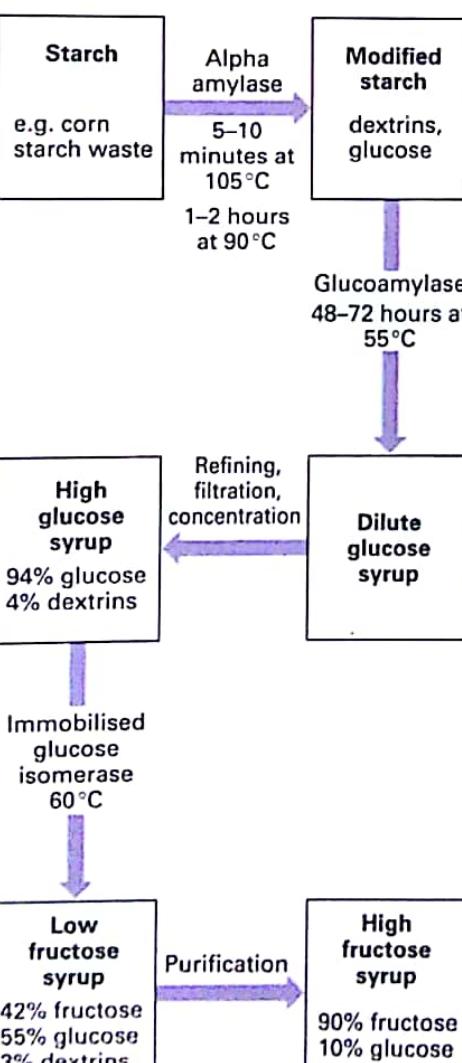


Figure 1 The enzymes used in the production of high fructose syrup.

Naturally occurring enzymes can catalyse only a limited number of reactions. Most of these enzymes are denatured by the organic solvents and high temperatures that are an integral part of many industrial processes. Existing enzymes are therefore being genetically modified to work under harsh industrial conditions and, using computer-aided design, completely new enzymes are being created to catalyse reactions that do not occur in nature.

### Biosensors

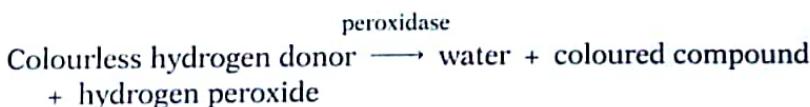
The fact that enzymes bind with a specific substance is used in diagnostic tools called biosensors. A **biosensor** usually consists of a **receptor** which uses a biochemical reaction to detect a specific substance or condition, a **transducer** which converts a biochemical signal into an electrical signal, and a system for converting the electrical signal into a reading or measurement (figure 2). The receptor is usually an immobilised living cell, tissue, or enzyme (see spread 3.6).

Biosensors are used extensively in industry, forensic science, agriculture, environmental science, and medicine to monitor specific chemicals accurately and rapidly.

Some biosensors use a colour change to detect a specific substance. One common biochemical device enables diabetics to monitor their own blood glucose levels. It consists of a plastic strip with a piece of special filter paper at one end. The filter paper contains two immobilised enzymes, glucose oxidase and a peroxidase, and a colourless hydrogen donor. When the paper is dipped in blood containing glucose, the glucose oxidase catalyses the conversion of the glucose to gluconic acid and hydrogen peroxide:



The peroxidase enzyme then catalyses a reaction between the hydrogen peroxide produced and the colourless hydrogen donor. The product of this second reaction is a coloured compound.



The type and intensity of the colour indicates the concentration of glucose in the blood.

### QUICK CHECK

- What is subtilisin?
- Why is the commercial value of glucose increased by converting it to fructose?
- What is the function of a transducer in a biosensor?

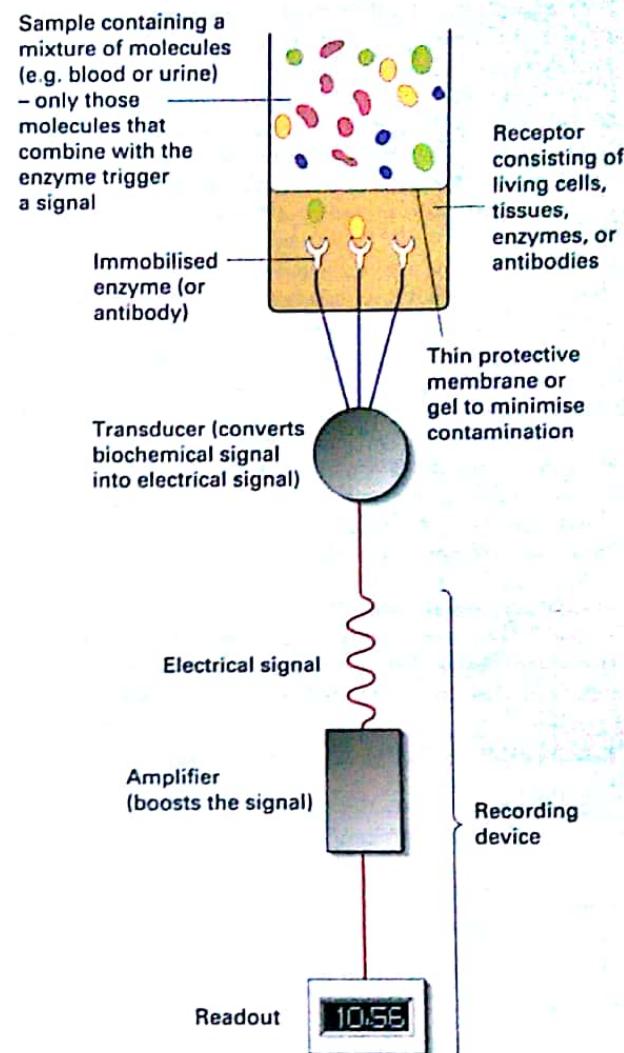


Figure 2 Plan of a typical biosensor.

### Food for thought

- One of the major uses of enzymes is in detergents. Suggest:
- why the first biological detergents were withdrawn from the market
  - which fabrics may be damaged by prolonged soaking in biological detergents
  - how biological detergents affect domestic energy consumption.

## Summary

**Metabolism** refers to the chemical reactions that take place in living organisms. There are two main types of reaction: **anabolic reactions** result in synthesis whereas **catabolic reactions** result in breakdown. Metabolic reactions obey the **laws of thermodynamics**: they neither create nor destroy energy, but when they transform energy from one form to another they always produce heat.

Before a metabolic reaction can get underway, **activation energy** is required to overcome an **energy barrier**. The amount of activation energy required depends on the type of reaction and the conditions in which it takes place. **Enzymes** are proteins which act as **biological catalysts**. They speed up specific chemical reactions by lowering the activation energy. The activity of an enzyme depends on the shape of its active site. According to the **lock-and-key theory**, the shape of the active site is rigid and fits that of its substrate precisely. In contrast, the **induced-fit theory** suggests that the active site is not rigid and that it takes up its most effective shape after binding with its substrate. In either case, the substrate fits neatly onto the active site to form an **enzyme-substrate complex**.

Factors affecting the rate of enzyme-catalysed reactions include **substrate concentration**, **enzyme concentration**, **incubation**

**time**, **temperature**, **hydrogen ion concentration**, **cofactors** and **inhibitors**. Extreme temperatures and pH can inactivate an enzyme by changing its **tertiary structure** so that the shape of active site does not fit that of its substrate. This process is called **denaturation**. Cofactors are non-protein substances, inorganic **activators** or organic coenzymes that enable enzymes to work better. Inhibitors slow down enzyme-catalysed reactions.

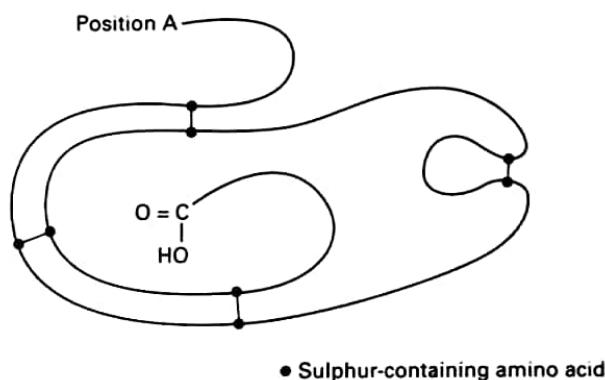
**Competitive inhibitors** compete with the normal substrate for the active site of the enzyme whereas **non-competitive inhibitors** alter the shape of the active site after binding onto another site on the enzyme. Inhibitors may be reversible or irreversible. **Irreversible inhibitors** include metabolic poisons such as cyanide. **Reversible inhibitors** include the final products of reaction pathways which regulate their own production by **end-product inhibition**.

Enzymes are classified into six major groups: **oxidoreductases**, **transferases**, **hydrolases**, **lysases**, **isomerases**, and **ligases**.

Enzymes have many industrial applications. They are used in the food and drinks industry, in detergents, and in **biosensors**. In many industrial processes, **immobilisation** of enzymes onto insoluble surfaces has improved their usefulness. The study of industrial enzymes and their uses is called **enzyme technology**.

## PRACTICE EXAM QUESTIONS

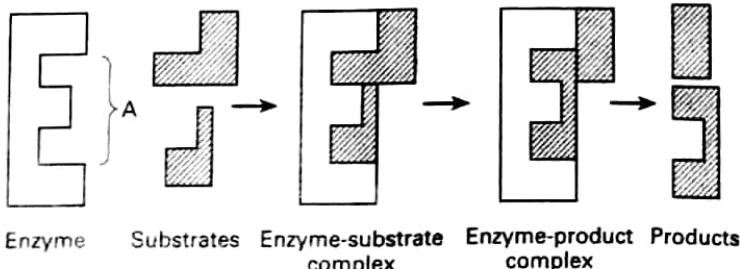
- 1 The diagram shows the tertiary structure of a molecule of the enzyme RNAase.



- a What is the name of the chemical group found in position A? [1]
- b i Explain what is meant by the *tertiary structure* of a protein. [1]
- ii The chemical mercaptoethanol breaks disulphide bonds (bridges). Describe and explain what would happen to the enzyme activity of RNAase if it were treated with mercaptoethanol. [3]

[Total 5 marks]

- 2 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. [Total 5 marks]

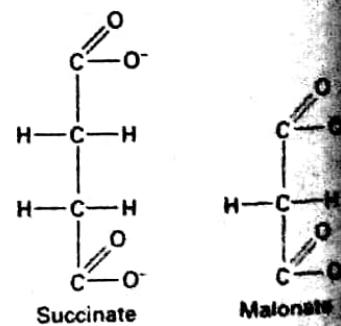
- 3 The **turnover number** of an enzyme is defined as the number of substrate molecules converted to product by one molecule of enzyme in one minute. In an experiment carried out at 20 °C the turnover number for an enzyme was found to be 2500 at the start of the experiment but dropped to 1000 after 5 minutes.

- a i Suggest why the turnover number decreased after 5 minutes.  
ii How would you expect the turnover number to differ from 2500 at the start of an identical experiment but carried out at 30 °C? Explain your answer.  
b Explain why it would be important to have a control in the experiment at 20 °C and at 30 °C. [Total 5 marks]

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

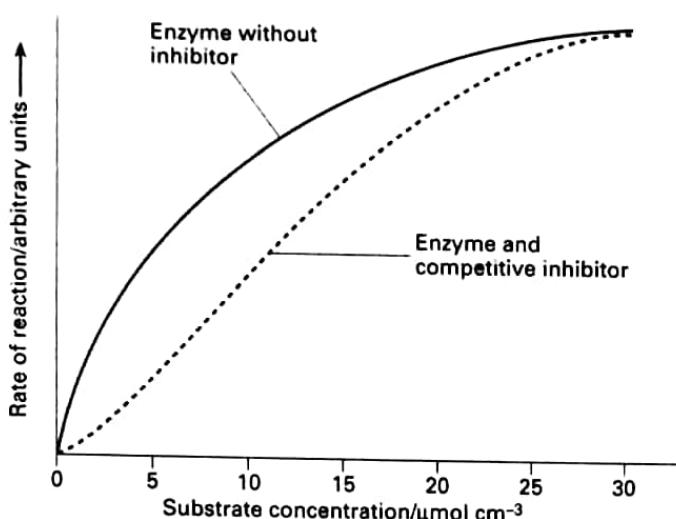
- a Use your knowledge of enzyme structure to explain why succinate dehydrogenase catalyses this reaction only.

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

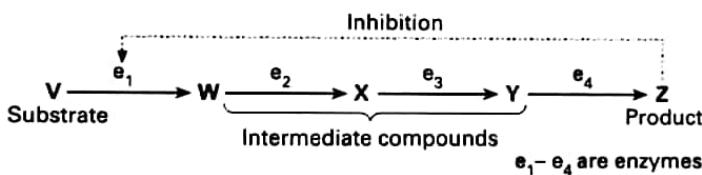


Use the information in the diagram to explain how malonate inhibits the enzyme. [Total 4 marks]

- 5 The graph shows the results of an investigation into the effect of a competitive inhibitor on an enzyme-controlled reaction over a range of substrate concentrations.



- a Give one factor which would need to be kept constant in this investigation. [1]
- b i Explain the difference in the rates of reaction at the substrate concentration of  $10 \mu\text{mol cm}^{-3}$ . [2]
- ii Explain why the rates of reaction are similar at the substrate concentration of  $30 \mu\text{mol cm}^{-3}$ . [1]
- c The diagram represents a metabolic pathway controlled by enzymes.

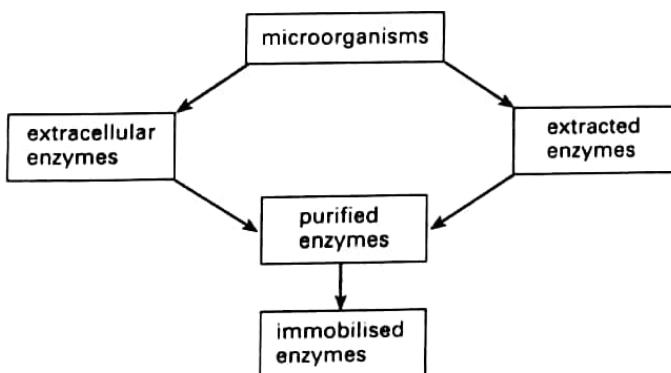


- i Name the type of control mechanism which regulates production of compound Z. [1]
- ii Explain precisely how an excess of compound Z will inhibit its further production. [2]

[Total 7 marks]

- 6 The flow diagram shows two ways in which enzymes may be produced for industrial processes.

- a Explain the advantages of using immobilised enzymes. [4]



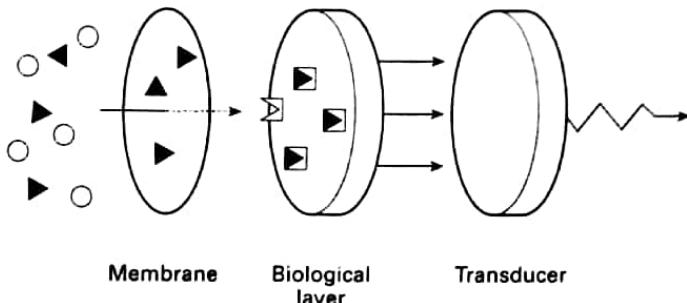
- b i Describe three ways by which enzymes or cells may be immobilised. [3]
- ii A bacterial enzyme used widely in the production of sweeteners for soft drinks is glucose isomerase which converts glucose to the much sweeter fructose. Outline how you would test whether

different ways of immobilising this enzyme affect the rate at which it converts glucose to fructose. [3]

- c Suggest why enzymes used for industrial processes may be obtained from microorganisms that are thermophilic (able to grow at high temperatures). [2]

[Total 12 marks]

- 7 The diagram shows the main components of a glucose biosensor.



- a i Name the receptor molecules in the biological layer. [1]
- ii What is the function of these receptor molecules? [1]
- b What is the function of the transducer? [1]
- c A glucose biosensor can be used to measure blood glucose levels in diabetics. Suggest two advantages of using a biosensor rather than the Benedict's test in measuring blood glucose levels. [2]

[Total 5 marks]

- 8 Answers should be written in continuous prose. Credit will be given for biological accuracy, the organisation and presentation of the information and the way in which the answer is expressed.

