Cell structure

In the early days of microscopy an English scientist, Robert Hooke, decided to examine thin slices of plant material. He chose cork as one of his examples. Looking down the microscope, he was struck by the regular appearance of the structure, and in 1665 he wrote a book containing the diagram shown in Figure 1.2. If you examine the diagram you will see the 'porelike' regular structures that Hooke called 'cells'. Each cell appeared to be an empty box surrounded by a wall. Hooke had discovered and described, without realising it, the fundamental unit of all living things. Although we now know that the cells of cork are dead, further observations of cells in living materials were made by Hooke and other scientists. However, it was not until almost 200 years later that a general cell theory emerged from the work of two German scientists. In 1838 Schleiden, a botanist, suggested that all plants are made of cells, and a year later Schwann, a zoologist, suggested the same for animals. The cell theory states that the basic unit of structure and function of all living organisms is the cell. Now, over 170 years later, this idea is one of the most familiar and important theories in biology. To it has been added Virchow's theory of 1855 that all cells arise from pre-existing cells by cell division.

Why cells?

A cell can be thought of as a bag in which the chemistry of life is allowed to occur, partially separated from the environment outside the cell. The thin membrane which surrounds all cells is essential in controlling exchange between the cell and its environment. It is a very effective barrier, but also allows a controlled traffic of materials across it in both directions. The membrane is therefore described as partially permeable. If it were freely permeable, life could not exist, because the chemicals of the cell would simply mix with the surrounding chemicals by diffusion.

Cell Biology and Microscopy

The study of cells has given rise to an important branch of biology known as cell biology. Cells can now be studied by many different methods, but scientists began simply by looking at them, using various types of microscope. There are two fundamentally different types of microscope now in use: the light microscope and the electron microscope. Both use a form of radiation in order to create an image of the specimen being examined. The light microscope uses light as a source of radiation, while the electron microscope uses electrons, for reasons which are discussed later.

Light microscopy

The 'golden age' of light microscopy could be said to be the 19th century. Microscopes had been available since the beginning of the 17th century but, when dramatic improvements were made in the quality of glass lenses in the early 19th century, interest among scientists became widespread. The fascination of the microscopic world that opened up in biology inspired rapid progress both in microscope design and, equally importantly, in preparing material for examination with microscopes. This branch of biology is known as cytology. Figure 1.3 shows how the light microscope works. By 1900, all the structures shown in Figures 1.4 and 1.5 had been discovered. Figure 1.4 shows the structure of a generalised animal cell and Figure 1.5 the structure of a generalised plant cell as seen with a light microscope. (A generalised cell shows

all the structures that are typically found in a cell.) Figure 1.6 shows some actual human cells and Figure 1.7 shows an actual plant cell taken from a leaf.

Animal and plant cells have features in common

In animals and plants each cell is surrounded by a very thin cell surface membrane. This is also sometimes referred to as the plasma membrane. Many of the cell contents are colourless and transparent so they need to be stained to be seen. Each cell has a nucleus, which is a relatively large structure that stains intensely and is therefore very conspicuous. The deeply staining material in the nucleus is called chromatin and is a mass of loosely coiled threads. This material collects together to form visible separate chromosomes during nuclear division (page 98). It contains DNA (deoxyribonucleic acid), a molecule which contains the instructions that control the activities of the cell (see Chapter 6). Within the nucleus an even more deeply staining area is visible, the nucleolus, which is made of loops of DNA from several chromosomes. The number of nucleoli is variable, with one to five being common in mammals. The material between the nucleus and the cell surface membrane is known as cytoplasm. Cytoplasm is an aqueous (watery) material, varying from a fluid to a jelly-like consistency. Many small structures can be seen within it. These have been likened to small organs and hence are known as organelles. An organelle can be defined as a functionally and structurally distinct part of a cell. Organelles themselves are often surrounded by membranes so that their activities can be separated from the surrounding cytoplasm. This is described as compartmentalisation. Having separate compartments is essential for a structure as complex as an animal or plant cell to work efficiently. Since each type of organelle has its own function, the cell is said to show a division of labour, a sharing of the work between different specialised organelles. The most numerous organelles seen with the light microscope are usually mitochondria (singular: mitochondrion). Mitochondria are only just visible, but films of living cells, taken with the aid of a light microscope, have shown that they can move about, change shape and divide. They are specialised to carry out aerobic respiration. The use of special stains containing silver enabled the Golgi apparatus to be detected for the first time in 1898 by Camillo Golgi. The Golgi apparatus is part of a complex internal sorting and distribution system within the cell (page 15). It is also sometimes called the Golgi body or Golgi complex.

Differences between animal and plant cells

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

Centrioles

Under the light microscope the centriole appears as a small structure close to the nucleus (Figure 1.4, page 3). Centrioles are discussed on page 18.

Cell walls and plasmodesmata

With a light microscope, individual plant cells are more easily seen than animal cells, because they are usually larger and, unlike animal cells, surrounded by a cell wall outside the cell surface membrane. This is relatively rigid because it contains fibres of cellulose, a polysaccharide which strengthens the wall. The cell wall gives the cell a definite shape. It prevents the cell from

bursting when water enters by osmosis, allowing large pressures to develop inside the cell (page 84). Cell walls may also be reinforced with extra cellulose or with a hard material called lignin for extra strength (page 141). Cell walls are freely permeable, allowing free movement of molecules and ions through to the cell surface membrane. Plant cells are linked to neighbouring cells by means of fine strands of cytoplasm called plasmodesmata (singular: plasmodesma), which pass through pore-like structures in their walls. Movement through the pores is thought to be controlled by the structure of the pores.

Vacuoles

Although animal cells may possess small vacuoles such as phagocytic vacuoles (page 87), which are temporary structures, mature plant cells often possess a large, permanent, central vacuole. The plant vacuole is surrounded by a membrane, the tonoplast, which controls exchange between the vacuole and the cytoplasm. The fluid in the vacuole is a solution of pigments, enzymes, sugars and other organic compounds (including some waste products), mineral salts, oxygen and carbon dioxide. Vacuoles help to regulate the osmotic properties of cells (the flow of water inwards and outwards) as well as having a wide range of other functions. For example, the pigments which colour the petals of certain flowers and parts of some vegetables, such as the red pigment of beetroots, may be located in vacuoles.

Chloroplasts

Chloroplasts are found in the green parts of the plant, mainly in the leaves. They are relatively large organelles and so are easily seen with a light microscope. It is even possible to see tiny ‘grains’ or grana (singular: granum) inside the chloroplasts using a light microscope. These are the parts of the chloroplast that contain chlorophyll, the green pigment which absorbs light during the process of photosynthesis, the main function of chloroplasts. Chloroplasts are discussed further on page 19.

Points to note

- You can think of a plant cell as being very similar to an animal cell, but with extra structures.
- Plant cells are often larger than animal cells, although cell size varies enormously.
- Do not confuse the cell wall with the cell surface membrane. Cell walls are relatively thick and physically strong, whereas cell surface membranes are very thin. Cell walls are freely permeable, whereas cell surface membranes are partially permeable. All cells have a cell surface membrane.
- Vacuoles are not confined to plant cells; animal cells may have small vacuoles, such as phagocytic vacuoles, although these are not usually permanent structures. We return to the differences between animal and plant cells as seen using the electron microscope on page 13.