Read the following passage.

Because they are proteins, enzymes are sensitive to changes in pH and temperature. The effects of temperature are complex but involve a balance between the movement of molecules and the structural stability of proteins. This results in an optimum rate of reaction. Inhibitors work by influencing the active site of the enzyme molecule, either directly or indirectly.

- a Describe how polypeptide chains may be folded to form protein molecules. [5]
- b Explain the effects of temperature on enzyme activity. [6]
- c Explain how inhibitors can alter the rate of enzyme-controlled reactions by acting either directly or indirectly. [6]

*Quality of language.* [3]

[Total 20 marks]

- 9 Explain why

- i enzymes which break down starch and cellulose are called hydrolases. [1]
- ii amylase will break down starch but it will not break down cellulose. [3]

[Total 4 marks]

# 4

## Cells

### CELL THEORY

#### 4.1

##### OBJECTIVES

By the end of this spread you should be able to:

- describe the main ideas of the cell theory
- compare the structures of animal and plant cells as seen with a light microscope.

##### Fact of life

Cells do not function in isolation. They have to be able to communicate with each other. Communication often involves **cell signalling**: the release by one cell of one or more substances that transmit information to other cells. Cell signalling occurs between unicellular organisms, for example prior to mating. It is also an essential feature in multicellular organisms, for example, enabling the different cells in a tissue to function in a coordinated manner and to respond appropriately to their microenvironment.

Calcium is one of many chemical signals that play an important role in regulating cellular activity and orchestrating the complexities of cellular communication. Disruption of signalling pathways in which calcium plays a key part can lead to diseases such as hypertension, cardiac arrhythmia and heart failure, cancer, and depressive illness. Cell signalling often involves complex multi-component signalling pathways that provide opportunities for feedback, signal amplification, and interactions between different signalling substances and different signalling pathways.

#### The discovery of cells

Cells were discovered in 1665 by the English scientist and inventor Robert Hooke. Hooke designed his own compound light microscope (see spread 4.2) to observe structures too small to be seen with the naked eye. Among the first structures he examined was a thin piece of cork (the outer surface of bark from a tree). Hooke described the cork as being made of hundreds of little boxes, giving it the appearance of a honeycomb (figure 1). He called these little boxes **cells**.

It soon became clear that virtually all living things are made of cells, and that these cells have certain features in common.

#### The cell theory

The concept that cells are the basic units of life became embodied in a theory called the **cell theory**, which embraces the following main ideas:

- cells form the building blocks of living organisms
- cells arise only by the division of existing cells
- cells contain inherited information which controls their activities
- the cell is the functioning unit of life; metabolism (the chemical reactions of life) takes place in cells
- given suitable conditions, cells are capable of independent existence.

#### A typical animal cell

Figure 2 shows the structure of a typical animal cell as seen with a light microscope.

- The cell has a **cell surface membrane** which encloses the cell contents.
- The contents consist of a central ball-shaped **nucleus** surrounded by material called **cytoplasm**.
- The nucleus contains a fibrous material called **chromatin**.
- This condenses to form **chromosomes** during cell division.
- Chromatin contains DNA, the inherited material which controls the various activities inside the cell.
- Scattered within the cytoplasm are **mitochondria**, small rod-like structures. They have been described as the 'power-houses' of the cell because they supply energy.
- Smaller dots within the cytoplasm are particles of stored food. Many consist of glycogen, which is a food storage polysaccharide.

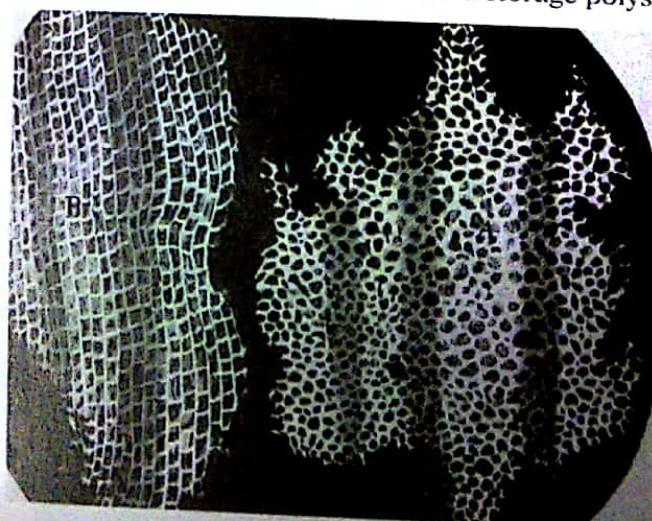
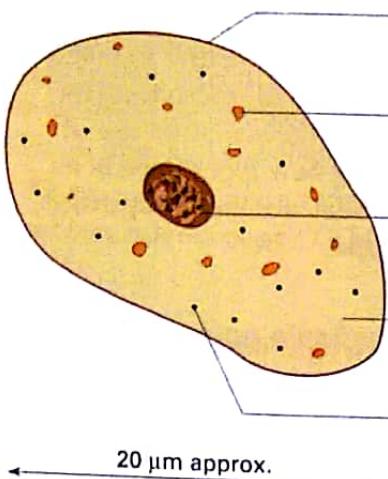
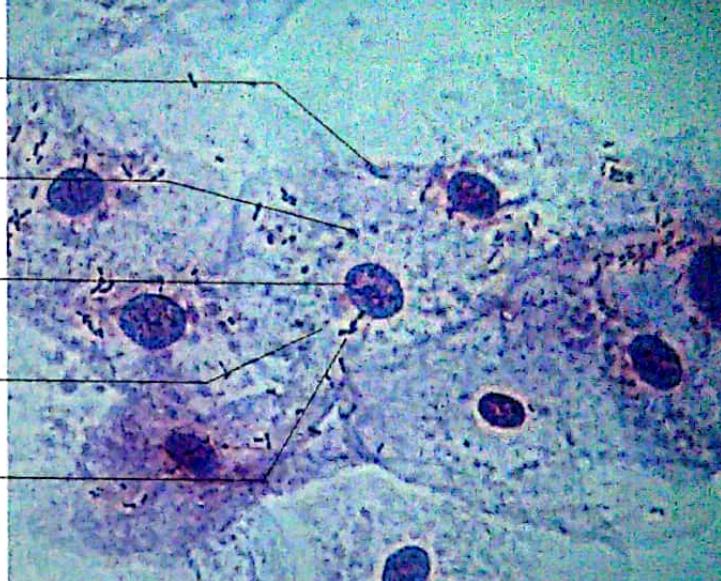


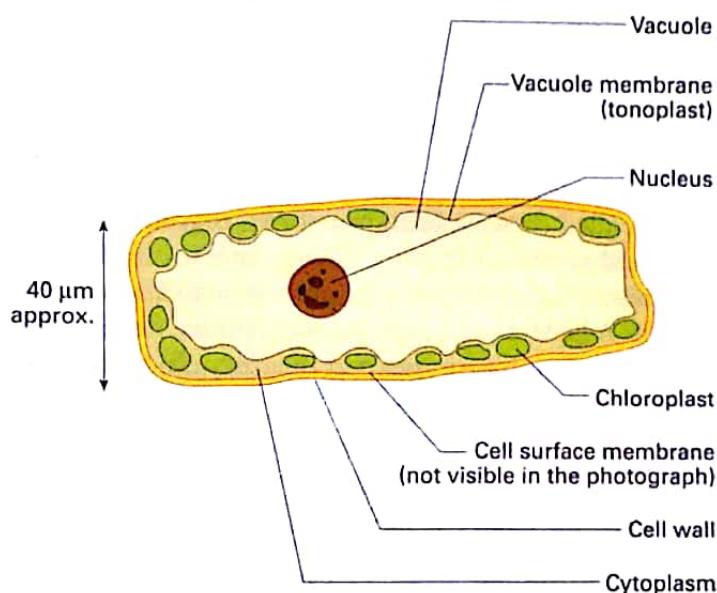
Figure 1 Cork cells drawn by Robert Hooke for his book *Micrographia*, first published in September 1665 by the Royal Society. In the book, Hooke was the first to use the word 'cell' as a biological term.



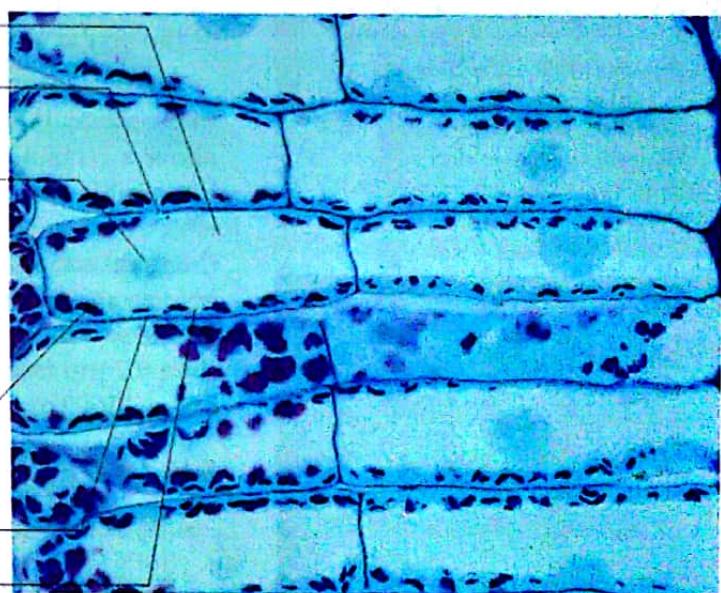
**Figure 2 (a)** Drawing of a typical animal cell as seen with a light microscope. Animal cells exist in many shapes and sizes. Most share the features shown in this diagram. They may also have vacuoles, but these are not permanent features as they are in plant cells.



**Figure 2 (b)** Some cheek cells as they appear under the light microscope ( $\times 2000$ ).



**Figure 3 (a)** Drawing of a typical plant cell as seen with a light microscope. A cell wall surrounds the cell surface membrane. The cell wall is made of cellulose, a tough rubbery material which is completely permeable to water. In some plant cells, the cellulose cell wall becomes impregnated with lignin, which makes it impermeable.



**Figure 3 (b)** Some palisade cells from a leaf as they appear under the light microscope ( $\times 500$ ). Palisade cells form a photosynthetic layer just beneath the surface of many leaves.

### A typical plant cell

Figure 3 shows the structure of a typical plant cell as seen with a light microscope. Like an animal cell, it has a cell surface membrane, cytoplasm, and a nucleus. However, plant cells differ from animal cells in several ways:

- Most plant cells have a large sap-filled cavity called the **vacuole**. Sap is a watery fluid containing salts and sugars. The vacuole is surrounded by a membrane called the **tonoplast**.
- The cytoplasm contains **starch grains**, the food storage products of plants.
- Many plant cells have **chloroplasts** in the cytoplasm. These contain the pigments used in photosynthesis. **Chlorophyll**, which is green, is the main pigment. Chloroplasts occur only in the parts of plants exposed to light – the green parts. They are absent from underground structures such as roots.

### QUICK CHECK

- 1 Briefly state the main concept of the cell theory.
- 2 Using figures 2 and 3, list the features:
  - a that only animal cells have
  - b that only plant cells have
  - c that both animal and plant cells have,

### Food for thought

Suggest why red blood cells (spread 7.8) appear to contradict the cell theory.

## O B J E C T I V E S

By the end of this spread you should be able to:

- describe the main features of a light microscope and an electron microscope
- distinguish between the terms magnification and resolving power
- give the approximate size of different biological structures using an appropriate unit of measurement.

### Fact of life

Since the invention of the first transmission electron microscopes (TEMs), several different types of electron microscope have been developed with special capabilities. For example, the scanning tunnelling microscope (STM) was developed in the 1980s to create images of surfaces at the atomic level. It earned its inventors Gerd Binnig and Heinrich Rohrer the Nobel Prize in Physics in 1986. The STM can achieve magnifications of more than 100 million. It has been used to view biological structures, such as DNA (figure 1), that are too small to be resolved by TEMs.

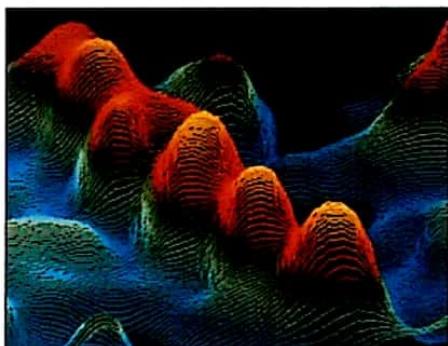


Figure 1 A scanning tunnelling micrograph. The orange and yellow peaks represent ridges of the DNA double helix (false colour;  $\times 2500000$ ).

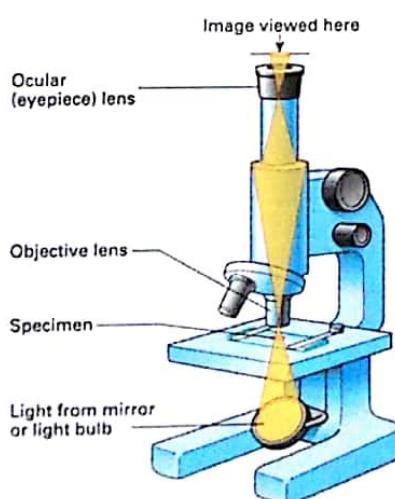


Figure 2 A compound light microscope has two lens systems: the objective lens

A microscope is used to produce a magnified image of an object or specimen. Anton van Leeuwenhoek (1632–1723) was the first to invent a microscope powerful enough to explore the world of microbes. His discoveries stimulated an explosion of interest in the scientific use of microscopes. Since the eighteenth century many new types have been invented, of which the most commonly used today are the **compound light microscope** and the **electron microscope**.

### The compound light microscope

The compound light microscope is also called a light microscope or optical microscope. Light passes through a specimen (the object) and then through two sets of glass lenses, the objective lens and the ocular or eyepiece lens. The lenses refract (bend) the light to give a magnified image of the object (figure 2). The image may be projected directly into the viewer's eye or onto photographic film. A photograph taken through a light microscope is called a **photomicrograph** or light micrograph.

### Magnification and resolution

The **magnification** of an instrument is the increase in the apparent size of the object. The total magnification of a compound microscope is worked out by multiplying the magnification of the objective lens by that of the ocular lens.

There is virtually no limit to the magnification produced by a light microscope; it depends on the power of the lenses used. However, above a certain magnification the image becomes blurred and it is impossible to distinguish structures lying close together. This limit of effective magnification is called the **resolving power** or **resolution** of the microscope. It is defined as the ability of a microscope to show two objects as separate. The resolving power of the light microscope is limited by the wavelength of light. A light microscope cannot resolve detail finer than  $0.2 \mu\text{m}$  (see figure 3). Light microscopes can magnify objects up to about 1500 times without losing clarity.

Table 1 Measurements and equivalents

|  |                               |                            |
|--|-------------------------------|----------------------------|
| 1 millimetre (mm)  | $= 10^{-3} \text{ metre (m)}$ | $= 1/1000 \text{ m}$       |
| 1 micrometre ( $\mu\text{m}$ )   | $= 10^{-6} \text{ metre (m)}$ | $= 1/1000000 \text{ m}$    |
| 1 nanometre (nm)   | $= 10^{-9} \text{ metre (m)}$ | $= 1/1000000000 \text{ m}$ |
| 1 metre (m) = $10^3 \text{ mm} = 10^6 \mu\text{m} = 10^9 \text{ nm}$ ; 1 kilometre (km) = $10^3 \text{ m}$ |                               |                            |

### Calculating magnifications

The **magnification** is the number of times larger an image is than the object (specimen). Therefore,

$$\text{Magnification} = \frac{\text{size of image}}{\text{size of object}}$$

It is essential that the same unit is used for the size of the image and the size of the object. For example, if an image measures 50 mm and the object measures 5  $\mu\text{m}$ , the size of the image should be converted to  $\mu\text{m}$ :

$$\text{Size of image} = 50 \text{ mm} = 50000 \mu\text{m}$$

$$\text{Therefore, magnification} = \frac{50000}{5} = 10000$$

Conversely, if the magnification is 50000 times, and the size of the image is 5 mm (5000  $\mu\text{m}$ ), the actual size of the object is equal to:

$$\frac{\text{Size of image}}{\text{magnification}} = \frac{5000}{50000} = 0.1 \mu\text{m}$$

## The electron microscope

Electron microscopes use a beam of electrons instead of a beam of light. Electron beams have a much smaller wavelength than light rays, so electron microscopes have greater resolving powers and can produce much higher effective magnifications than light microscopes. Figure 3 shows the sizes of objects that can be viewed with the naked eye, the light microscope, and the electron microscope.