Units of measurement

In order to measure objects in the microscopic world, we need to use very small units of measurement, which are unfamiliar to most people. According to international agreement, the International System of Units (SI units) should be used. In this system, the basic unit of length is

the metre (symbol, m). Additional units can be created in multiples of a thousand times larger or smaller, using standard prefixes. For example, the prefix kilo means 1000 times. Thus 1 kilometre = 1000 metres. The units of length relevant to cell studies are shown in Table 1.1

It is difficult to imagine how small these units are, but, when looking down a microscope and seeing cells clearly, we should not forget how amazingly small the cells actually are. The smallest structure visible with the human eye is about 50–100 μm in diameter. Your body contains about 60 million million cells, varying in size from about 5 μm to 40 μm. Try to imagine structures like mitochondria, which have an average diameter of 1 μm. The smallest cell organelles we deal with in this book, ribosomes, are only about 25 nm in diameter! You could line up about 20,000 ribosomes across the full stop at the end of this sentence.

Electron microscopy

As we said on page 3, by 1900 almost all the structures shown in Figures 1.4 and 1.5 (pages 3 and 4) had been discovered. There followed a time of frustration for microscopists, because they realised that no matter how much the design of light microscopes improved, there was a limit to how much could ever be seen using light. In order to understand why this is, it is necessary to know something about the nature of light itself and to understand the difference between magnification and resolution.

Magnification

Magnification is the number of times larger an image is, than the real size of the object.
magnification = observed size of the image actual size
Or
M = I/A
Here I = observed size of the image (that is, what you can measure with a ruler) and A = actual size (that is, the real size – for example, the size of a cell before it is magnified). If you know two of these values, you can work out the third one. For example, if the observed size of the image and the magnification are known, you can work out the actual size: A = I × M . If you write the formula in a triangle as shown on the right and cover up the value you want to find, it should be obvious how to do the right calculation. Some worked examples are now provided.

Background information

Biological material may be examined live or in a preserved state. Prepared slides contain material that has been killed and preserved in a life-like condition. This material is often cut into thin sections to enable light to pass through the structures for viewing with a light microscope. The sections are typically stained and ‘mounted’ on a glass slide, forming a permanent preparation. Temporary preparations of fresh material have the advantage that they can be made rapidly and are useful for quick preliminary investigations. Sectioning and staining may still be carried out if required. Sometimes macerated (chopped up) material can be used, as when examining the structure of wood (xylem). A number of temporary stains are commonly used. For example, iodine in potassium iodide solution is useful for plant specimens. It stains starch blue-black and will also colour nuclei and cell walls a pale yellow. A dilute solution of methylene blue can be used to stain animal cells such as cheek cells. Viewing specimens

yourself with a microscope will help you to understand and remember structures more fully. This can be reinforced by making a pencil drawing on good quality plain paper, using the guidance given later in Chapter 7 (Box 7.1, page 129). Remember always to draw what you see, and not what you think you should see.

Procedure
The material is placed on a clean glass slide and one or two drops of stain added. A cover slip is carefully lowered over the specimen to protect the microscope lens and to help prevent the specimen from drying out. A drop of glycerine mixed with the stain can also help prevent drying out. Suitable animal material: human cheek cells Suitable plant material: onion epidermal cells, lettuce epidermal cells, Chlorella cells, moss leaves

Resolution
Look again at Figure 1.9 (page 8). Figure 1.9a is a light micrograph (a photograph taken with a light microscope, also known as a photomicrograph). Figure 1.9b is an electron micrograph of the same specimen taken at the same magnification (an electron micrograph is a picture taken with an electron microscope). You can see that Figure 1.9b, the electron micrograph, is much clearer. This is because it has greater resolution. Resolution can be defined as the ability to distinguish between two separate points. If the two points cannot be resolved, they will be seen as one point. In practice, resolution is the amount of detail that can be seen – the greater the resolution, the greater the detail. The maximum resolution of a light microscope is 200nm. This means that if two points or objects are closer together than 200 nm they cannot be distinguished as separate. It is possible to take a photograph such as Figure 1.9a and to magnify (enlarge) it, but we see no more detail; in other words, we do not improve resolution, even though we often enlarge photographs because they are easier to see when larger. With a microscope, magnification up to the limit of resolution can reveal further detail, but any further magnification increases blurring as well as the size of the image.

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

Magnification is the number of times greater that an image is than the actual object; magnification = image size ÷ actual (real) size of the object.

The electromagnetic spectrum
How is resolution linked with the nature of light? One of the properties of light is that it travels in waves. The length of the waves of visible light varies, ranging from about 400 nm (violet light) to about 700 nm (red light). The human eye can distinguish between these different wavelengths, and in the brain the differences are converted to colour differences. (Colour is an invention of the brain!) The whole range of different wavelengths is called the electromagnetic spectrum. Visible light is only one part of this spectrum. Figure 1.11 shows some of the parts of the electromagnetic spectrum. The longer the waves, the lower their frequency (all the waves travel at the same speed, so imagine them passing a post: shorter waves pass at higher frequency). In theory, there is no limit to how short or how long the waves can be. Wavelength changes with

energy: the greater the energy, the shorter the wavelength. Now look at Figure 1.12, which shows a mitochondrion, some very small cell organelles called ribosomes (page 15) and light of 400 nm wavelength, the shortest visible wavelength. The mitochondrion is large enough to interfere with the light waves. However, the ribosomes are far too small to have any effect on the light waves. The general rule is that the limit of resolution is about one-half the wavelength of the radiation used to view the specimen. In other words, if an object is any smaller than half the wavelength of the radiation used to view it, it cannot be seen separately from nearby objects. This means that the best resolution that can be obtained using a microscope that uses visible light (a light microscope) is 200nm, since the shortest wavelength of visible light is 400 nm (violet light). In practice, this corresponds to a maximum useful magnification of about 1500 times. Ribosomes are approximately 25 nm in diameter and can therefore never be seen using light.

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

The electron microscope

Biologists, faced with the problem that they would never see anything smaller than 200 nm using a light microscope, realised that the only solution would be to use radiation of a shorter wavelength than light. If you study Figure 1.11, you will see that ultraviolet light, or better still X-rays, look like possible candidates. Both ultraviolet and X-ray microscopes have been built, the latter with little success partly because of the difficulty of focusing X-rays. A much better solution is to use electrons. Electrons are negatively charged particles which orbit the nucleus of an atom. When a metal becomes very hot, some of its electrons gain so much energy that they escape from their orbits, like a rocket escaping from Earth's gravity. Free electrons behave like electromagnetic radiation. They have a very short wavelength: the greater the energy, the shorter the wavelength. Electrons are a very suitable form of radiation for microscopy for two major reasons. Firstly, their wavelength is extremely short (at least as short as that of X-rays). Second, because they are negatively charged, they can be focused easily using electromagnets (a magnet can be made to alter the path of the beam, the equivalent of a glass lens bending light). Using an electron microscope, a resolution of 0.5 nm can be obtained, 400 times better than a light microscope.

Transmission and scanning electron microscopes

Two types of electron microscope are now in common use. The transmission electron microscope, or TEM, was the type originally developed. Here the beam of electrons is passed through the specimen before being viewed. Only those electrons that are transmitted (pass through the specimen) are seen. This allows us to see thin sections of specimens, and thus to see inside cells. In the scanning electron microscope (SEM), on the other hand, the electron beam is used to scan the surfaces of structures, and only the reflected beam is observed. An example of a scanning electron micrograph is shown in Figure 1.13. The advantage of this microscope is that surface structures can be seen. Also, great depth of field is obtained so that much of the specimen is in focus at the same time and a three-dimensional appearance is achieved. Such a picture would be impossible to obtain with a light microscope, even using the

same magnification and resolution, because you would have to keep focusing up and down with the objective lens to see different parts of the specimen. The disadvantage of the SEM is that it cannot achieve the same resolution as a TEM. Using an SEM, resolution is between 3 nm and 20 nm.

Viewing specimens with the electron microscope

Figure 1.14 shows how an electron microscope works and Figure 1.15 shows one in use. It is not possible to see an electron beam, so to make the image visible the electron beam has to be projected onto a fluorescent screen. The areas hit by electrons shine brightly, giving overall a black and white picture. The stains used to improve the contrast of biological specimens for electron microscopy contain heavy metal atoms, which stop the passage of electrons. The resulting picture is like an X-ray photograph, with the more densely stained parts of the specimen appearing blacker. 'False-colour' images can be created by colouring the standard black and white image using a computer.

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

Ultrastructure of an animal cell

The fine (detailed) structure of a cell as revealed by the electron microscope is called its ultrastructure. Figure 1.16 shows the appearance of typical animal cells as seen with an electron microscope, and Figure 1.17 is a diagram based on many other such micrographs.

Structures and functions of organelles

Compartmentalisation and division of labour within the cell are even more obvious with an electron microscope than with a light microscope. We will now consider the structures and functions of some of the cell components in more detail.

Nucleus

The nucleus (Figure 1.18) is the largest cell organelle. It is surrounded by two membranes known as the nuclear envelope. The outer membrane of the nuclear envelope is continuous with the endoplasmic reticulum (Figure 1.17)

The nuclear envelope has many small pores called nuclear pores. These allow and control exchange between the nucleus and the cytoplasm. Examples of substances leaving the nucleus through the pores are mRNA and ribosomes for protein synthesis. Examples of substances entering through the nuclear pores are proteins to help make ribosomes, nucleotides, ATP (adenosine triphosphate) and some hormones such as thyroid hormone T3. Within the nucleus, the chromosomes are in a loosely coiled state known as chromatin (except during nuclear division, Chapter 5). Chromosomes contain DNA, which is organised into functional units called

genes. Genes control the activities of the cell and inheritance; thus the nucleus controls the cell's activities. When a cell is about to divide, the nucleus divides first so that each new cell will have its own nucleus (Chapters 5 and 16). Also within the nucleus, the nucleolus makes ribosomes, using the information in its own DNA.

Endoplasmic reticulum and ribosomes

When cells were first seen with the electron microscope, biologists were amazed to see so much detailed structure. The existence of much of this had not been suspected. This was particularly true of an extensive system of membranes running through the cytoplasm, which became known as the endoplasmic reticulum (ER) (Figures 1.18, 1.19 and 1.22). The membranes form an extended system of flattened compartments, called sacs, spreading throughout the cell. Processes can take place inside these sacs, separated from the cytoplasm. The sacs can be interconnected to form a complete system (reticulum) – the connections have been compared to the way in which the different levels of a parking lot are connected by ramps. The ER is continuous with the outer membrane of the nuclear envelope (Figure 1.17). There are two types of ER: rough ER and smooth ER. Rough ER is so called because it is covered with many tiny organelles called ribosomes. These are just visible as black dots in Figures 1.18 and 1.19. At very high magnifications they can be seen to consist of two subunits: a large and a small subunit. Ribosomes are the sites of protein synthesis (page 119). They can be found free in the cytoplasm as well as on the rough ER. They are very small, only about 25 nm in diameter. They are made of RNA (ribonucleic acid) and protein. Proteins made by the ribosomes on the rough ER enter the sacs and move through them. The proteins are often modified in some way on their journey. Small sacs called vesicles can break off from the ER and these can join together to form the Golgi body. They form part of the secretory pathway because the proteins can be exported from the cell via the Golgi vesicles (Figure 1.2). Smooth ER, so called because it lacks ribosomes, has a completely different function. It makes lipids and steroids, such as cholesterol and the reproductive hormones oestrogen and testosterone.

Golgi body (Golgi apparatus or Golgi complex)

The Golgi body is a stack of flattened sacs (Figure 1.20). More than one Golgi body may be present in a cell. The stack is constantly being formed at one end from vesicles that bud off from the ER, and broken down again at the other end to form Golgi vesicles. The stack of sacs together with the associated vesicles is referred to as the Golgi apparatus or Golgi complex. The Golgi body collects, processes and sorts molecules (particularly proteins from the rough ER), ready for transport in Golgi vesicles either to other parts of the cell or out of the cell (secretion). Two examples of protein processing in the Golgi body are the addition of sugars to proteins to make molecules known as glycoproteins, and the removal of the first amino acid, methionine, from newly formed proteins to make a functioning protein. In plants, enzymes in the Golgi body convert sugars into cell wall components. Golgi vesicles are also used to make lysosomes.

Lysosomes

Lysosomes (Figure 1.21) are spherical sacs, surrounded by a single membrane and having no internal structure. They are commonly 0.1– 0.5 μm in diameter. They contain digestive

(hydrolytic) enzymes which must be kept separate from the rest of the cell to prevent damage from being done. Lysosomes are responsible for the breakdown (digestion) of unwanted structures such as old organelles or even whole cells, as in mammary glands after lactation (breastfeeding). In white blood cells, lysosomes are used to digest bacteria (see endocytosis, page 87). Enzymes are sometimes released outside the cell – for example, in the replacement of cartilage with bone during development. The heads of sperm contain a special lysosome, the acrosome, for digesting a path to the ovum (egg).

Mitochondria

Structure

The structure of the mitochondrion as seen with the electron microscope is visible in Figures 1.16, 1.22, 12.13 and 12.14. Mitochondria (singular: mitochondrion) are usually about 1 μm in diameter and can be various shapes, often sausage-shaped as in Figure 1.22. They are surrounded by two membranes (an envelope). The inner of these is folded to form finger-like cristae which project into the interior solution, or matrix. The space between the two membranes is called the intermembrane space. The outer membrane contains a transport protein called porin, which forms wide aqueous channels allowing easy access of small, water-soluble molecules from the surrounding cytoplasm into the intermembrane space. The inner membrane is a far more selective barrier and controls precisely what ions and molecules can enter the matrix. The number of mitochondria in a cell is very variable. As they are responsible for aerobic respiration, it is not surprising that cells with a high demand for energy, such as liver and muscle cells, contain large numbers of mitochondria. A liver cell may contain as many as 2000 mitochondria. If you exercise regularly, your muscles will make more mitochondria.

Function of mitochondria and the role of ATP

As we have seen, the main function of mitochondria is to carry out aerobic respiration, although they do have other functions, such as the synthesis of lipids. During respiration, a series of reactions takes place in which energy is released from energy-rich molecules such as sugars and fats. Most of this energy is transferred to molecules of ATP. ATP (adenosine triphosphate) is the energy-carrying molecule found in all living cells. It is known as the universal energy carrier. The reactions of respiration take place in solution in the matrix and in the inner membrane (cristae). The matrix contains enzymes in solution, including those of the Krebs cycle (Chapter 12) and these supply the hydrogen and electrons to the reactions that take place in the cristae. The flow of electrons along the precisely placed electron carriers in the membranes of the cristae is what provides the power to generate ATP molecules, as explained in Chapter 12. The folding of the cristae increases the efficiency of respiration because it increases the surface area available for these reactions to take place. Once made, ATP leaves the mitochondrion and, as it is a small, soluble molecule, it can spread rapidly to all parts of the cell where energy is needed. Its energy is released by breaking the molecule down to ADP (adenosine diphosphate). This is a hydrolysis reaction. The ADP can then be recycled into a mitochondrion for conversion back to ATP during aerobic respiration.