There are two main types of electron microscope: the **transmission electron microscope** (TEM), and the **scanning electron microscope** (SEM).

### The transmission electron microscope

The TEM (Figure 4) is used to study the details of the internal structure (the ultrastructure) of cells. Extremely thin samples of the specimen are needed. To make these the specimen is supported in a resin block to prevent it collapsing during cutting, and is sliced with a diamond or glass knife. The section is then impregnated with a heavy-metal stain, such as osmium tetroxide.

As the beam passes through the specimen, electrons are absorbed by heavily stained parts but pass readily through the lightly stained parts. **Electromagnets** bend the electron beam to focus an image onto a fluorescent screen or photographic film. A photograph taken through an electron microscope is called an **electron micrograph**.

The most modern TEMs can distinguish objects as small as 0.2 nm. This means that they can produce clear images magnified up to 250 000 times. The magnification is varied by changing the strength of the electromagnets.

### The scanning electron microscope

The SEM is used to produce three-dimensional images of the surface of specimens. Electrons are reflected from the surface of a specimen stained with a heavy metal. This enables the SEM to produce images of whole specimens: cells, tissues, or even organisms.

Although electron microscopes have revolutionised cell biology, they have not completely replaced light microscopes. Light microscopes are used to examine living and unstained specimens. Preparation of specimens for electron microscopy is complicated and time-consuming. Electron microscopes are very expensive and can be used only to study dead specimens stained with heavy metal, which might well produce artefacts (see spread 4.5).

#### QUICK CHECK

- 1 How is the magnification varied in:
  - a a light microscope
  - b an electron microscope?
- 2 Why is the resolving power of an electron microscope so much better than that of a light microscope?
- 3 What is the approximate size of the smallest structure that can be observed with a light microscope?

#### Food for thought

Suggest which unit should be used when calculating the diameter of the DNA molecule in figure 1. From the information given in the figure, what is its actual diameter? Why might there be a discrepancy between the actual diameter and that estimated from the scanning tunnelling micrograph?

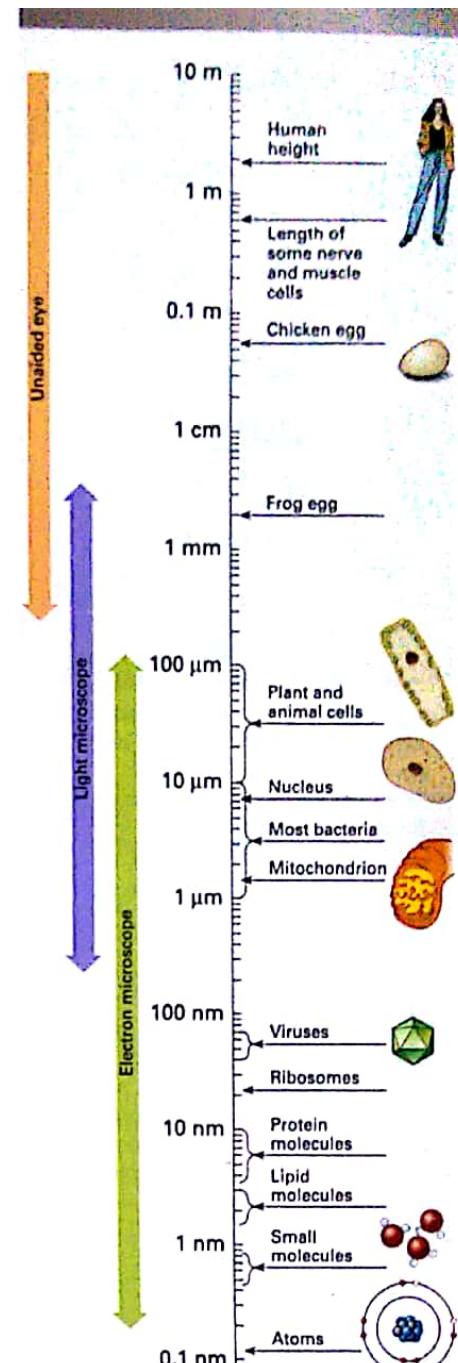


Figure 3 The sizes of various biological structures.

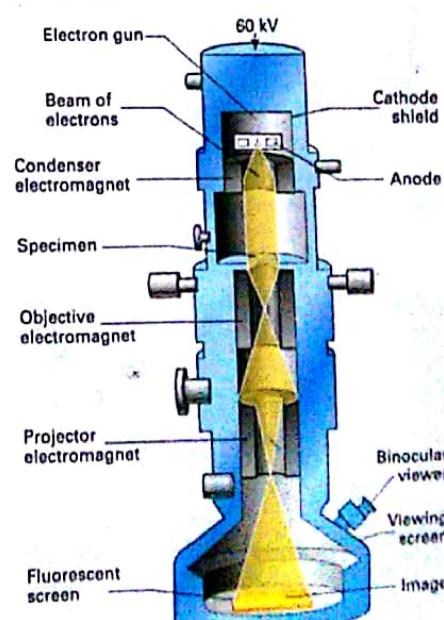


Figure 4 A transmission electron microscope. The beam of electrons is emitted from the electron gun, a heated filament. Electromagnets bend the beam.

## OBJECTIVES

By the end of this spread you should be able to:

- describe the ultrastructure of animal cells and the functions of their organelles.

## Fact of life

Protein degradation is a vital function of cells. It eliminates damaged and unwanted proteins, removes excess enzymes, and helps to supply amino acids for fresh protein synthesis.

**Proteasomes** are organelles that break down unwanted, abnormal, or misfolded endogenous proteins (that is, proteins synthesised inside the cell). A typical human cell contains about 30 000 proteasomes. Each proteasome is a protein complex folded to form a barrel-shaped structure open at both ends. Degradation takes place on the internal surface of the proteasome, shielded from the rest of the cell. Proteins to be degraded can gain entry into the proteasome only if they are tagged with ubiquitin, a small regulatory protein that has been found in nearly all eukaryotic cells. A molecular lock at one end of the 'barrel' recognises the tagged proteins and admits them for disassembly once the ubiquitin label has been removed. The peptides and amino acids formed by degradation are released from the other end of the proteasome. Proteasomes require energy from ATP to work.



Figure 2 False-colour transmission electron micrograph of part of the nucleus of an animal cell ( $\times 4000$ ).

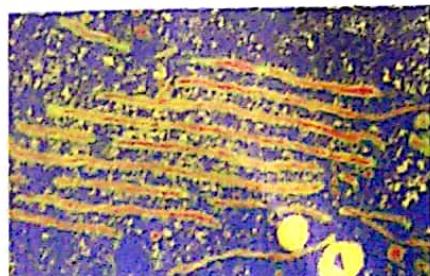


Figure 3 False colour transmission electron micrograph of mammalian smooth endoplasmic reticulum ( $\times 4500$ ). (In figure 2 above, the red areas covered with black dots are rough endoplasmic reticulum.)

## THE ULTRASTRUCTURE OF ANIMAL CELLS

A typical animal cell viewed under a light microscope appears to have a simple structure: a ball-shaped nucleus is surrounded by a relatively uniform clear cytoplasm, which is bounded by a cell surface membrane. However, the electron microscope reveals that the cell is highly complex and contains structures called **organelles** which carry out specific functions within the cell. The detailed internal structure of a cell is known as the **ultrastructure** (figure 1).

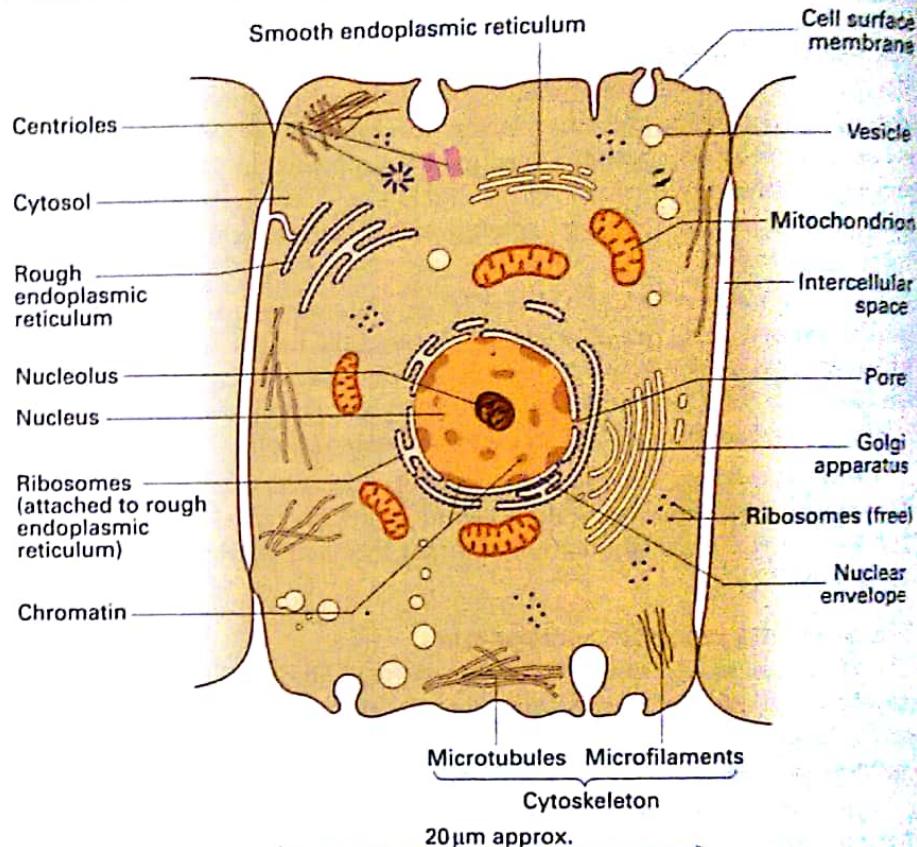


Figure 1 A generalised animal cell showing structures visible under an electron microscope. There is an enormous variety of animal cells. The size and form of each cell is adapted to carry out specific functions. However, despite differences in detail most animal cells have the same basic ultrastructure.

The **cell surface membrane** is selectively permeable and controls the exchange of substances between the cell and its environment. The membrane consists mainly of lipid and protein (see spread 4.6).

The **cytoplasm** refers to all the living parts of the cell, excluding the nucleus. It consists of membrane-bound organelles and the **cytosol**, the fluid part of the cytoplasm. The cytosol contains numerous small molecules in solution and large molecules in suspension.

The **cytoskeleton** consists of microtubules, filaments, and fibres, which give the cytoplasm physical support. The cytoskeleton is also involved in cell movement.

## The nucleus

The **nucleus** (figure 2) is the largest cell organelle. It is enclosed by a **nuclear envelope**, which consists of two membranes perforated by **nuclear pores**. These pores control the exchange of materials between the nucleus and cytoplasm. The nucleus contains **chromatin**, the form that chromosomes take when the cell is not dividing. Chromatin contains DNA, the molecule of inheritance which controls the activity of the cell. Two forms of chromatin are visible: **euchromatin** stains lightly and is thought to contain active DNA; **heterochromatin** stains deeply and is thought to contain inactive DNA. The nucleus also contains one or more **nucleoli**. These manufacture ribosomes (see below).

## The endoplasmic reticulum and ribosomes

The **endoplasmic reticulum (ER)** (figure 3) is a system of flattened membrane-bound sacs forming tubes and sheets. The sacs, called **cisternae**, are full of fluid. Some regions of the ER are covered with bead-like structures called ribosomes. This type of ER is called **rough ER (RER)**. Smooth ER (**SER**) has no ribosomes.

- The cisternae of the RER transport proteins made by the ribosomes.
- The SER synthesises, secretes, and stores carbohydrates, lipids, and other non-protein products.
- The SER of liver cells also contains enzymes which break down many chemicals.

**Ribosomes** are tiny organelles consisting of two subunits, one smaller than the other. Ribosomes are made of roughly equal parts of protein and RNA, and they are the sites of protein synthesis. They may be either attached to the RER or may lie free in the cytoplasm.

## Mitochondria

**Mitochondria** (figure 4) are cigar-shaped organelles, usually 1–2 µm long. They are involved in generating ATP by aerobic metabolism. Each mitochondrion is bounded by an envelope consisting of two membranes. To maximise its surface area, the inner membrane is deeply folded into shelves called **cristae**. The watery matrix inside contains DNA, ribosomes, calcium phosphate granules, and enzymes.

## The Golgi apparatus and lysosomes

The **Golgi apparatus** or Golgi body (figure 5) consists of a stack of flattened membrane-bound sacs, called **cisternae**. New membrane is continuously added to one end of the Golgi apparatus and buds off as vesicles (small sacs) at the other end. The stack often forms an extensive network in animal cells. (In plant cells it is better defined and is called a **dictyosome**.)

The Golgi apparatus functions as a processing and packaging structure. It enables cell materials such as enzymes to be secreted from the cell in vesicles, and is involved in the formation of **lysosomes**.

Lysosomes (figure 6) contain enzymes that break down proteins derived from outside the cell, eliminate worn-out mitochondria and other redundant organelles, digest the contents of vacuoles ingested by white blood cells, and are involved in autolysis and apoptosis. **Autolysis** is the process by which a cell self-destructs when it dies or is injured. **Apoptosis** involves a genetically programmed series of biochemical events that leads to cell death (see spread 4.10). It occurs during the normal development of multicellular organisms, for example, when a tadpole loses its tail.

Other structures commonly found in animal cells include **centrioles**, **flagella**, and **cilia**. Like the cytoskeleton, these all consist of microtubules. Centrioles are involved in cell division (see spread 4.11). Cilia and flagella probably originated as locomotory structures in single-celled organisms (see spread 21.4).



Figure 4 False-colour scanning electron micrograph of a mitochondrion (x2000).



Figure 5 False-colour transmission electron micrograph of Golgi apparatus (x4000).

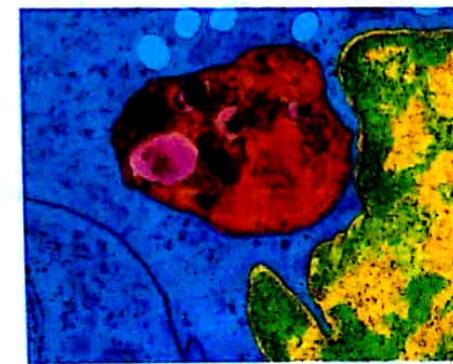


Figure 6 Transmission electron micrograph showing a lysosome (x20000). The lysosome (red) is a simple spherical sac bound by a single membrane. It contains hydrolytic enzymes which break down intracellular materials.

## QUICK CHECK

- 1 Which organelle:
  - carries out aerobic respiration
  - is the site of protein synthesis
  - contains hydrolytic enzymes
  - contains chromatin?

## Food for thought

Organelles are defined as distinct structures in a cell in which or on which certain functions and processes are localised. They acquired their name because they were regarded as analogous to organs in a multicellular organism (organelle means 'little organ'). For example, the cell surface membrane is regarded by some biologists as an organelle because it is analogous to the skin. Suggest which parts of the human body carry out equivalent functions to each of the other organelles.

## OBJECTIVES

By the end of this spread you should be able to:

- describe the ultrastructure of a typical plant cell
- describe the ultrastructure of a typical bacterial cell
- distinguish between prokaryotes and eukaryotes.

## Fact of life

Mitochondria, chloroplasts, and bacteria share an uncanny resemblance to each other: they all self-replicate, and they all contain circular DNA and similar ribosomes. These striking similarities led to the theory of **endosymbiosis**. This theory suggests that mitochondria and chloroplasts evolved from bacteria. These bacteria were endosymbionts – they were whole organisms that lived inside other cells in a mutually beneficial relationship. Eventually, the relationship became permanent. The large host cell became a eukaryotic cell containing chloroplasts derived from the photosynthesising bacteria and/or mitochondria derived from the bacteria that could use oxygen for respiration. Cells having both types of bacteria became plant cells; those with only the respiring bacteria became animal cells.