The endosymbiont theory

In the 1960s, it was discovered that mitochondria and chloroplasts contain ribosomes which are slightly smaller than those in the cytoplasm and are the same size as those found in bacteria. The size of ribosomes is measured in 'S units', which are a measure of how fast they sediment in a centrifuge. Cytoplasmic ribosomes are 80S, while those of bacteria, mitochondria and ribosomes are 70S. It was also discovered in the 1960s that mitochondria and chloroplasts contain small, circular DNA molecules, also like those found in bacteria. It was later proved that mitochondria and chloroplasts are, in effect, ancient bacteria which now live inside the larger cells typical of animals and plants (see prokaryotic and eukaryotic cells, page 21). This is known as the endosymbiont theory. 'Endo' means 'inside' and a 'symbiont' is an organism which lives in a mutually beneficial relationship with another organism. The DNA and ribosomes of mitochondria and chloroplasts are still active and responsible for the coding and synthesis of certain vital proteins, but mitochondria and chloroplasts can no longer live independently. Mitochondrial ribosomes are just visible as tiny dark orange dots in the mitochondrial matrix in Figure 1.22.

Cell surface membrane

The cell surface membrane is extremely thin (about 7 nm). However, at very high magnifications, at least × 100 000, it can be seen to have three layers, described as a tri-laminar appearance. This consists of two dark lines (heavily stained) either side of a narrow, pale interior (Figure 1.23). The membrane is partially permeable and controls exchange between the cell and its environment. Membrane structure is discussed further in Chapter 4.

Microvilli

Microvilli (singular: microvillus) are finger-like extensions of the cell surface membrane, typical of certain epithelial cells (cells covering surfaces of structures). They greatly increase the surface area of the cell surface membrane (Figure 1.17 on page 14). This is useful, for example, for absorption in the gut and for reabsorption in the proximal convoluted tubules of the kidney (page 308).

Microtubules and microtubule organising centres (MTOCs)

Microtubules are long, rigid, hollow tubes found in the cytoplasm. They are very small, about 25nm in diameter. Together with actin filaments and intermediate filaments (not discussed in this book), they make up the cytoskeleton, an essential structural component of cells which helps to determine cell shape. Microtubules are made of a protein called tubulin. Tubulin has two forms, α-tubulin (alpha-tubulin) and β-tubulin (beta-tubulin). α- and β-tubulin molecules combine to form dimers (double molecules). These dimers are then joined end to end to form long 'protofilaments'. This is an example of polymerisation. Thirteen protofilaments then line up alongside each other in a ring to form a cylinder with a hollow centre. This cylinder is the microtubule. Figure 1.24 (overleaf) shows the helical pattern formed by neighbouring α- and β-tubulin molecules.

Apart from their mechanical function of support, microtubules have a number of other functions. Secretory vesicles and other organelles and cell components can be moved along the outside surfaces of the microtubules, forming an intracellular transport system. Membrane-bound

organelles are held in place by the cytoskeleton. During nuclear division (Chapter 5), the spindle used for the separation of chromatids or chromosomes is made of microtubules, and microtubules form part of the structure of centrioles. The assembly of microtubules from tubulin molecules is controlled by special locations in cells called microtubule organising centres (MTOCs). These are discussed further in the following section on centrioles. Because of their simple construction, microtubules can be formed and broken down very easily at the MTOCs, according to need.

Centrioles and centrosomes

The extra resolution of the electron microscope reveals that just outside the nucleus of animal cells there are really two centrioles and not one as it appears under the light microscope (compare Figures 1.4 and 1.17). Th ey lie close together and at right angles to each other in a region known as the centrosome. Centrioles and the centrosome are absent from most plant cells. A centriole is a hollow cylinder about 500 nm long, formed from a ring of short microtubules. Each centriole contains nine triplets of microtubules (Figures 1.25 and 1.26).
The function of the centrioles remains a mystery. Until recently, it was believed that they acted as MTOCs for the assembly of the microtubules that make up the spindle during nuclear division (Chapter 5). It is now known that this is done by the centrosome, but does not involve the centrioles. Centrioles found at the bases of cilia (page 189) and flagella, where they are known as basal bodies, do act as MTOCs. The microtubules that extend from the basal bodies into the cilia and flagella are essential for the beating movements of these organelles.

Ultrastructure of a plant cell

All the structures so far described in animal cells are also found in plant cells, with the exception of centrioles and microvilli. The plant cell structures that are not found in animal cells are the cell wall, the large central vacuole, and chloroplasts. These are all shown clearly in Figures 1.27 and 1.28. The structures and functions of cell walls and vacuoles have been described on page 5.

Chloroplasts

The structure of the chloroplast as seen with the electron microscope is visible in Figures 1.27–1.29 and at a higher resolution in Figure 13.6. Chloroplasts tend to have an elongated shape and a diameter of about 3 to 10 μm (compare 1 μm diameter for mitochondria). Like mitochondria, they are surrounded by two membranes, forming the chloroplast envelope. Also like mitochondria, chloroplasts replicate themselves independently of cell division by dividing into two. The main function of chloroplasts is to carry out photosynthesis. Chloroplasts are an excellent example of how structure is related to function, so a brief understanding of their function will help you to understand their structure. During the first stage of photosynthesis (the light-dependent stage) light energy is absorbed by photosynthetic pigments, particularly the green pigment chlorophyll. Some of this energy is used to manufacture ATP from ADP. An essential stage in the process is the splitting of water into hydrogen and oxygen. The hydrogen is used as the fuel which is oxidised to provide the energy to make the ATP. This process, as in mitochondria, requires electron transport in membranes. This explains why chloroplasts contain a complex system of membranes. The membrane system is highly organised. It consists of fluid-filled sacs called thylakoids which spread out like sheets in three dimensions. In places, the

thylakoids form flat, disc-like structures that stack up like piles of coins many layers deep, forming structures called grana (from their appearance in the light microscope; ‘grana’ means grains). These membranes contain the photosynthetic pigments and electron carriers needed for the light-dependent stage of photosynthesis. Both the membranes and whole chloroplasts can change their orientation within the cell in order to receive the maximum amount of light. The second stage of photosynthesis (the light-independent stage) uses the energy and reducing power generated during the first stage to convert carbon dioxide into sugars. This requires a cycle of enzyme-controlled reactions called the Calvin cycle and takes place in solution in the stroma (the equivalent of the matrix in mitochondria). The sugars made may be stored in the form of starch grains in the stroma (Figures 1.27 and 13.6). The lipid droplets also seen in the stroma as black spheres in electron micrographs (Figure 1.29) are reserves of lipid for making membranes or from the breakdown of membranes in the chloroplast. Like mitochondria, chloroplasts have their own protein-synthesising machinery, including 70S ribosomes and a circular strand of DNA. In electron micrographs, the ribosomes can just be seen as small black dots in the stroma (Figure 13.6, page 291). Fibres of DNA can also sometimes be seen in small, clear areas in the stroma. As with mitochondria, it has been shown that chloroplasts originated as endosymbiotic bacteria, in this case photosynthetic blue-green bacteria. The endosymbiont theory is discussed in more detail on page 17.