It is easy to imagine how the original relationship could be mutually beneficial. The host cell could provide shelter, some protection against predators, and raw materials for its smaller partners. The ancestral chloroplasts could use sunlight and simple chemicals to provide the host with organic molecules, and the ancestral mitochondria could provide the cell with a bountiful supply of ATP.

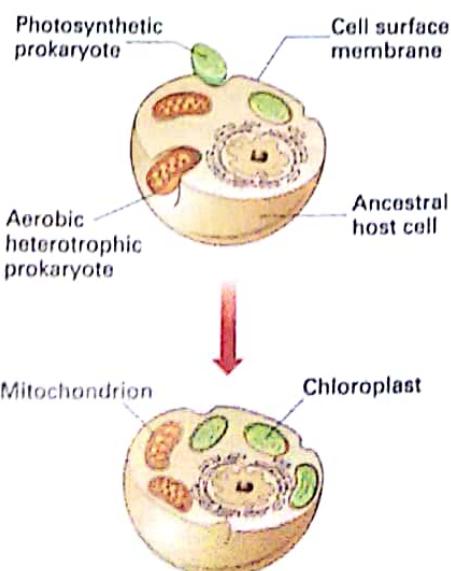


Figure 1 Mitochondria and chloroplasts may have evolved from endosymbiotic bacteria.

## THE ULTRASTRUCTURE OF PLANT AND BACTERIAL CELLS

A typical plant cell has many features in common with a typical animal cell (figure 2). It has mitochondria, ribosomes, an endoplasmic reticulum, Golgi apparatus, and a well defined nucleus bounded by a nuclear membrane. However, there are differences between plant and animal cells:

- Each plant cell is surrounded by a rigid cellulose cell wall. This gives plant cells a more uniform and regular shape than that of animal cells.
- Animal cells have to obtain their food from an outside source. Many cells in the green parts of plants contain chloroplasts, which enable plants to make their own food by photosynthesis.
- Plant cells may contain a large fluid-filled sac called a vacuole. Vacuoles occur in some animal cells, but they are not usually permanent features.

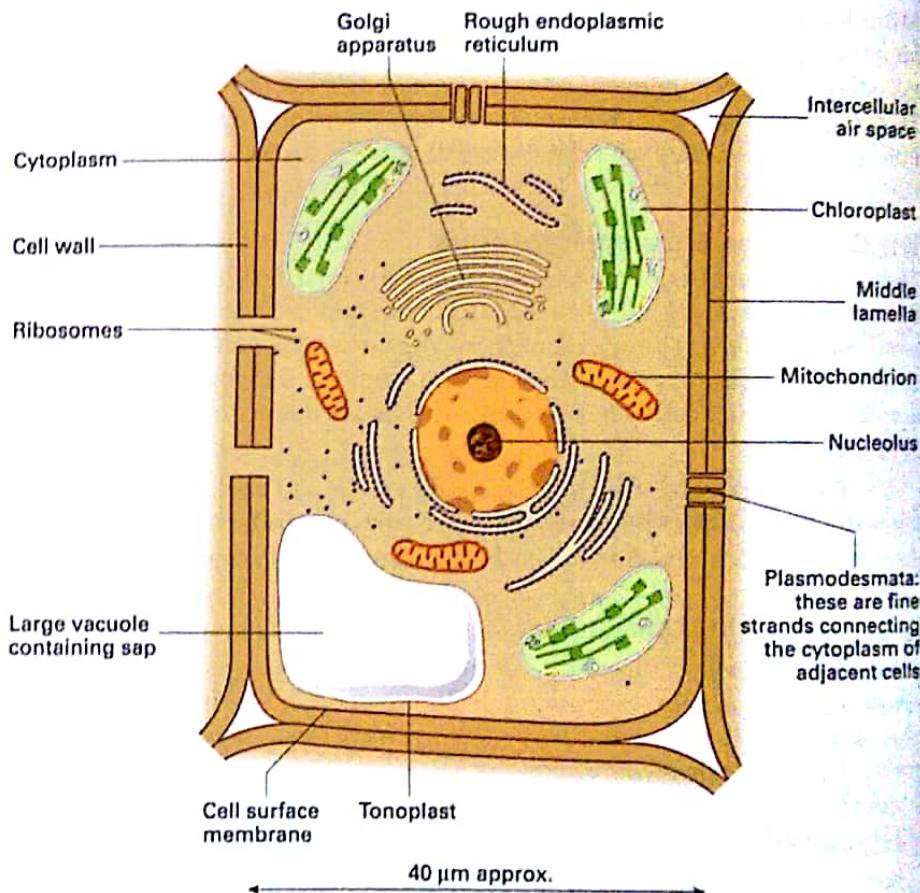


Figure 2 Diagram of a palisade plant cell from a leaf showing structures visible under an electron microscope. Although there is no such thing as a 'typical' plant cell, palisade cells have features commonly seen in most plant cells.

Chloroplasts, a cellulose cell wall, and a vacuole are visible under the light microscope, but the ultrastructure of a plant cell becomes clear only with the aid of an electron microscope.

A rigid **cell wall** surrounds each plant cell. This consists of cellulose microfibrils running through a matrix of other complex polysaccharides. Cell walls of neighbouring cells are cemented together by a thin layer of pectic substances (calcium and magnesium pectates) which form the **middle lamella**.

Most cell walls are not continuous but are perforated by fine membrane-lined pores through which pass cytoplasmic threads called **plasmodesmata**. These threads link the cytoplasm of two adjoining cells. Each plasmodesma has a central tubular core, usually connected at each end to the endoplasmic reticulum. Plasmodesmata enable substances to

be transported easily between neighbouring cells.

Cell walls allow plant cells to become full of water without bursting, and to develop a turgor pressure which helps support the plant. Most cell walls are completely permeable to water and provide a pathway for the free flow of water and mineral salts. Some cell walls become modified by substances such as lignin. Lignin strengthens cell walls but may make them impermeable.

A **chloroplast** (spread 5.1, figure 2) is a large organelle (usually 5–10 µm long) in which photosynthesis takes place. It has an envelope of two membranes and contains a gel-like matrix called the **stroma**, and a system of membranes. The stroma contains ribosomes, DNA, and photosynthetic enzymes. It also stores lipid droplets and starch. The internal chloroplast membranes are piled up in places to form stacks called **grana**. The membranes contain photosynthetic pigments, including the green pigment **chlorophyll**, responsible for harvesting light energy for photosynthesis.

A typical plant cell has a large central **vacuole**. This is a sac bounded by a membrane called the **tonoplast**. The vacuole contains **sap**, a watery solution of various substances including sugars, mineral salts, pigments, and enzymes. When full of sap, the vacuole causes the cell surface membrane to press against the cell wall. This exerts a turgor pressure which helps to support the plant. Sometimes the vacuole functions as a lysosome.

## Eukaryotes and prokaryotes

Plants, animals, fungi, and protists (a group that includes single-celled organisms such as *Amoeba*) all have cells containing double-membraned structures and with the genetic material enclosed within a well defined nucleus. These organisms are known as **eukaryotes**, and are made up of **eukaryotic cells**. Organisms such as bacteria that lack double-membraned organelles and do not have a membrane-bound nucleus are known as **prokaryotes** (figure 3).

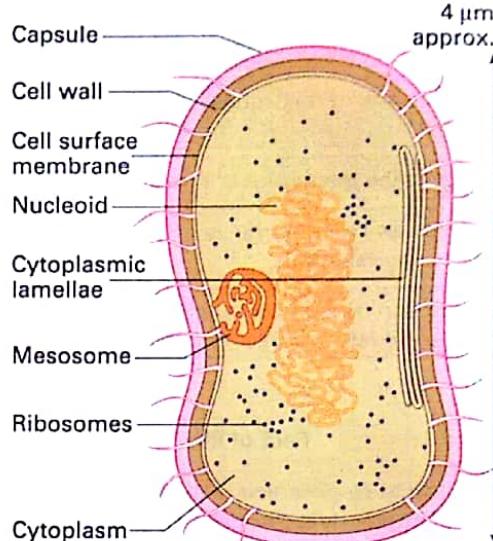
The biology of bacteria is covered in more detail in spreads 17.2 to 17.4, and a fuller comparison of prokaryotes and eukaryotes is given in spread 21.2. Viruses, which are neither prokaryotes nor eukaryotes, are described in spread 17.1.

### QUICK CHECK

- 1 What are plasmodesmata?
- 2 a Which structure in a bacterial cell resembles a nucleus?  
b How does it differ from the nucleus of a eukaryotic cell?
- 3 Do double-membraned organelles occur in prokaryotes or eukaryotes?

### Food for thought

A plant cell has been described as 'an animal cell crawling inside a cardboard box'. Is this an apt description?



**Figure 3** A generalised bacterial cell (prokaryotic cell). In common with animal and plant cells, a typical bacterial cell has a cell surface membrane enclosing cytoplasm that contains enzymes, ribosomes, and food granules. The membrane is surrounded by a cell wall, and this may in turn be enclosed in a capsule. However, a bacterial cell lacks the high level of organisation of an animal or plant cell. It has no Golgi apparatus or endoplasmic reticulum. The flagella (if they occur) are simple; they have no complex assembly of microtubules. The genetic material of a bacterium is a single strand of DNA, usually coiled up into the centre of the cell to form a nucleoid. This nucleoid has no double-membraned nuclear envelope, so is often described as an 'ill-defined nucleus'. Some bacterial cells possess additional circular pieces of genetic material called plasmids within the cell. Respiration generally takes place on a mesosome, an infolding of the cell surface membrane, but there are no mitochondria. Photosynthesising bacterial cells such as cyanobacteria (commonly known as blue-green algae) have a special form of chlorophyll, but it is not enclosed in a double-membraned chloroplast.

**OBJECTIVES**

- By the end of this spread you should be able to:
- describe the nature of artefacts
  - discuss the importance of freeze-fracturing
  - explain how cell organelles can be isolated by cell fractionation.

**Fact of life**

Structures observed in electron micrographs are not always what they seem to be. When tiny vesicles surrounded by ribosomes were first noticed on electron micrographs, cell biologists thought that they had discovered a new type of organelle. They classified these structures, along with other small organelles, as **microsomes** (small bodies). However, these newly discovered microsomes turned out to be artefacts: they do not really exist. They were fragments of endoplasmic reticulum produced during the preparation of material.

**REVEALING THE****ULTRASTRUCTURE OF CELLS**

The invention of the electron microscope allowed cell biologists to look at structures that were invisible under the light microscope. However, some cell biologists were sceptical: they believed that many features observed in electron micrographs were **artefacts**, that is, features not present in life.

**Artifacts**

An artefact may result during the preparation of a specimen. Air bubbles trapped under a coverslip are obvious artefacts produced during light microscopy.

Artefacts produced in electron microscopy are less obvious. For example, cells may be distorted during dehydration, so that they appear very different from the original living material. During the staining process, chemicals from outside the cell may appear as solid deposits which can look like intracellular structures.

**Freeze-fracturing**

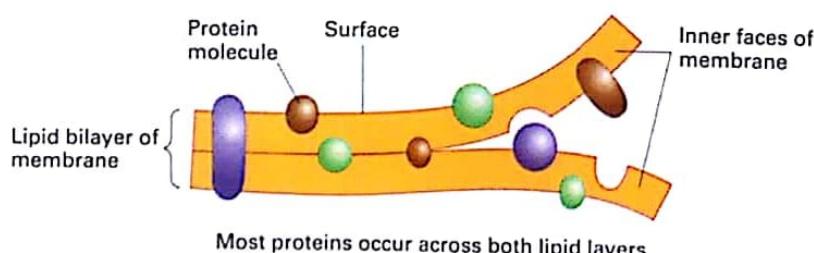
The existence of structures in an electron micrograph can be checked by examining cells using a different technique. **Freeze-fracturing** (sometimes called freeze-etching) allows biologists to check structures observed by transmission electron microscopy (TEM).

- Living material is plunged into liquid nitrogen at  $-196^{\circ}\text{C}$  and pushed against a sharp blade in a precise way.
- The frozen tissue splits along lines of weakness, often in the middle of a membrane.
- The fractured surfaces are 'etched' with heavy metal so that the specimen can be examined by TEM.

Freeze-fracturing has confirmed the existence of structures in cells, and has also revealed new features. For example, when cell membranes fracture between the two lipid layers, tiny dots are revealed, which are thought to be protein molecules (figure 1). This provides compelling evidence that proteins form a mosaic in cell membranes (see spread 4.6).

**Cell fractionation**

**Cell fractionation** is a technique used to prepare samples of the various cell organelles so that their functions can be studied. The organelles



**Figure 1 (a)** Freeze-fracturing. An artist's impression of how freeze-fracturing splits the bilipid layer of a cell surface membrane to reveal proteins embedded in the membrane.



**Figure 1 (b)** A scanning electron micrograph of a freeze-fractured cell surface membrane. The spherical structures are thought to be proteins.

are separated into fractions according to their size (using **differential centrifugation**) or density (using **density gradient centrifugation**). The main steps of cell fractionation are shown in figure 2. For either method, the tissue is first cut into small pieces and then placed in a chilled, isotonic, and buffered solution.

- The temperature of the solution is kept low to slow down metabolism and minimise self-digestion of the organelles.
- The salt concentration of the solution is made isotonic so that organelles do not change volume. (A very salty solution causes organelles to shrink, whereas very watery solutions cause them to swell.)
- The solution is buffered to minimise changes in pH during the process. This prevents enzymes in the organelles from becoming denatured.

These conditions minimise the risk of damage to isolated organelles and improve the chance of organelles functioning normally during investigations.

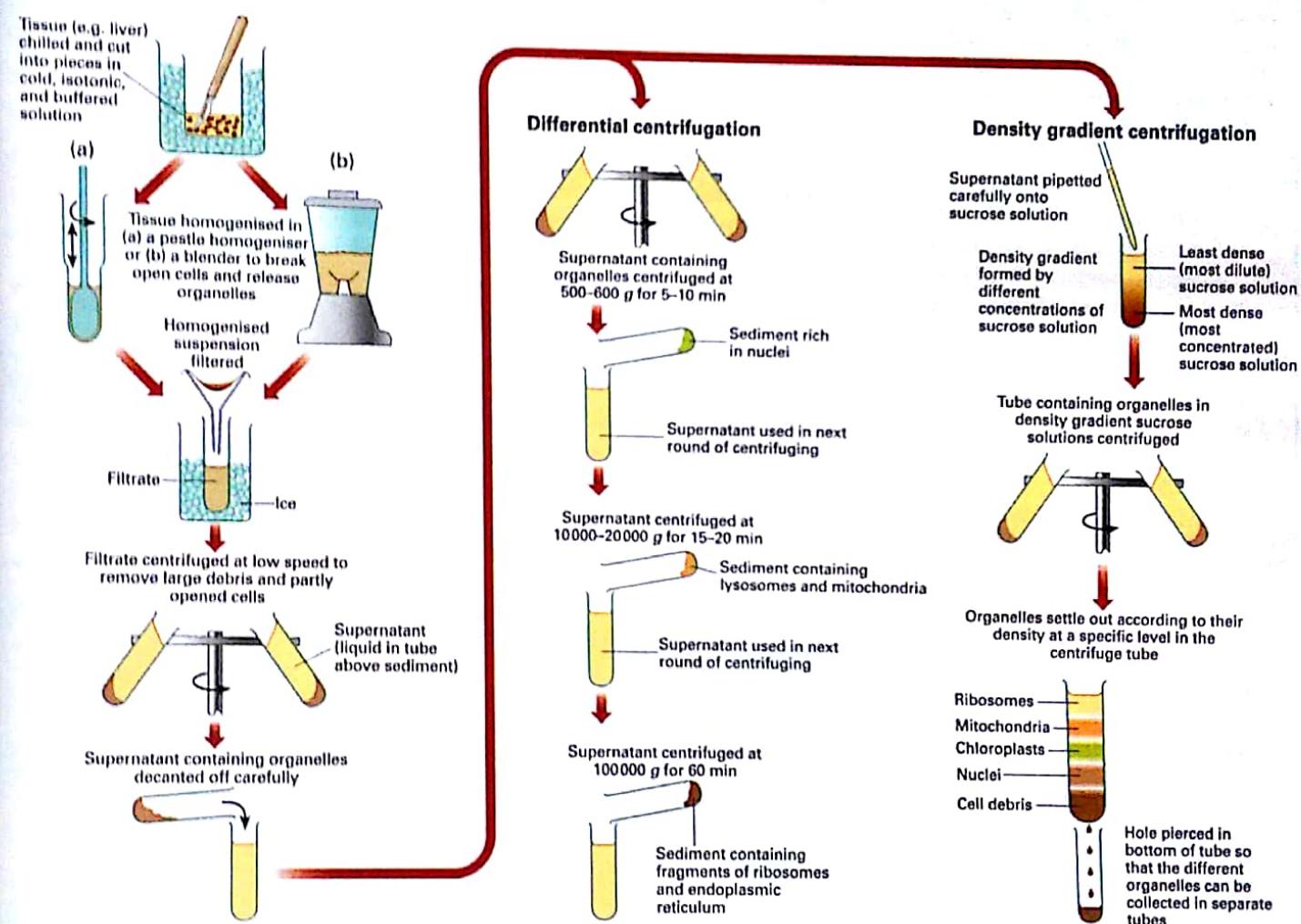


Figure 2 A flow diagram summarising the main stages of cell fractionation by two methods: differential centrifugation and density gradient centrifugation.

### QUICK CHECK

- Name two possible causes of artefacts in the preparation of specimens for the transmission electron microscope.
- Which component of cell membranes, not visible in electron micrographs taken with a transmission electron microscope, is revealed by freeze-fracturing?
- Why is tissue chilled during cell fractionation?

### Food for thought

Suggest different methods you could use to ensure that a structure seen in an electron micrograph is a real structure and not an artefact.

## OBJECTIVES

By the end of this spread you should be able to:

- list the functions of cell membranes
- describe the fluid-mosaic structure of cell membranes
- explain the roles of the different components of cell membranes.

## Fact of life

Cholesterol is infamous for contributing to heart disease. This lipid, however, is a vital component of human cell membranes, which contain almost as much cholesterol as phospholipid. Without cholesterol, our membranes would be too fluid at our relatively high body temperature, and would be liable to burst like soap bubbles.

Membranes cover the surface of every living cell, form an intricate network within the cytoplasm, and surround most cell organelles. These membranes are barely visible under a light microscope, but studies using electron microscopes, freeze-fracturing, and other modern techniques reveal that cell membranes have a complex structure and carry out a host of vital functions.

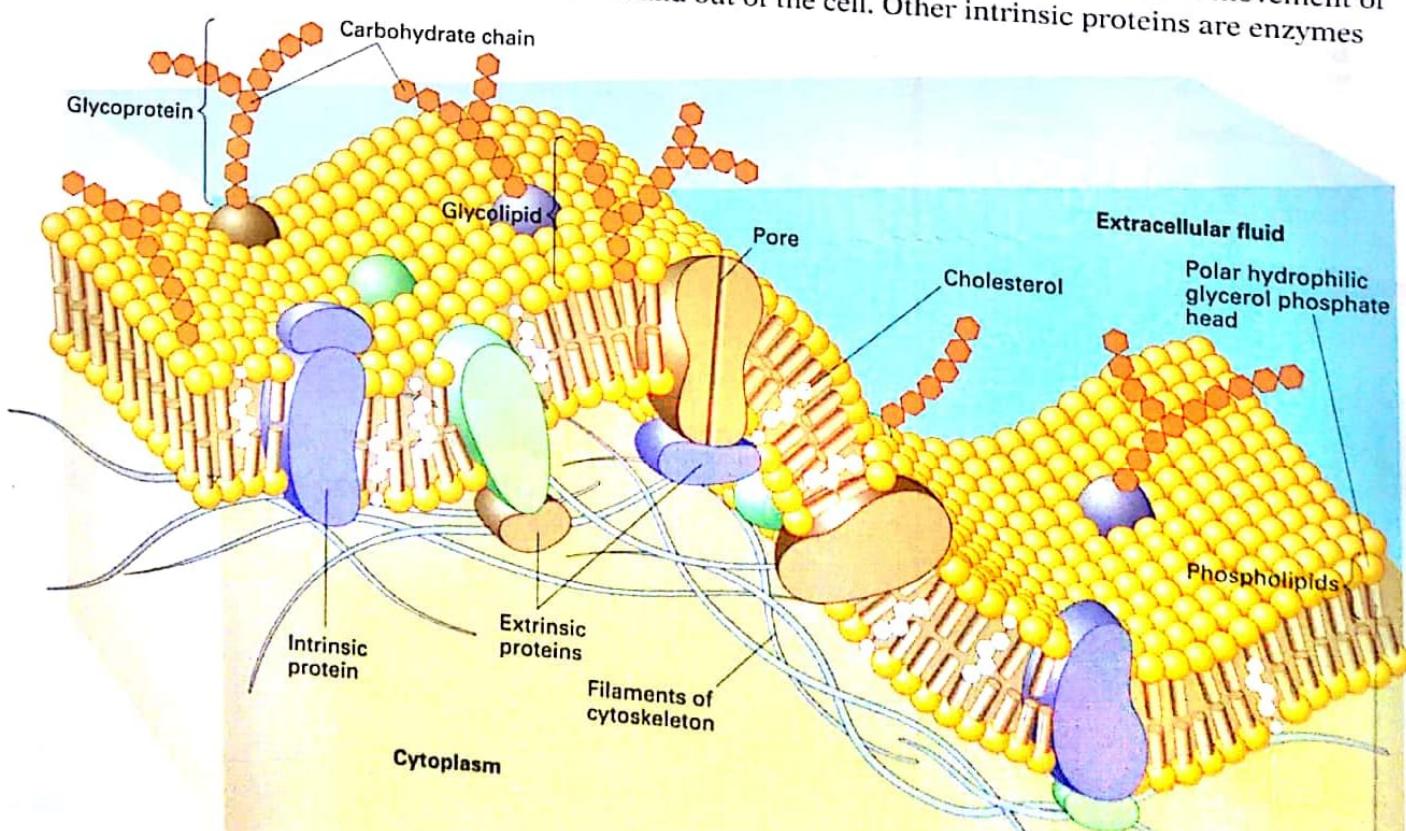
## The structure of the cell membrane

Electron micrograph studies indicate that membranes are 7–8 nm wide and have three layers (figure 1). This was originally (and wrongly) interpreted as indicating a lipid layer sandwiched between two layers of protein. Biochemical analyses show that **cell surface membranes** (membranes around the outsides of cells) are approximately 45 per cent lipid, 45 per cent protein, and 10 per cent carbohydrate. Most of the lipid is **phospholipid** (see spread 2.8). Each phospholipid molecule consists of a hydrophobic tail of two fatty acids, and a hydrophilic phosphate head. In cell membranes, phospholipids arrange themselves in a layer two molecules thick (a **bilayer**), with their hydrophobic tails pointing inwards, away from the water both inside and outside the cell.

In 1972, Jonathan Singer and Garth Nicolson proposed the **fluid-mosaic model** of cell membrane structure. They proposed this model after realising that membranes must have a complex structure to carry out their many and varied activities (see below). In their model (figure 1), individual protein molecules shift and move on a fluid bilayer of phospholipid. The protein molecules are variable in structure and function, but they all contribute to the mechanical strength of membranes.

Some, called **intrinsic proteins**, span the width of the membrane; others, **extrinsic proteins**, are confined to the outer or inner surface. Some intrinsic proteins act as carrier molecules, transporting substances across the membrane, or as more passive routes for the movement of material in and out of the cell. Other intrinsic proteins are enzymes

**Figure 1** Illustration of the fluid-mosaic model of the cell surface membrane.



which catalyse specific metabolic reactions at a particular location within the cell.

Many extrinsic proteins combine with carbohydrate groups to form **glycoproteins**. The carbohydrate groups usually extend from the cell surface like antennae or feelers and act along with **glycolipids** (lipid molecules joined to a carbohydrate group) as the chemical receptors of the cell. Some proteins on the inner surface of the cell surface membrane attach onto the cytoskeleton to anchor the membrane in place.

The phospholipid bilayer is oily, giving membranes flexibility and fluidity. The phospholipids also allow the passage of certain lipid-soluble substances through the membrane.

### Functions of cell surface membranes

A cell surface membrane forms the barrier between a cell and its surroundings. It has to be strong to offer mechanical support, and flexible to allow cells to move, grow, and divide. It must also be self-sealing so that the cell can divide without bursting. Some other functions of cell surface membranes are listed below along with a reference to the spread in which you can find out more about them.

- controlling the passage of materials in and out of cells (4.7 to 4.9)
- recognition of other cells (15.5)
- receptor sites for hormones and neurotransmitters (10.3 and 10.6)
- transmission of nerve impulses (10.4 and 10.5)
- insulation of nerves (10.5)

### Functions of membranes inside cells

Intracellular membranes (the membranes inside cells) have a structure very similar to that of cell surface membranes. However, the proportions of the molecular components vary considerably. The membranes enveloping chloroplasts, for example, contain very little carbohydrate.

Nearly every cellular process involves intracellular membranes. A few of the functions of these membranes are given below, together with the spread containing more information about them.

- acting as a reaction surface (6.4)
- acting as an intracellular transport system (4.3)
- providing separate intracellular compartments, isolating different chemical reactions (4.3)

### QUICK CHECK

1 Why must cell membranes be partially permeable?

2 Explain why the fluid-mosaic model is so called.

3 The human immunodeficiency virus which causes AIDS attacks certain white blood cells (T cells), interfering with a person's immune response. The virus attaches to specific receptor sites on the cell surface membranes of T cells. Suggest which components of the cell surface membrane act as receptor sites, and how the receptor site is specific to T cells.

### Food for thought

Cholesterol is an essential component of cell surface membranes. Suggest why having cell surface membranes with too much cholesterol is harmful.

### Cell membrane models

Like all scientific knowledge, our understanding of the structure of the cell membrane is tentative. Models have changed with the discovery and publication in scientific journals of new evidence that provides a better explanation of scientific observations. Early models, based on the observation that lipids and lipid-soluble substances can pass through membranes more readily than can substances insoluble in lipid, suggested that membranes are made of lipids. The discovery of protein in membranes isolated from red blood cells led Hugh Davson and James Danielli to propose a new model in 1935. In this model, cell membranes consist of a phospholipid core sandwiched between two layers of globular protein. The **Davson-Danielli** model was supported by early electron micrographs in which cell membranes appeared to consist of three layers (figure 2). Similar electron micrographs of cell membranes from a wide variety of organisms gave rise to the **unit membrane theory** which proposed that all cell membranes have this same basic structure. Detailed analysis of the electron micrographs revealed that not all membranes are identical or symmetrical. The unit membrane theory was rejected and the fluid-mosaic model proposed.

However, this is not the end of the story. The fluid-mosaic model has been refined since it was proposed in 1972 by Singer and Nicolson, and will continue to evolve as new scientific evidence comes to light.



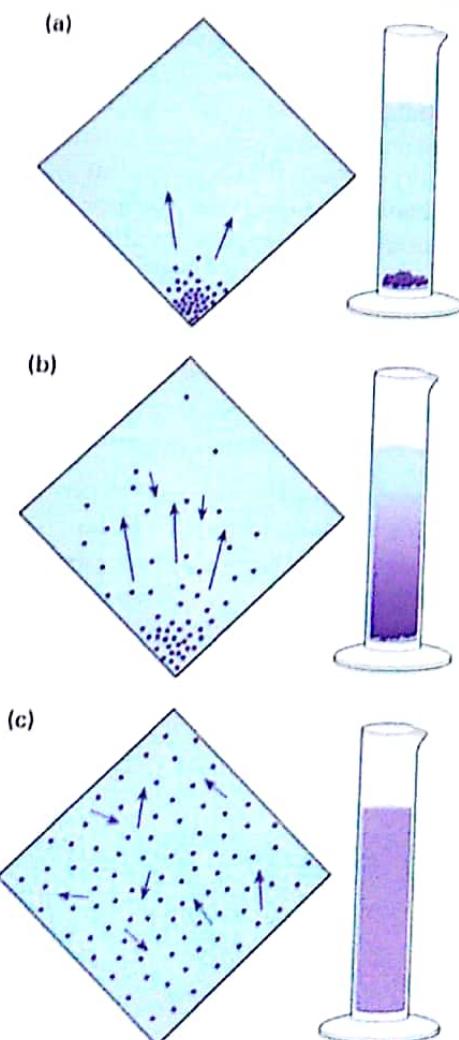
Figure 2 An electron micrograph of part of a cell. The arrow is pointing to the cell surface membrane which appears to have three layers: two dark layers

## OBJECTIVES

- By the end of this spread you should be able to:
- define diffusion
  - list the factors that affect the rate of diffusion
  - compare the surface area to volume ratio for objects of different sizes.

## Fact of life

The time a substance takes to diffuse increases with the square of the average distance it has to cover. This means that although diffusion may be fast over short distances, it becomes impossibly slow over long distances. For example, it would take about three years for oxygen to diffuse the one metre or so from the lungs to the extremities of the human body. Clearly diffusion alone is not suitable for transport of gases within the human body.



**Figure 1** A model of diffusion. Each dot represents a dye molecule moving randomly. In (a) and (b) there is a net movement from the bottom of the measuring cylinder to the top. In (c) an equilibrium has been established and there is no net movement; the molecules remain evenly distributed and there is an even concentration of dye throughout the cylinder.

## Moving down a concentration gradient

Particles are never still; they keep moving and bumping into each other all the time. When a drop of dye is added to water (figure 1), the molecules mix so that the dye spreads through the water even if it is not stirred. Dye molecules move in every direction, but the net effect is that they spread from where they are plentiful to where they are less plentiful.

This type of movement is called **diffusion**. It is defined as: *the net movement of molecules or ions from a region of their high concentration to a region of their lower concentration.*

Diffusion occurs in living and non-living systems wherever there is a concentration gradient. It continues until the particles are distributed evenly throughout the system. When a uniform concentration is reached, equilibrium is established. The molecules still move, but there is no net movement in any particular direction.

Diffusion is the mechanism by which gases are exchanged across respiratory surfaces, such as gills in fish and alveoli in mammals.

## The rate of diffusion

The rate of diffusion in a given direction across the exchange surface:

- is directly proportional to the area of the surface
- is directly proportional to the concentration gradient
- is inversely proportional to the distance (the length of the diffusion pathway).

This is known as **Fick's law** and can be summarised simply as:

$$\text{Rate of diffusion is proportional to } \frac{\text{surface area} \times \text{difference in concentration}}{\text{length of diffusion path}}$$

Increasing the factors on the top line of the equation will make diffusion faster, while increasing that on the bottom will slow it down.

Fick's law applies to situations where there is no barrier to the movement of substances. Like the diffusion of a dye in a container of water, the diffusion of a substance into or out of a cell is a form of **passive transport**: it does not require the expenditure of energy. Diffusion through a cell membrane is affected by the nature of the membrane (for example, its permeability) and the size and type of molecule or ion diffusing through it.

## Adaptation for diffusion

As would be expected from Fick's law, cellular diffusion is a very slow process unless there is a large concentration gradient over a short distance. Tissues such as those in the lungs and small intestine are especially adapted to maximise the rate of diffusion by:

- maintaining a steep concentration gradient
- having a high surface area to volume ratio
- being thin, minimising the distance over which the diffusion takes place.

## Membrane permeability

Cell membranes are **partially permeable**: many substances can pass through them, but some substances cannot. Some substances, such as steroid hormones, oxygen, and carbon dioxide, dissolve readily in the inner oily phospholipid bilayer of cell membranes (see spread 4.6, figure 1). Consequently these substances diffuse readily through a cell membrane down a concentration gradient.

## Facilitated diffusion

Glucose, nucleic acids, amino acids, and proteins are not soluble in

lipids and do not pass readily through the phospholipid bilayer of cell membranes. Instead, these substances pass through the membrane by a process called **facilitated diffusion**.