Two fundamentally different types of cell

At one time it was common practice to try to classify all living organisms as either animals or plants. With advances in our knowledge of living things, it has become obvious that the living world is not that simple. Fungi and bacteria, for example, are very different from animals and plants, and from each other. Eventually it was discovered that there are two fundamentally different types of cell. The most obvious difference between these types is that one possesses a nucleus and the other does not. Organisms that lack nuclei are called prokaryotes (‘pro’ means before; ‘karyon’ means nucleus). They are, on average, about 1000 to 10,000 times smaller in volume than cells with nuclei, and are much simpler in structure – for example, their DNA lies free in the cytoplasm. Organisms whose cells possess nuclei are called eukaryotes (‘eu’ means true). Their DNA lies inside a nucleus. Eukaryotes include animals, plants, fungi and a group containing most of the unicellular eukaryotes known as protoctists. Most biologists believe that eukaryotes evolved from prokaryotes, 1500 million years after prokaryotes first appeared on Earth. We mainly study animals and plants in this book, but all eukaryotic cells have certain features in common. A generalised prokaryotic cell is shown in Figure 1.30. A comparison of prokaryotic and eukaryotic cells is given in Table 1.2.

Viruses

In 1852, a Russian scientist discovered that certain diseases could be transmitted by agents that, unlike bacteria, could pass through the finest filters. This was the first evidence for the existence of viruses, tiny ‘organisms’ which are much smaller than bacteria and are on the boundary between what we think of as living and non-living. Unlike prokaryotes and eukaryotes, viruses do not have a cell structure. In other words, they are not surrounded by a partially permeable membrane containing cytoplasm with ribosomes. They are much simpler in structure. Most consist only of:

- a self-replicating molecule of DNA or RNA which acts as its genetic code
- a protective coat of protein molecules.

Prokaryotes

- average diameter of cell is 0.5–5 μm
- DNA is circular and lies free in the cytoplasm
- DNA is naked
- slightly smaller (70S) ribosomes (about 20 nm diameter) than those of eukaryotes
- no ER present
- very few cell organelles – no separate membrane-bound compartments unless formed by infolding of the cell surface membrane
- cell wall present – wall contains murein, a peptidoglycan (a polysaccharide combined with amino acids)

Eukaryotes

- cells commonly up to 40 μm diameter and commonly 1000–10 000 times the volume of prokaryotic cells
- DNA is not circular and is contained in a nucleus – the nucleus is surrounded by an envelope of two membranes
- DNA is associated with protein, forming structures called chromosomes
- slightly larger (80S) ribosomes (about 25 nm diameter) than those of prokaryotes
- ER present, to which ribosomes may be attached
- many types of cell organelle present (extensive compartmentalisation and division of labour):
    - some organelles are bounded by a single membrane, e.g. lysosomes, Golgi body, vacuoles
    - some are bounded by two membranes (an envelope), e.g. nucleus, mitochondrion, chloroplast
    - some have no membrane, e.g. ribosomes, centrioles, microtubules
- cell wall sometimes present, e.g. in plants and fungi – contains cellulose or lignin in plants, and chitin (a nitrogen-containing polysaccharide similar to cellulose) in fungi

Figure 1.31 shows the structure of a simple virus. It has a very symmetrical shape. Its protein coat (or capsid) is made up of separate protein molecules, each of which is called a capsomere. Viruses range in size from about 20–300 nm (about 50 times smaller on average than bacteria). All viruses are parasitic because they can only reproduce by infecting and taking over living cells. The virus DNA or RNA takes over the protein-synthesising machinery of the host cell, which then helps to make new virus particles.

Summary

- The basic unit of life, the cell, can be seen clearly only with the aid of microscopes. The light microscope uses light as a source of radiation, whereas the electron microscope

uses electrons. The electron microscope has greater resolution (allows more detail to be seen) than the light microscope, because electrons have a shorter wavelength than light.

- With a light microscope, cells may be measured using an eyepiece graticule and a stage micrometer. Using the formula I A = M the actual size of an object (A) or its magnification (M) can be found if its observed (image) size (I) is measured and A or M, as appropriate, is known.
- All cells are surrounded by a partially permeable cell surface membrane that controls exchange between the cell and its environment. All cells contain genetic material in the form of DNA, and ribosomes for protein synthesis.
- The simplest cells are prokaryotic cells, which are thought to have evolved before, and given rise to, the much more complex and much larger eukaryotic cells. Prokaryotic cells lack a true nucleus and have smaller (70S) ribosomes than eukaryotic cells. They also lack membrane-bound organelles. Their DNA is circular and lies naked in the cytoplasm.
- All eukaryotic cells possess a nucleus containing one or more nucleoli and DNA. The DNA is linear and bound to proteins to form chromatin.
- The cytoplasm of eukaryotic cells contains many membrane-bound organelles providing separate compartments for specialised activities (division of labour). Organelles of eukaryotic cells include endoplasmic reticulum (ER), 80S ribosomes, mitochondria, Golgi apparatus and lysosomes. Animal cells also contain a centrosome and centrioles. Plant cells may contain chloroplasts, often have a large, permanent, central vacuole and have a cell wall containing cellulose.

Biological molecules

The study of biological molecules forms an important branch of biology known as molecular biology. The importance of the subject is clear from the relatively large number of Nobel prizes that have been awarded in this field. It has attracted some of the best scientists, even from other disciplines like physics and mathematics. Molecular biology is closely linked with biochemistry, which looks at the chemical reactions of biological molecules. The sum total of all the biochemical reactions in the body is known as metabolism. Metabolism is complex, but it has an underlying simplicity. For example, there are only 20 common amino acids used to make naturally occurring proteins, whereas theoretically there could be millions. Why so few? One possibility is that all the manufacture and reactions of biological molecules must be controlled and regulated and, the more there are, the more complex the control becomes. (Control and regulation by enzymes is examined in Chapter 3.) Another striking principle of molecular biology is how closely the structures of molecules are related to their functions. This will become clear in this chapter and in Chapter 3. Our understanding of how structure is related to function may lead to the creation of a vast range of ‘designer’ molecules to carry out such varied functions as large-scale industrial reactions and precise targeting of cells in medical treatment.

The building blocks of life

The four most common elements in living organisms are, in order of abundance, hydrogen, carbon, oxygen and nitrogen. They account for more than 99% of the atoms found in all living things. Carbon is particularly important because carbon atoms can join together to form long

chains or ring structures. They can be thought of as the basic skeletons of organic molecules to which groups of other atoms are attached. Organic molecules always contain carbon and hydrogen. It is believed that, before life evolved, there was a period of chemical evolution in which thousands of carbon-based molecules evolved from the more simple molecules that existed on the young planet Earth. Such an effect can be artificially created reasonably easily today given similar raw ingredients, such as methane (CH_4), carbon dioxide (CO_2), hydrogen (H_2), water (H_2O), nitrogen (N_2), ammonia (NH_3) and hydrogen sulfide (H_2S), and an energy source – for example, an electrical discharge. These simple but key biological molecules, which are relatively limited in variety, then act as the building blocks for larger molecules. The main ones are shown in Figure 2.2.

Monomers, polymers and macromolecules

The term macromolecule means giant molecule. There are three types of macromolecule in living organisms, namely polysaccharides, proteins (polypeptides) and nucleic acids (polynucleotides). The prefix 'poly' means many, and these molecules are polymers, meaning that they are made up of many repeating subunits that are similar or identical to each other. These subunits are referred to as monomers. They are joined together like beads on a string. Making such molecules is relatively simple because the same reaction is repeated many times. The monomers from which polysaccharides, proteins and nucleic acids are made are monosaccharides, amino acids and nucleotides respectively, as shown in Figure 2.2. Figure 2.2 also shows two types of molecule which, although not polymers, are made up of simpler biochemicals. These are lipids and nucleotides.

Natural examples of polymers are cellulose and rubber. There are many examples of industrially produced polymers, such as polyester, polythene, PVC (polyvinyl chloride) and nylon. All these are made up of carbon-based monomers and contain thousands of carbon atoms joined end to end. We shall now take a closer look at some of the small biological molecules and the larger molecules made from them. Organic bases, nucleotides and nucleic acids are dealt with in Chapter 6.

Carbohydrates

All carbohydrates contain the elements carbon, hydrogen and oxygen. The 'hydrate' part of the name comes from the fact that hydrogen and oxygen atoms are present in the ratio of 2 : 1, as they are in water ('hydrate' refers to water). The general formula for a carbohydrate can therefore be written as $C_x(H_2O)_y$. Carbohydrates are divided into three main groups, namely monosaccharides, disaccharides and polysaccharides. The word 'saccharide' refers to a sugar or sweet substance.

Monosaccharides

Monosaccharides are sugars. Sugars dissolve easily in water to form sweet-tasting solutions. Monosaccharides have the general formula $(CH_2O)_n$ and consist of a single sugar molecule ('mono' means one). The main types of monosaccharides, if they are classified according to the number of carbon atoms in each molecule, are trioses (3C), pentoses (5C) and hexoses (6C).

The names of all sugars end with -ose. Common hexoses are glucose, fructose and galactose. Two common pentoses are ribose and deoxyribose.

A macromolecule is a large biological molecule such as a protein, polysaccharide or nucleic acid.
A monomer is a relatively simple molecule which is used as a basic building block for the synthesis of a polymer; many monomers are joined together to make the polymer, usually by condensation reactions; common examples of molecules used as monomers are monosaccharides, amino acids and nucleotides.

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

Molecular and structural formulae
The formula for a hexose can be written as $C_6H_{12}O_6$. This is known as the molecular formula. It is also useful to show the arrangements of the atoms, which can be done using a diagram known as the structural formula. Figure 2.3 shows the structural formula of glucose, a hexose, which is the most common monosaccharide.

Ring structures
One important aspect of the structure of pentoses and hexoses is that the chain of carbon atoms is long enough to close up on itself and form a more stable ring structure. This can be illustrated using glucose as an example. When glucose forms a ring, carbon atom number 1 joins to the oxygen on carbon atom number 5 (Figure 2.4). The ring therefore contains oxygen, and carbon atom number 6 is not part of the ring. You will see from Figure 2.4 that the hydroxyl group, –OH, on carbon atom 1 may be above or below the plane of the ring. The form of glucose where it is below the ring is known as α-glucose (alpha-glucose) and the form where it is above the ring is β-glucose (beta-glucose). The same molecule can switch between the two forms. Two forms of the same chemical are known as isomers, and the extra variety provided by the existence of α- and β-isomers has important biological consequences, as we shall see in the structures of starch, glycogen and cellulose.

Roles of monosaccharides in living organisms
Monosaccharides have two major functions. First, they are commonly used as a source of energy in respiration. This is due to the large number of carbon–hydrogen bonds. These bonds can be broken to release a lot of energy, which is transferred to help make ATP (adenosine triphosphate) from ADP (adenosine diphosphate) and phosphate. The most important monosaccharide in energy metabolism is glucose. Secondly, monosaccharides are important as building blocks for larger molecules. For example, glucose is used to make the polysaccharides starch, glycogen and cellulose. Ribose (a pentose) is one of the molecules used to make RNA (ribonucleic acid) and ATP. Deoxyribose (also a pentose) is one of the molecules used to make DNA (Chapter 6).

Disaccharides and the glycosidic bond

Disaccharides, like monosaccharides, are sugars. They are formed by two monosaccharides joining together. The three most common disaccharides are maltose (glucose + glucose), sucrose (glucose + fructose) and lactose (glucose + galactose). Sucrose is the transport sugar in plants and the sugar commonly bought in shops. Lactose is the sugar found in milk and is therefore an important constituent of the diet of young mammals. The joining of two monosaccharides takes place by a process known as condensation. Two examples are shown in Figure 2.5. In Figure 2.5a two molecules of α-glucose combine to make the disaccharide maltose. In Figure 2.5b α-glucose and β-fructose combine to make the disaccharide sucrose. Notice that fructose has a different ring structure to glucose.

A monosaccharide is a molecule consisting of a single sugar unit with the general formula $(CH_2O)_n$.

A disaccharide is a sugar molecule consisting of two monosaccharides joined together by a glycosidic bond.

A polysaccharide is a polymer whose subunits are monosaccharides joined together by glycosidic bonds.

For each condensation reaction, two hydroxyl (–OH) groups line up alongside each other. One combines with a hydrogen atom from the other to form a water molecule. This allows an oxygen 'bridge' to form between the two molecules, holding them together and forming a disaccharide ('di' means two). The bridge is called a glycosidic bond. In theory any two –OH groups can line up and, since monosaccharides have many –OH groups, there are a large number of possible disaccharides. The shape of the enzyme controlling the reaction determines which –OH groups come alongside each other. Only a few of the possible disaccharides are common in nature. The reverse of condensation is the addition of water, which is known as hydrolysis (Figure 2.5). This takes place during the digestion of disaccharides and polysaccharides, when they are broken down to monosaccharides.

1 Reducing sugars – background information

The reducing sugars include all monosaccharides, such as glucose, and some disaccharides, such as maltose. The only common non-reducing sugar is sucrose. Reducing sugars are so called because they can carry out a type of chemical reaction known as reduction. In the process they are oxidised. This is made use of in the Benedict's test using Benedict's reagent. Benedict's reagent is copper(II) sulfate in an alkaline solution and has a distinctive blue colour. Reducing sugars reduce soluble blue copper sulfate, containing copper(II) ions, to insoluble brickred copper oxide, containing copper(I). The copper oxide is seen as a brick-red precipitate.

reducing sugar + Cu^{2+} → oxidised sugar + Cu^{+} blue red-brown

Procedure

Add Benedict's reagent to the solution you are testing and heat it in a water bath. If a reducing sugar is present, the solution will gradually turn through green, yellow and orange to red-brown as the insoluble copper(I) oxide forms a precipitate. As long as you use excess Benedict's reagent (more than enough to react with all of the sugar present), the intensity of the red colour is related to the concentration of the reducing sugar. You can then estimate the concentration using colour standards made by comparing the colour against the colours obtained in tests done with reducing sugar solutions of known concentration. You could also measure the time taken for the colour to change. Alternatively, you can use a colorimeter to measure subtle differences in colour precisely.