This is the passive movement of molecules down a concentration gradient but it involves special carrier proteins in the cell membrane. These proteins may have hydrophilic channels that function as pores along which solutes can pass (figure 2). Alternatively, they may be small globular proteins that move in the membrane, shuttling their load back and forth, like a ferry carrying cars.

Facilitated diffusion of relatively large molecules such as glucose appears to be very specific and may involve a protein called a **permease**. This protein changes its shape when glucose binds to it. The solute is moved across the membrane as the protein alternates between its two shapes (figure 3). Although net movement is always down a concentration gradient, the protein can transport the solute in either direction. Like enzymes, permeases can be inhibited by substances that resemble the normal substrate.

### Surface area to volume relationship

Table 1 shows that as the length of the sides of a cube is increased, the ratio of its surface area to its volume decreases. A similar relationship applies to organisms: as the size of an organism increases, its surface area to volume ratio decreases.

**Table 1** Surface area to volume ratios of a cube.

| Length of side (mm) | Area of side (mm <sup>2</sup> ) | Surface area of cube (mm <sup>2</sup> ) | Volume of cube (mm <sup>3</sup> ) | Surface area to volume ratio |
|---------------------|---------------------------------|---|-----------------------------------|------------------------------|
| 1                   | 1                               | 6                                       | 1                                 | 6:1                          |
| 2                   | 4                               | 24                                      | 8                                 | 3:1                          |
| 5                   | 25                              | 150                                     | 125                               | 1.2:1                        |
| 10                  | 100                             | 600                                     | 1000                              | 0.6:1                        |
| 100                 | 10 000                          | 60 000                                  | 1 000 000                         | 0.06:1                       |

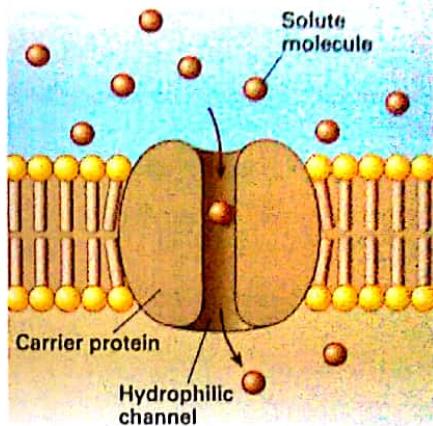
This relationship has important consequences for organisms. Some processes such as the rate of diffusion depend on surface area; other processes such as metabolic rate depend on volume. Consequently a small organism such as the single-celled *Amoeba* can satisfy its need for oxygen by simple diffusion from its watery environment. However, simple diffusion cannot supply the oxygen needed by large organisms. It would take several years for oxygen to diffuse into the centre of a whale because its surface area to volume ratio is more than one million times smaller than that of the single-celled organism. To compensate for their relatively small body surface areas, large organisms have evolved special exchange surfaces and transport systems, such as lungs and gills to exchange respiratory gases, and blood circulatory systems to transport the gases.

### QUICK CHECK

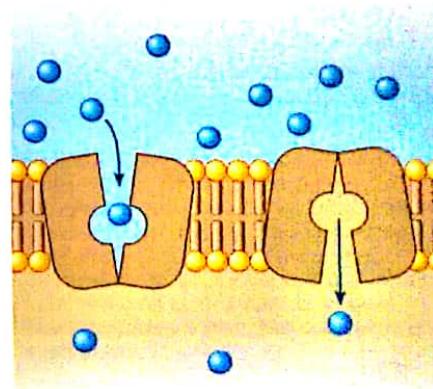
- Define diffusion.
- List the factors that affect the rate of diffusion of a molecule into a cell.
- Calculate the surface area to volume ratio of a cube with sides 4 mm long.

### Food for thought

Tissues involved in gaseous exchange are moist. It is a common misconception that they have to be moist in order to carry out diffusion. This is not true. The tissues are moist because they are very thin and are permeable to water and other substances. Their moistness is a result of their ability to allow diffusion, not a cause of it. Nevertheless, gills, for example, are no longer able to carry out gaseous exchange if they become dry. Suggest reasons for this loss of ability.



**Figure 2** Facilitated diffusion: a protein provides a functional pore in the membrane for the passage of a solute.



**Figure 3** Facilitated diffusion: a protein acts as a specific carrier molecule in the membrane.

## OBJECTIVES

By the end of this spread you should be able to:

- describe the process of active transport
- distinguish between endocytosis and exocytosis.

## Fact of life

Seaweeds can concentrate inside themselves trace metals that are present in only minute quantities in the surrounding sea. For example, *Pelvetia canaliculata*, *Ascophyllum nodosum*, and *Fucus spiralis* (brown seaweeds common on British rocky shores) concentrate titanium

1000–10 000 times, zinc 1000–1400 times, nickel 600–1000 times, and strontium 8–20 times.

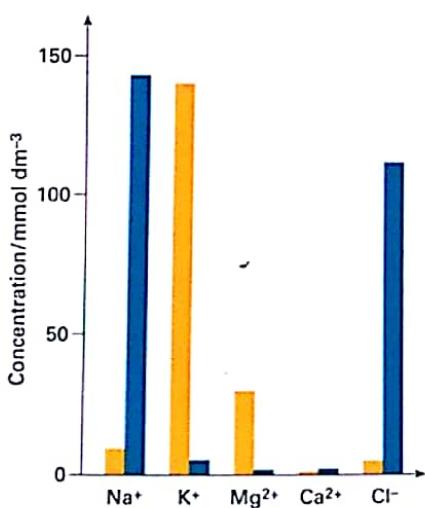


Figure 1 The concentrations of some ions inside cells (yellow bars) and outside cells (blue bars).

## ACTIVE TRANSPORT

## Maintaining an internal environment

Before sending space probes to Mars, NASA asked James Lovelock to suggest features that they could use in their search for life. Lovelock was the originator of the Gaia hypothesis concerning the environmental conditions on Earth and the organisms that live there (see spread 22.1). He concluded that extraterrestrial life may not share the characteristic features of life on Earth. He proposed, however, that to stay alive, any organism would have to keep its internal environment relatively constant and different from its external environment (a process called homeostasis).

The concentrations of ions inside a cell of any Earth organism are kept at a different level from the concentrations outside (figure 1). To maintain these concentrations, a cell must import some substances and export others.

## Active transport: using energy to import and export

Cells import and export their substances by **active transport**. Active transport is:

- the movement of substances,
- usually against a concentration gradient (from a region of lower to a region of higher concentration),
- across a cell membrane.
- It involves the expenditure of energy.

If a cell cannot carry out active transport, it dies as concentrations inside and outside the cell become equal.

The energy for active transport usually comes from ATP, provided by respiration in the mitochondria (see spread 2.11). Cells that carry out active transport on a large scale have an unusually high number of mitochondria and high concentrations of ATP. Active transport ceases if ATP production is prevented by metabolic poisons such as cyanide, or lack of oxygen.

## How active transport happens

Several types of active transport involve membrane proteins which carry specific molecules or ions. These carriers may move:

- a single substance in a single direction (**uniport carriers**)
- two substances in the same direction (**symport carriers**)
- two substances in opposite directions (**antiport carriers**).

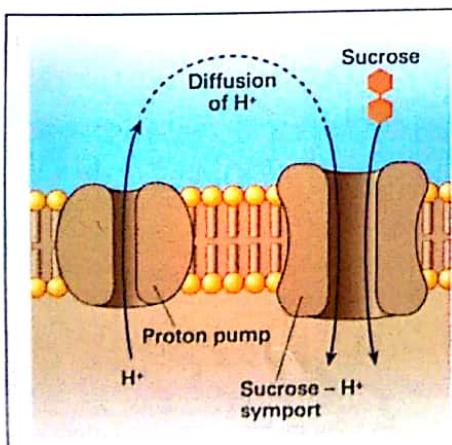
The precise mechanism of active transport is unclear. One hypothesis suggests that some actively transported substances can hitch a ride on a proton (hydrogen ion) in a process called **cotransport** (see box). Another

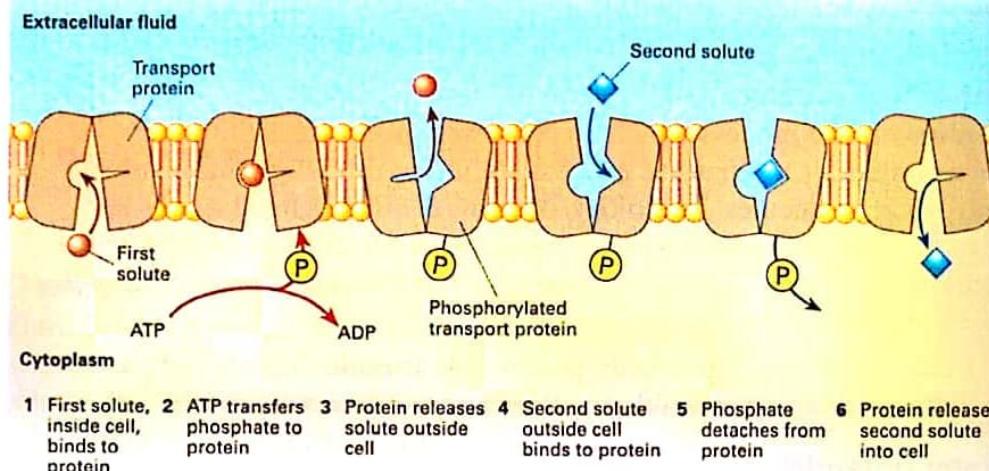
## Cotransport

Cotransport is a relatively complex form of active transport in which the pumping of one substance indirectly drives the transport of one or more other substances against a concentration gradient. In plant cells which store sucrose, for example, an ATP-driven proton pump actively transports hydrogen ions (protons) out of the cell. This creates a concentration gradient down which the protons can diffuse. Diffusion of the protons back into the cell via a symport carrier enables sucrose to be carried into the cell at the same time (figure 2).

A similar process is used to transport glucose and amino acids into cells lining the digestive tract in mammals. In this case, absorption of the nutrients is dependent on a sodium pump.

Figure 2 Cotransport: sucrose moves into plant cells along with protons. The protons travel down a concentration gradient set up by a proton pump.





hypothesis suggests that protein molecules change shape to transport solutes from one side of the membrane to the other (figure 3). To do this ATP is hydrolysed to ADP. The terminal phosphate group attaches directly onto the protein, causing it to change shape. Active transport systems that move two solutes together are quite common. One of the most important is the sodium-potassium pump which pumps sodium ions out of the cell and potassium ions in. This is important in generating impulses in nerve cells.

### Cytosis: movement with vesicles and vacuoles

Most cells can carry out **cytosis**: active transport which involves infolding or outfolding of sections of the cell surface membrane. This appears to involve the contractile proteins in cellular microfilaments and microtubules.

Cytosis may result in the bulk transport of material into a cell (**endocytosis**) or out of a cell (**exocytosis**). There are two main forms of endocytosis: **phagocytosis** and **pinocytosis**. A third form, **receptor-mediated endocytosis**, is shown in figure 4.

- In phagocytosis (cellular eating) solid substances, sometimes whole organisms, are brought inside the cell by invagination (an infolding of the cell surface membrane). A vacuole is formed, the inner surface of which is derived from the outer surface of the cell surface membrane. Many unicellular organisms such as *Amoeba* (figure 5) and certain white blood cells called phagocytes perform phagocytosis.
- Pinocytosis (cellular drinking) is a similar process, but the infoldings are much smaller. Liquids or large macromolecules such as proteins are taken in via a small vesicle.
- Exocytosis appears to be endocytosis in reverse. Vesicles and vacuoles move to the cell surface membrane, fuse with it and release their cargo to the outside world.

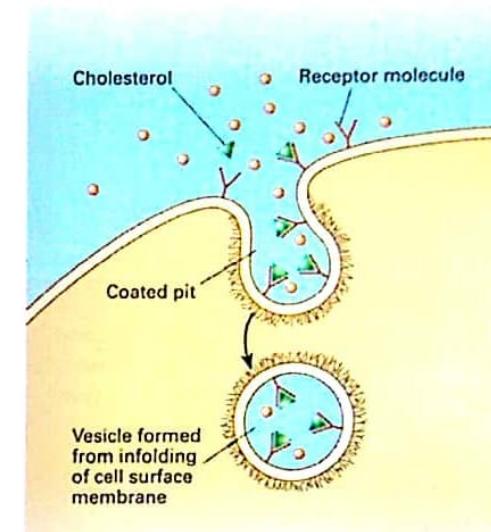
### QUICK CHECK

- Describe active transport, mentioning ATP, concentration gradient, and oxygen consumption.
- Suggest why active transport is affected by changes in oxygen concentration but diffusion is not.
- Distinguish between pinocytosis and phagocytosis.

### Food for thought

To study mineral uptake, a botanist used isolated roots bathed in solutions containing potassium ions and different oxygen concentrations. Suggest why the botanist found that:

- potassium ions were present in the roots even when the percentage of oxygen was zero
- the concentration of potassium ions in the roots increased rapidly with increasing oxygen concentrations up to 20 per cent
- the rate of sugar consumption by the roots rose with increases in oxygen concentration.



## OBJECTIVES

By the end of this spread you should be able to:

- define water potential
- explain how osmosis takes place
- distinguish between isotonic, hypertonic, and hypotonic solutions and their effects on animal and plant cells.

## Fact of life

Brine shrimps (*Artemia spp*) are found in tremendous numbers in salt lakes and coastal evaporation ponds (ponds used to obtain salt by evaporation of sea water). Brine shrimps can live in water with solute concentrations ranging from about 0.3 to about  $300 \text{ g dm}^{-3}$  because they can pump salts actively out of their bodies. At  $300 \text{ g dm}^{-3}$ , salt water is more than eight times saltier than normal sea water; it is so salty that the salts crystallise out of solution.

## OSMOSIS

## Defining osmosis

Osmosis is the process by which cells exchange water with their environment. It is a passive process similar to diffusion, but involving only water molecules. In biology, osmosis is often defined as: *the net movement of freely moving water molecules from a region of their higher concentration to a region of their lower concentration through a partially permeable membrane*.

In the definition, a **partially permeable membrane** refers to a membrane that is permeable to water and to certain solutes.

## Water potential

The pressure exerted by freely moving water molecules in a system is called the **water potential**, measured in **kilopascals (kPa)**. By convention, the water potential of pure water is given the value of 0 kPa. A solution with a high water potential has a high number of freely moving water molecules.

Consider a solution with a higher water potential separated from a solution with a lower water potential by a partially permeable membrane. More water molecules move from the region of higher water potential to the region of lower water potential than in the other direction (figure 1a). The movement continues until the water potentials on both sides of the membrane are equal. Water molecules continue to move in both directions after equalisation, but there is no *net* movement (figure 1c).

## The effect of solutes

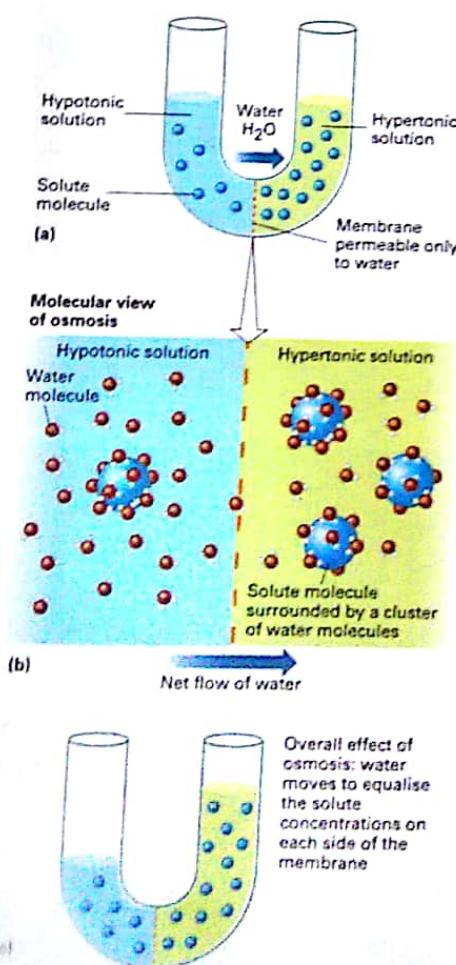
The water potential of a solution falls when solutes are added because water molecules cluster around the solute molecules (figure 1b). As the concentration of solutes increases, clustering also increases, further lowering the water potential.

The contribution of solutes to the water potential of a system is called the **solute potential** of that system. Because it always lowers water potential, solute potential is always negative. It becomes more negative as more solutes are added to the system.

## Osmosis in animal cells

A single-celled organism living in the sea is bathed in fluid which has the same water potential as its **cytosol** (the liquid part of the cytoplasm). The sea water is **isotonic** with the cytosol because it causes no change in the volume of the cell. This is because the water potential inside and outside the cell is the same and there is no net flow of water in or out (figure 2a). A similar situation applies to organisms, such as the malarial parasite *Plasmodium*, which live in blood.

An animal cell placed in fresh water has to deal with a very dilute medium which has a higher water potential than the cytosol. The fresh water is **hypotonic** to the cytosol. It tends to enter the cell, causing the cell volume to increase. If the cell cannot eliminate the excess water, as a red blood cell cannot, it will burst (a process known as lysis) (figure 2b). However, if fresh water is the cell's natural environment, it will have a mechanism for eliminating excess water which has entered by osmosis. Amoebae that live in ponds, for example, have a **contractile vacuole** which collects and gets rid of excess water, keeping the volume of the cell constant.



**Figure 1 Osmosis.** (a) A hypotonic (weak) solution is separated from a hypertonic (strong) solution by a membrane that is permeable only to water. (b) Water molecules are free to move in the hypertonic solution. (c) Consequently there is a net movement of water molecules from the hypotonic solution to the hypertonic solution, causing the fluid level to rise on the right-hand side.

An animal cell placed in a very salty solution has to cope with the risk of losing water. If the external solution is of a sufficiently high solute concentration, its water potential will be lower than that of the cell – it is a **hypertonic** solution. Water will tend to be drawn out of the cell, causing it to shrink and shrivel (figure 2c).

### Osmosis in plant cells

Plant cells behave in the same way as animal cells in an isotonic solution: they neither gain nor lose water (figure 2a). However, because a plant cell has a rigid cellulose cell wall, it behaves differently from an animal cell in hypotonic and hypertonic solutions.

In a hypotonic solution, water enters the plant cell, filling the vacuole to capacity. The cell surface membrane pushes against the cell wall, making the cell very rigid. A cell in this condition is said to be **turgid** (figure 2b). There is no danger of the cell bursting because the pressure of the cell wall against the cell surface membrane restricts the inflow of water.

In a hypertonic solution, water moves out of a plant cell, the cell vacuole shrinks, and the cell surface membrane pulls away from the cell wall (figure 2c). The cell becomes **flaccid** because the contents are no longer pushing against the cell wall. A cell in this condition is said to be **plasmolysed**.

### Pressure potential

The water potential of a plant cell is affected by both the pressure of the cell wall against the cellular contents and the solute potential of the cell. The contribution made by the cell wall is called the **pressure potential**. This always has a positive value when the cell wall is turgid, but equals 0 kPa when the cell is flaccid. Thus the water potential of a plant cell is the sum of its solute potential and pressure potential:

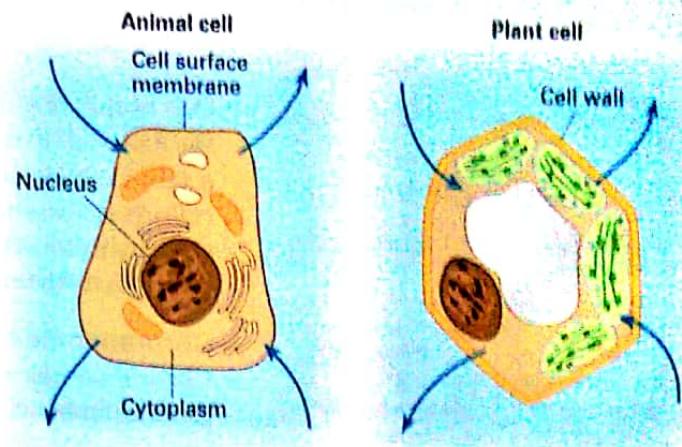
$$\Psi_{\text{cell}} = \Psi_s + \Psi_p$$

$$\text{water potential of plant cell} = \text{solute potential} + \text{pressure potential}$$

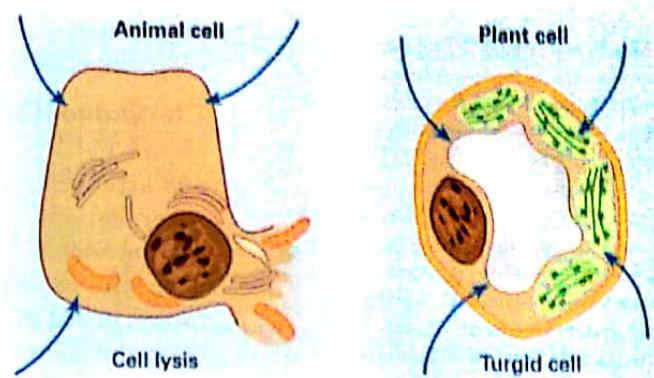
The water potential of an animal cell is determined primarily by its solute potential. The effect of the cell membrane is usually so small that it is ignored.

### QUICK CHECK

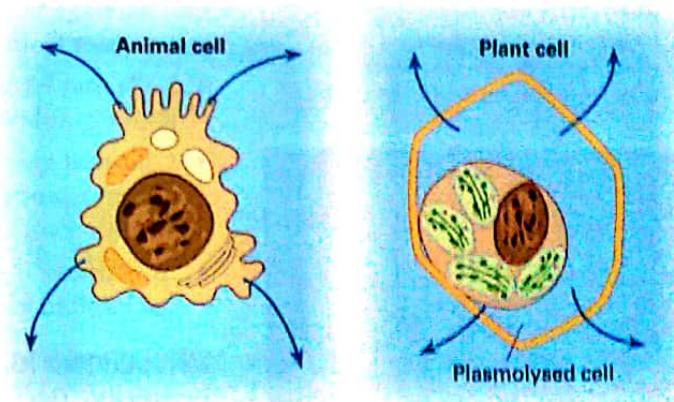
- What is the water potential of pure water at standard pressure and temperature?
- Plant cell A has a solute potential of -300 kPa and a pressure potential of 200 kPa. Lying next to it is plant cell B which has a solute potential of -400 kPa and a pressure potential of 100 kPa. Use the water potential equation to predict the direction of net movement of water in each cell.
- What type of replacement drink should a heavily perspiring athlete take: hypertonic, isotonic, or hypotonic? Give reasons for your answer.



(a) Isotonic solution: no net flow of water



(b) Hypotonic solution: net inflow of water



(c) Hypertonic solution: net outflow of water

**Figure 2** The effects of different solutions on animal and plant cells.

### Food for thought

There are many different groups of amoeba. Some inhabit freshwater and marine environments; others are internal parasites which infect human intestines and cause dysentery. Suggest why contractile vacuoles appear to be absent from some marine and parasitic groups of amoeba.

# INTRODUCTION TO CELL DIVISION

## OBJECTIVES

By the end of this spread you should be able to:

- describe the main stages of the cell cycle
- distinguish between mitosis and meiosis.

### Fact of life

The normal growth and development of a multicellular organism is determined by a fine balance between cell addition by mitosis and cell deletion by apoptosis (programmed cell death). Apoptosis controls cell quality and quantity in all multicellular organisms. It is the 'default setting' of each cell produced by mitosis. A new cell will automatically self-destruct unless signals from neighbouring cells prevent it from doing so.

Apoptosis removes cells that are no longer required and infected, worn, or damaged cells that need to be replaced. Unlike uncontrolled cell death (**necrosis**), apoptosis takes place in an orderly way. Cells to be destroyed are selected by specific cell signals. Then cell metabolism is closed down and the residual debris is dismantled and recycled within the organism. In most cases, one of a number of triggers causes mitochondria to release a signalling molecule (e.g. cytochrome c) which sets in train the self-destruction programme. The mechanisms that control the cell cycle are probably linked to those that control apoptosis.

One of the most important concepts in biology is that cells arise only by the division of existing cells. Cell division is essential to all life. It enables a multicellular organism to grow and to replace worn out or damaged cells. It is also the basis of reproduction in every organism. Cell division starts with the division of the nucleus. There are two forms of nuclear division: **mitosis** and **meiosis**.

### Chromosomes: carrying information

**Chromosomes** are the structures that provide continuity between one generation of cells and the next. Their name comes from the Greek: *chroma* = coloured, *soma* = body, because of their affinity for certain stains used in microscopy. Chromosomes consist of DNA, the genetic material of the cell, wrapped in protein. They become visible in the nucleus where the more dispersed chromatin existed before (see spread 4.3). Whole chromosomes can be examined microscopically after breaking a dividing cell open and staining it with a suitable dye.

### Chromosomes form homologous pairs

Figure 1 shows a photomicrograph of human chromosomes cut out and arranged into matching pairs according to their size and certain other features. These are called **homologous pairs**. Apart from the sex chromosomes, both chromosomes in a pair normally contain the same genes (for example, for eye colour). However, these may be different forms, called **alleles**, of the gene (for example, one chromosome may carry the allele for green eyes and the other may carry the allele for brown eyes). Alleles are described more fully in spread 19.3.

Human cells each have 46 chromosomes (23 pairs). Other species have different numbers, for example, chimpanzee cells each have 48 (24 pairs) and cabbage plant cells each have 18 (9 pairs). One chromosome in each pair comes from the individual's mother and the other from the father.

- Cells that have the normal two sets of chromosomes are called **diploid** (usually abbreviated  $2n$ ).
- Cells that give rise to gametes (eggs and sperm) have only one chromosome of each pair, so they have half the normal number of chromosomes. Such cells are called **haploid** ( $n$ ).
- In humans,  $n = 23$ , so normal diploid cells have 46 chromosomes and the haploid gametes have 23 chromosomes.

### Mitosis: two identical daughter cells

In **mitosis**, the nucleus divides once and produces two identical nuclei. The new daughter cells are genetically identical to the parental cell (unless their DNA has been changed in some way, for example by a mutation) (figure 2). So mitosis doubles the number of cells without changing the genetic information. New cells for growth of a multicellular organism, asexual reproduction, and wound healing, for example, are produced by mitosis.

### The cell cycle

The **cell cycle** is the sequence of events that occurs between one cell division and the next. It consists of three main stages (figure 3):

- 1 During interphase, the cell grows, carries out its functions, and replicates its DNA (see spread 18.3). After the DNA is replicated, new protein becomes attached to it. The chromosome now consists of two strands called **sister chromatids** which contain identical genetic information. Sister chromatids are joined at some point along their length by a **centromere**. These become visible under the light microscope only during mitosis. Typically, interphase lasts for about 90 per cent of the cell cycle.

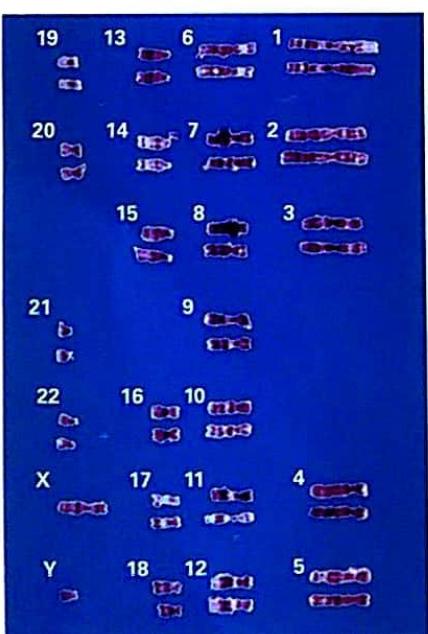


Figure 1 A karyotype: human chromosomes arranged in matching pairs. Each chromosome consists of two sister chromatids joined by a centromere. Twenty-two pairs of human chromosomes are numbered. The other pair are the sex chromosomes denoted by the letters X and Y. Usually, males have one X and one Y chromosome; females have two X chromosomes.

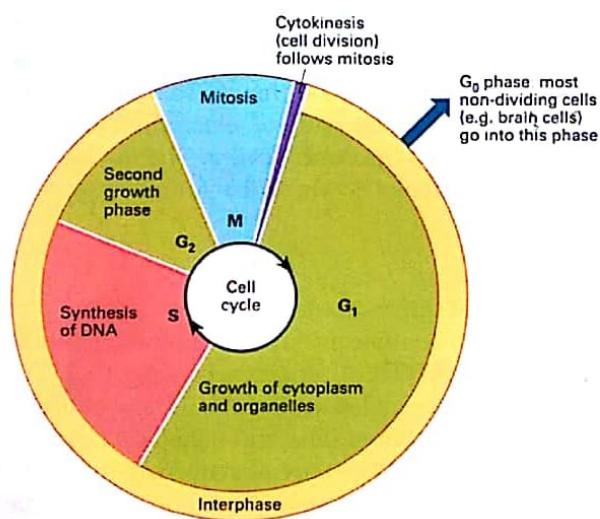
- 2 Nuclear division takes place during mitosis. The chromatids containing replicated DNA are separated from each other and are redistributed as chromosomes in the nuclei of the two new daughter cells.
- 3 In **cell division** (also called **cytokinesis**) the cytoplasm divides to form two daughter cells.

The duration of the cell cycle varies with conditions such as temperature and cell type. Mitosis of eukaryotic cells is controlled by the interaction of extracellular growth factors, such as growth hormone and insulin in humans, with intracellular cyclin-dependent kinases.

### Meiosis: four different daughter cells

In meiosis, the nucleus divides twice. This produces four haploid nuclei (figure 4). The number of chromosomes is therefore halved during meiosis. Moreover, homologous chromosomes within a pair can exchange genetic material before being separated. The daughter cells are therefore genetically different from the parent cell (and from each other).

Meiosis is the basis of sexual reproduction, occurring at some point in the life cycle of organisms that reproduce sexually. The haploid gametes produced by meiosis fuse during fertilisation. This means that the new fertilised cell has the diploid number of chromosomes. Without meiosis in the life cycle, the number of chromosomes of a sexually reproducing species would be doubled in each generation (see chapter 12).



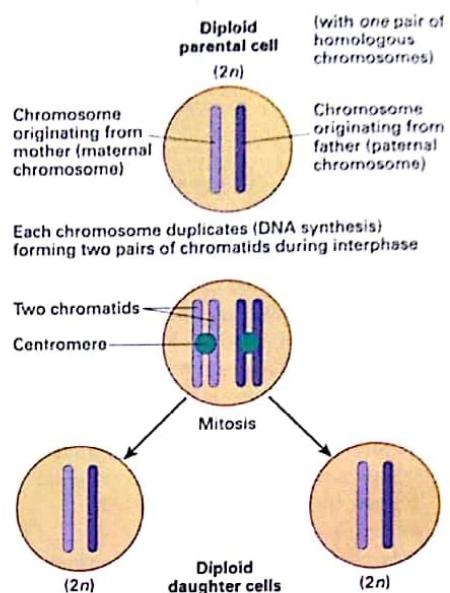
**Figure 3** The stages of the cell cycle. Normal cellular metabolism takes place during the three stages of interphase ( $G_1$ ,  $S$ , and  $G_2$ ). Active division of the nucleus takes place during mitosis (M) after which cells usually divide (cytokinesis). The stages of the cell cycle are described more fully in spread 4.11.