2 Non-reducing sugars – background information

Some disaccharides, such as sucrose, are not reducing sugars, so you would get a negative result from Benedict's test. In such a case, a brick-red precipitate in the test described below will tell you that a non-reducing sugar is present. If both a reducing sugar and a non-reducing sugar are present, the precipitate obtained in the test below will be heavier than the one obtained in Benedict's test. In the non-reducing sugars test, the disaccharide is first broken down into its two monosaccharide constituents. The chemical reaction is hydrolysis and can be brought about by hydrochloric acid. The constituent monosaccharides will be reducing sugars and their presence can be tested for using Benedict's test after the acid has been neutralised.

Procedure

Heat the sugar solution with hydrochloric acid. This will release free monosaccharides. Benedict's reagent needs alkaline conditions to work, so you need to neutralise the test solution now by adding an alkali such as sodium hydroxide. Add Benedict's reagent and heat as before and look for the colour change. If the solution goes red now but didn't in the first stage of the test, there is non-reducing sugar present. If there is still no colour change, then there is no sugar of any kind present.

Polysaccharides

Polysaccharides are polymers whose subunits (monomers) are monosaccharides. They are made by joining many monosaccharide molecules by condensation. Each successive monosaccharide is added by means of a glycosidic bond, as in disaccharides. The final molecule may be several thousand monosaccharide units long, forming a macromolecule. The most important polysaccharides are starch, glycogen and cellulose, all of which are polymers of glucose. Polysaccharides are not sugars. Since glucose is the main source of energy for cells, it is important for living organisms to store it in an appropriate form. If glucose itself accumulated in cells, it would dissolve and make the contents of the cell too concentrated, which would seriously affect its osmotic properties (page 82). Glucose is also a reactive molecule and would interfere with normal cell chemistry. These problems are avoided by converting glucose, by condensation reactions, to a storage polysaccharide, which is a convenient, compact, inert (unreactive) and insoluble molecule. The storage polysaccharide formed is starch in plants and glycogen in animals. Glucose can be made available again quickly by an enzyme-controlled reaction.

Starch and glycogen

Starch is a mixture of two substances – amylose and amylopectin. Amylose is made by condensations between α-glucose molecules, as shown in Figure 2.5a. In this way, a long, unbranching chain of several thousand 1,4 linked glucose molecules is built up. ('1,4 linked' means they are linked between carbon atoms 1 and 4 of successive glucose units.) The chains are curved (Figure 2.6) and coil up into helical structures like springs, making the final molecule more compact. Amylopectin is also made of many 1,4 linked α-glucose molecules, but the chains are shorter than in amylose, and branch out to the sides. The branches are formed by 1,6 linkages, as shown in Figure 2.7. Mixtures of amylose and amylopectin molecules build up into relatively large starch grains, which are commonly found in chloroplasts and in storage organs such as potato tubers and the seeds of cereals and legumes (Figure 2.8). Starch grains are easily seen with a light microscope, especially if stained; rubbing a freshly cut potato tuber on a glass slide and staining with iodine–potassium iodide solution (Box 2.2) is a quick method of preparing a specimen for viewing. Starch is never found in animal cells. Instead, a substance with molecules very like those of amylopectin is used as the storage carbohydrate. This is called glycogen. Glycogen, like amylopectin, is made of chains of 1,4 linked α-glucose with 1,6 linkages forming branches (Figure 2.7b). Glycogen molecules tend to be even more branched than amylopectin molecules. Glycogen molecules clump together to form granules, which are visible in liver cells and muscle cells, where they form an energy reserve.

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

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

Cellulose
Cellulose is the most abundant organic molecule on the planet, due to its presence in plant cell walls and its slow rate of breakdown in nature. It has a structural role, being a mechanically strong molecule, unlike starch and glycogen. However, the only difference between cellulose and starch and glycogen is that cellulose is a polymer of β-glucose, not α-glucose. Remember that in the β-isomer, the –OH group on carbon atom 1 projects above the ring (Figure 2.4 on page 30). In order to form a glycosidic bond with carbon atom 4, where the –OH group is below the ring, one glucose molecule must be upside down (rotated 180°) relative to the other. Thus successive glucose units are linked at 180° to each other, as shown in Figure 2.9. This arrangement of β-glucose molecules results in a strong molecule because the hydrogen atoms of –OH groups are weakly attracted to oxygen atoms in the same cellulose molecule (the oxygen of the glucose ring) and also to oxygen atoms of –OH groups in neighbouring molecules. These hydrogen bonds (page 35) are individually weak, but so many can form, due to the large number of –OH groups, that collectively they provide enormous strength. Between

60 and 70 cellulose molecules become tightly cross-linked to form bundles called microfibrils. Microfibrils are in turn held together in bundles called fibres by hydrogen bonding.

A cell wall typically has several layers of fibres, running in different directions to increase strength (Figure 2.10). Cellulose makes up about 20–40% of the average cell wall; other molecules help to cross-link the cellulose fibres, and some form a glue-like matrix around the fibres, which further increases strength. Cellulose fibres have a very high tensile strength, almost equal to that of steel. This means that if pulled at both ends they are very difficult to stretch or break, and makes it possible for a cell to withstand the large pressures that develop within it as a result of osmosis (page 82). Without the wall, the cell would burst when in a dilute solution. These pressures help provide support for the plant by making tissues rigid, and are responsible for cell expansion during growth. The arrangement of fibres around the cell helps to determine the shape of the cell as it grows. Despite their strength, cellulose fibres are freely permeable, allowing water and solutes to reach or leave the cell surface membrane.

Dipoles and hydrogen bonds

When atoms in molecules are held together by covalent bonds, they share electrons with each other. Each shared pair of electrons forms one covalent bond. For example, in a water molecule, two hydrogen atoms each share a pair of electrons with an oxygen atom, forming a molecule with the formula H_2O.

However, the electrons are not shared absolutely equally. In water, the oxygen atom gets slightly more than its fair share, and so has a small negative charge, written $\delta-$ (delta minus). The hydrogen atoms get slightly less than their fair share, and so have a small positive charge, written $\delta+$ (delta plus). This unequal distribution of charge is called a dipole.

In water, the negatively charged oxygen of one molecule is attracted to a positively charged hydrogen of another, and this attraction is called a hydrogen bond (see diagram below). It is much weaker than a covalent bond, but still has a very significant effect. You will find out how hydrogen bonds affect the properties of water on pages 46–47.

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

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

Lipids

It is difficult to define precisely what we mean by a ‘lipid’ because lipids are a very varied group of chemicals. They are all organic molecules which are insoluble in water. The most familiar lipids are fats and oils. Fats are solid at room temperature and oils are liquid at room

temperature – chemically they are very similar. We could say that true lipids are esters formed by fatty acids combining with an alcohol.

Fatty acids

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

The tails of some fatty acids have double bonds between neighbouring carbon atoms, like this: –C C–. Such fatty acids are described as unsaturated because they do not contain the maximum possible amount of hydrogen. They form unsaturated lipids. Double bonds make fatty acids and lipids melt more easily – for example, most oils are unsaturated. If there is more than one double bond, the fatty acid or lipid is described as polyunsaturated; if there is only one it is monounsaturated. Animal lipids are often saturated (no double bonds) and occur as fats, whereas plant lipids are often unsaturated and occur as oils, such as olive oil and sunflower oil.

Alcohols and esters

Alcohols are a series of organic molecules which contain a hydroxyl group, –OH, attached to a carbon atom. Glycerol is an alcohol with three hydroxyl groups (Figure 2.12). The reaction between an acid and an alcohol produces a chemical known as an ester. The chemical link between the acid and the alcohol is known as an ester bond or an ester linkage.