### QUICK CHECK

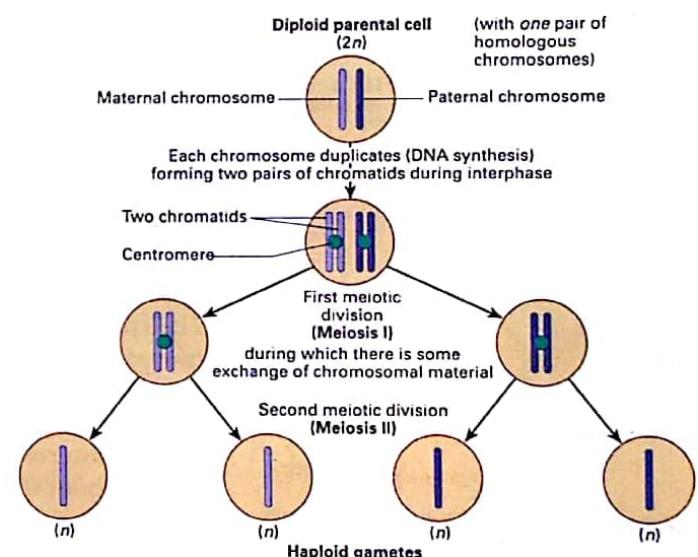
- 1 a List the main stages of mitosis, starting with interphase.  
b At which stage is DNA replicated?
- 2 Compare mitosis and meiosis in terms of number of cell divisions and number of daughter cells.

### Food for thought

Apoptosis is responsible for removing tail cells when tadpoles metamorphose into frogs, and during human embryo development for the removal of cells that form the webbing between digits. It is also responsible for destroying brain cells that have not interconnected during child development. Suggest the possible implications of this for learning a new skill such as music or a foreign language.



**Figure 2** Outline of mitosis. Duplication of a chromosome is followed by nuclear division and cell division. Mitosis is explained in more detail in the next spread.



**Figure 4** Outline of meiosis. Duplication of a chromosome is followed by two nuclear divisions and cell divisions. Meiosis is explained in more detail in spread 4.12.

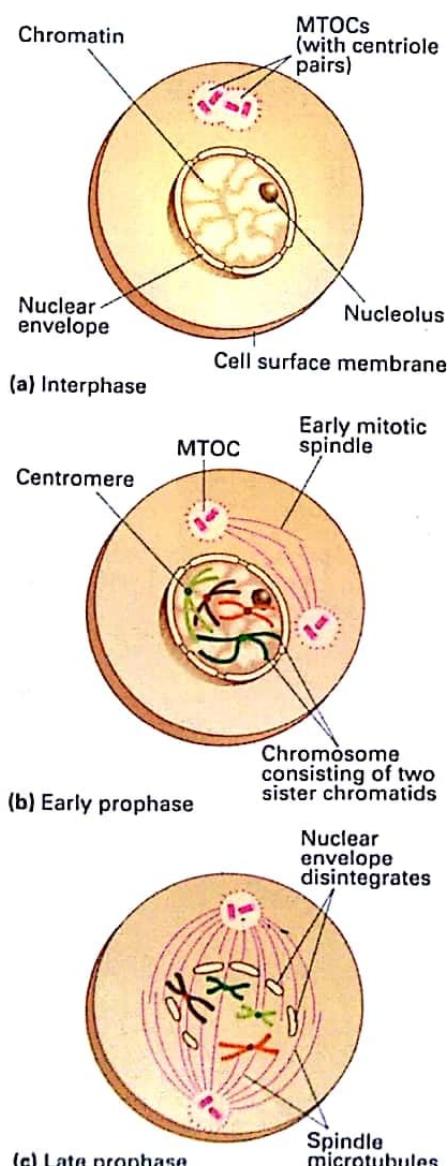
**OBJECTIVES**

By the end of this spread you should be able to:

- describe the main stages of mitosis.

**Fact of life**

When studying sea urchin cell cycles, Sir Tim Hunt discovered a new family of proteins involved in cell signalling. He named these proteins **cyclins** because their concentrations varied in a cyclical fashion during the cycle. Different cyclins control the progress of the cycle by being active at different stages. Mitosis of eukaryotic cells is regulated by the interaction of intracellular cyclin-dependent kinases and extracellular growth factors, such as those associated with human growth hormone and insulin. Cyclin-dependent kinases are enzymes that, when cyclin is bound to the kinase, transfer phosphate groups from high-energy donor molecules, such as ATP, to specific substrates.



**Figure 1** Interphase; and prophase, the first stage of mitosis.

# MITOSIS

## The stages of interphase

Mitosis is preceded by **interphase** (figure 1a). This was once called the resting stage because, under the light microscope, little seems to be happening apart from growth. In fact, interphase is a period of intense activity. DNA replicates, and energy in the form of ATP is built up ready for use during mitosis.

Interphase has three stages: G<sub>1</sub>, S, and G<sub>2</sub> (see spread 4.10, figure 3).

- During stage G<sub>1</sub>, the cell is very active, growing and carrying out its metabolic functions.
- During stage S, DNA replicates and two sister chromatids form from each chromosome.
- During G<sub>2</sub>, the mitochondria divide and the cell continues to grow until mitosis begins. In plants, chloroplasts also divide during G<sub>2</sub>.

Mitosis is a continuous process, but is described in four main stages: prophase, metaphase, anaphase, and telophase.

## Prophase

Mitosis starts with **prophase** (figure 1b). The chromosomes condense, becoming more tightly coiled and folded and so appear shorter and fatter as prophase progresses. As soon as the chromosomes start to condense, the DNA becomes inactive. The condensation of the chromosomes into separate structures enables them to be moved easily; during interphase they are diffuse and would become entangled if they were moved about the nucleus. In the later stages of prophase pairs of sister chromatids can be seen, attached at a point called the **centromere**. The nucleoli disappear, the nuclear membrane breaks down, and a spindle apparatus appears.

## The spindle apparatus

The **spindle apparatus** is made of microtubules which control the movements of the chromosomes. The microtubules originate from two **microtubule-organising centres (MTOCs)**. In animal cells each MTOC contains two barrel-shaped structures called **centrioles**. These centrioles have an internal structure similar to that of cilia and flagella. Little is known about the function of centrioles. They are absent from most plant cells and experimental destruction of animal centrioles appears to have little effect on mitosis.

By late prophase (figure 1c), the MTOCs (with or without their centrioles) have moved to opposite ends (poles) of the cell. Some microtubules of the spindle apparatus make contact with the centromeres of the chromosomes; others make contact with microtubules from the opposite pole. The chromosomes move towards the equator (an imaginary line equidistant between the poles) of the cell.

## Metaphase

In **metaphase** (figure 2a) the centromeres of all the chromosomes are lined up on the equator and divide. The spindle apparatus is fully formed. One sister chromatid from each chromosome is attached by microtubules to one pole; the other is attached to the opposite pole.

## Anaphase

**Anaphase** (figure 2b) begins with the separation of the centromeres. The sister chromatids are drawn apart to opposite poles of the cell. Once the sister chromatids are separated they are referred to as **daughter chromosomes**. The separation and movement is brought about by the shortening of the microtubules that connect the chromosomes to the poles, and the lengthening of the pole-to-pole microtubules. The poles move further apart, lengthening the cell.

# CARBOHYDRATES: POLYSACCHARIDES

## OBJECTIVES

By the end of this spread you should be able to:

- describe the differences between sugars and polysaccharides
- explain how the structures of starch, glycogen, and cellulose are related to their functions.

### Fact of life

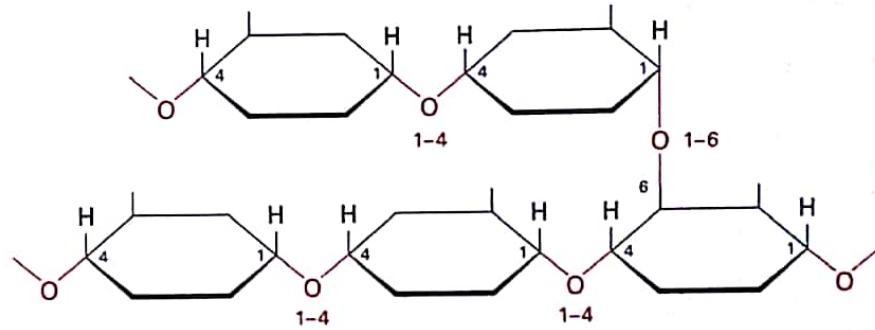
Cellulose is the principal structural material of plants. As such, it is the most abundant organic compound in the world.

Polysaccharides are polymers made up of many monosaccharide units (monomers), which are linked by condensation reactions (see spread 2.6). They have the general formula  $(C_6H_{10}O_5)_n$ . Polysaccharides are relatively insoluble in water; are not sweet, and cannot be crystallised.

The monosaccharides can join together in different ways to form chains or ring structures. Chains may be straight, helical, coils (spirals), or branched. The properties of a polysaccharide depend on the number and type of monomer it contains, and how these are joined together.

By convention, each carbon atom in a monosaccharide unit is identified by a number (see spread 2.6, figure 1).

- A condensation reaction between the hydroxyl groups at carbon 1 of one monosaccharide and carbon 4 of the other results in a bond called a **1-4 glycosidic bond**.
- If the reaction is between the hydroxyl groups on carbon 1 and carbon 6, a **1-6 glycosidic bond** is formed.
- Straight chains are formed by monomers linked by 1-4 glycosidic bonds; branched chains have one or more 1-6 glycosidic bonds (figure 1).



**Figure 1** Part of a glycogen molecule showing a straight-chained section of alpha glucose units interconnected by 1-4 glycosidic bonds, and a branch formed by a 1-6 glycosidic bond. In glycogen, the 1-6 branches occur at intervals of 8–10 glucose units.

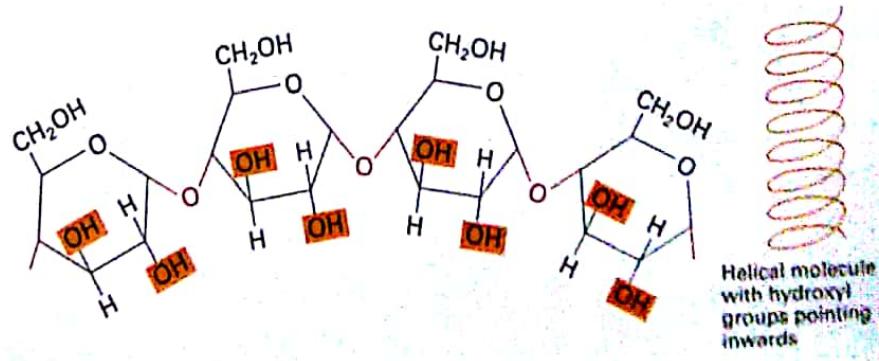
In amylopectin (a constituent of starch which has a similar structure to glycogen), the branches occur at intervals of 12–20 glucose units.

### Starch: energy storage in plants

Starch is a polysaccharide formed from alpha glucose units. It consists of two components: amylose and amylopectin. Both are formed by the condensation of alpha glucose units, but the units are arranged differently. In amylose, the units are linked mainly by 1-4 glycosidic bonds which form unbranched chains. Amylopectin has many more 1-6 glycosidic bonds, producing highly branched chains.

Because of its structure (figure 2) starch is compact which is ideal for a storage product. In flowering plants, starch granules are confined to double-membraned organelles called **plastids**. These may be colourless **amyloplasts** in storage structures such as potato tubers (figure 3), or green **chloroplasts** in leaves.

Starch is broken down to glucose for respiration. It is also a source of organic carbon for making other substances.



**Figure 2** The structure of a starch molecule. Amylose and amylopectin are laid down in successive rings to form starch granules.

The long chains of alpha glucose units are coiled into a helix, forming a cylinder-like structure with most of the hydroxyl groups pointing inwards.

## Glycogen: energy storage in animal cells

Glycogen is also a storage polysaccharide. It is found as small granules (figure 4) and is particularly abundant in liver and muscle cells. Glycogen has a similar role and structure to starch. (It is sometimes called 'animal starch'.) However, it is much more branched because it has many more 1–6 glycosidic bonds. This makes glycogen less dense and more soluble than starch. Glycogen can be also hydrolysed readily by enzymes and is broken down more rapidly than starch. This is reflected in the higher metabolic rate in animals compared with plants.

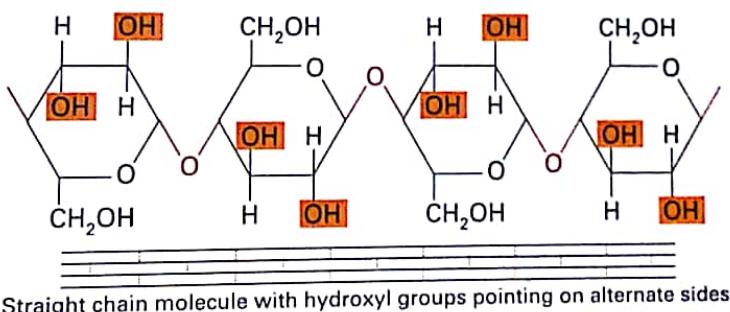
### Structural materials: cellulose

**Cellulose** is a tough structural polysaccharide. It is the major constituent of plant cell walls. The structure of cellulose is shown in figures 5 and 6. Cellulose is completely permeable; it allows water and dissolved substances to enter and leave plant cells freely. The cells swell when they take in water by osmosis (spread 4.9) and the cell wall prevents the cells from bursting when this happens. Cellulose becomes impermeable when the gaps between fibres are filled with impermeable substances.

Unlike starch and glycogen, cellulose cannot be hydrolysed easily. Herbivores such as cows and elephants are able to digest grass because microorganisms in their guts (spread 9.10) produce **cellulase**, the enzyme that digests cellulose. Humans and most other animals do not produce cellulase so they cannot obtain the nutrient content from plant cells.

### Lignin

In wood, cellulose is further strengthened by **lignin**. Lignin is a highly complex non-carbohydrate polymer. It impregnates the cell walls of the water-transporting tubes (xylem) to form an impermeable lining, a process called lignification. Xylem cells die when they become completely impregnated or lignified. In addition to strengthening cells, lignin also helps prevent rot, infection, and decay.



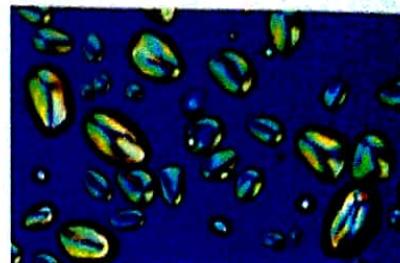
**Figure 5** The structure of a cellulose molecule. Cellulose is formed from beta glucose units linked by 1–4 glycosidic bonds. The hydroxyl groups alternate on either side of the molecule. This forms straight chains, giving cellulose a fibrous structure. Cellulose fibres are strengthened further by hydrogen bonds that link adjacent chains.

### QUICK CHECK

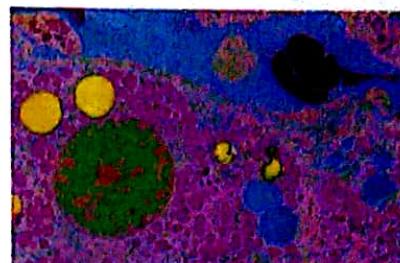
- 1 List three differences between a sugar and a polysaccharide such as starch.
- 2 a What is the main function of starch?  
b What is the main function of cellulose?  
c Outline the essential structural differences between starch and cellulose.

### Food for thought

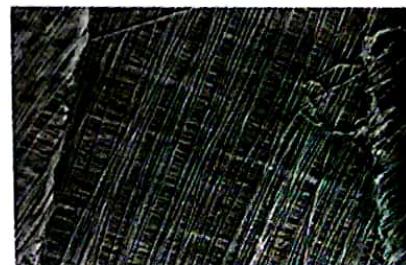
Sea squirts are marine animals which belong to the chordates, the same phylum as ourselves. They have a larval stage which resembles a tadpole, but the adults are barrel-shaped creatures that no longer move around. Typically, sea squirts remain attached by one end to a rock and filter feed. The body of a sea squirt is covered by a special mantle called a tunic. Curiously, the principal component of most tunics is a type of cellulose called tunicine. Suggest the advantages to sea squirts of having cellulose in their tunics.



**Figure 3** A photomicrograph of potato cells showing starch granules (x300).



**Figure 4** A photomicrograph of a liver cell showing glycogen granules which appear as pink-red packages (x3400).



**Figure 6** An electron micrograph of a cell wall of the seaweed *Chaetomorpha*. The cell wall is made up of microfibrils which are laid down in layers. Each microfibril is a rope-like structure, about 30–40 nm in diameter, consisting of a mass of cellulose chains. Microfibrils of one layer lie roughly parallel, but at an angle to those in other layers. Some of the microfibrils are interwoven, adding strength to the cell wall.