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

Triglycerides

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

Triglycerides are insoluble in water but are soluble in certain organic solvents, including ether, chloroform and ethanol. This is because of the non-polar nature of the hydrocarbon tails: they have no uneven distribution of electrical charge. Consequently, they will not mix freely with water molecules and are described as hydrophobic (water-hating). Figure 2.13 shows a simplified diagram of a triglyceride.

Roles of triglycerides

Lipids make excellent energy reserves because they are even richer in carbon–hydrogen bonds than carbohydrates. A given mass of lipid will therefore yield more energy on oxidation than the

same mass of carbohydrate (it has a higher calorific value), an important advantage for a storage product. Fat is stored in a number of places in the human body, particularly just below the dermis of the skin and around the kidneys. Below the skin it also acts as an insulator against loss of heat. Blubber, a lipid found in sea mammals like whales, has a similar function, as well as providing buoyancy. An unusual role for lipids is as a metabolic source of water. When oxidised in respiration they are converted to carbon dioxide and water. The water may be of importance in very dry habitats. For example, the desert kangaroo rat (Figure 2.14) never drinks water and survives on metabolic water from its fat intake.

Phospholipids

Phospholipids are a special type of lipid. Each molecule has the unusual property of having one end which is soluble in water. This is because one of the three fatty acid molecules is replaced by a phosphate group, which is polar (page 35) and can therefore dissolve in water. The phosphate group is hydrophilic (water-loving) and makes the head of a phospholipid molecule hydrophilic, although the two remaining tails are still hydrophobic (Figure 2.15). This allows the molecules to form a membrane around a cell, where the hydrophilc heads lie in the watery solutions on the outside of the membrane, and the hydrophobic tails form a layer that is impermeable to hydrophilic substances. The biological significance of this will become apparent when we study membrane structure (Chapter 4).

Background information

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

Procedure

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

Further information

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

Proteins

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

- all enzymes are proteins
- proteins are essential components of cell membranes – their functions in membranes, such as receptor proteins and signalling proteins, are discussed in Chapter 4
- some hormones are proteins – for example, insulin and glucagon
- the oxygen-carrying pigments haemoglobin and myoglobin are proteins

- antibodies, which attack and destroy invading microorganisms, are proteins
- collagen, another protein, adds strength to many animal tissues, such as bone and the walls of arteries
- hair, nails and the surface layers of skin contain the protein keratin
- actin and myosin are the proteins responsible for muscle contraction
- proteins may be storage products – for example, casein in milk and ovalbumin in egg white.

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

Amino acids

Figure 2.16 shows the general structure of all amino acids and of glycine, the simplest amino acid. They all have a central carbon atom which is bonded to an amine group, –NH2 , and a carboxylic acid group, –COOH. It is these two groups which give amino acids their name. The third component that is always bonded to the carbon atom is a hydrogen atom.

The only way in which amino acids differ from each other is in the remaining, fourth, group of atoms bonded to the central carbon. This is called the R group. There are 20 different amino acids which occur in the proteins of living organisms, all with a different R group. You can see their molecular formulae in Appendix 1. (You do not need to remember all the different R groups.) Appendix 1 also shows the three-letter abbreviations commonly used by scientists for the names of the amino acids. Many other amino acids have been synthesised in laboratories.

The peptide bond

Figure 2.17 shows how two amino acids can join together. One loses a hydroxyl (–OH) group from its carboxylic acid group, while the other loses a hydrogen atom from its amine group. This leaves a carbon atom of the first amino acid free to bond with the nitrogen atom of the second. The link is called a peptide bond. The oxygen and two hydrogen atoms removed from the amino acids form a water molecule. We have seen this type of reaction, a condensation reaction, in the formation of glycosidic bonds (Figure 2.5 on page 31) and in the synthesis of triglycerides (Figure 2.12 on page 37). The new molecule which has been formed, made up of two linked amino acids, is called a dipeptide. Any number of extra amino acids could be added to the chain in a series of condensation reactions. A molecule made up of many amino acids linked together by peptide bonds is called a polypeptide. A polypeptide is another example of a polymer and a macromolecule, like a polysaccharide. A complete protein molecule may contain just one polypeptide chain, or it may have two or more chains which interact with each other. In living cells, ribosomes are the sites where amino acids are joined together to form polypeptides. The reaction is controlled by enzymes. You can read more about this on pages 119–121.
Polypeptides can be broken down to amino acids by breaking the peptide bonds. This is a hydrolysis reaction, involving the addition of water (Figure 2.17), and happens naturally in the stomach and small intestine during digestion. Here, protein molecules in food are hydrolysed into amino acids before being absorbed into the blood.

Primary structure

A polypeptide or protein molecule may contain several hundred amino acids linked into a long chain. The particular amino acids contained in the chain, and the sequence in which they are joined, is called the primary structure of the protein. Figure 2.18 shows the primary structure of the protein ribonuclease, an enzyme. There are an enormous number of different possible primary structures. Even a change in one amino acid in a chain made up of thousands may completely alter the properties of the polypeptide or protein.

Secondary structure

The amino acids in a polypeptide chain have an effect on each other even if they are not directly next to each other. A polypeptide chain, or part of it, often coils into a corkscrew shape called an α-helix (Figure 2.19a). This secondary structure is due to hydrogen bonding between the oxygen of the –CO– group of one amino acid and the hydrogen of the –NH– group of the amino acid four places ahead of it. Each amino acid has an –NH– and a –CO– group, and Figure 2.19a shows that all these groups are involved in hydrogen bonding in the α-helix, holding the structure firmly in shape. Hydrogen bonding is a result of the polar characteristics of the –CO– and –NH– groups (page 36). Sometimes hydrogen bonding can result in a much looser, straighter shape than the α-helix, which is called a β-pleated sheet (Figure 2.19b). Hydrogen bonds, although strong enough to hold the α-helix and β-pleated sheet structures in shape, are easily broken by high temperatures and pH changes. As you will see, this has important consequences for living organisms. Some proteins or parts of proteins show no regular arrangement at all. It all depends on which R groups are present and therefore what attractions occur between amino acids in the chain. In diagrams of protein structure, α-helices can be represented as coils or cylinders, β-sheets as arrows, and random coils as ribbons (Figures 2.20 and 2.21).

Tertiary structure

In many proteins, the secondary structure itself is coiled or folded. Figure 2.20 shows the complex way in which a molecule of the protein lysozyme folds. Here the α-helices are represented as coils, while in Figure 2.21, which shows the secondary and tertiary structure of myoglobin, the α-helices are shown as cylinders. At first sight, the myoglobin and lysozyme molecules look like disorganised tangles, but this is not so. The shape of the molecules is very precise, and the molecules are held in these exact shapes by bonds between amino acids in different parts of the chain. The way in which a protein coils up to form a precise three-dimensional shape is known as its tertiary structure.

Primary structure is the sequence of amino acids in a polypeptide or protein.

Secondary structure is the structure of a protein molecule resulting from the regular coiling or folding of the chain of amino acids, e.g. an α-helix or β-pleated sheet.

Tertiary structure is the compact structure of a protein molecule resulting from the three-dimensional coiling of the already-folded chain of amino acids.

Figure 2.22 shows the four types of bond which help to keep folded proteins in their precise shapes. Hydrogen bonds can form between a wide variety of R groups. Disulfide bonds form between two cysteine molecules, which contain sulfur atoms. (Can you spot the four disulfide bonds in ribonuclease in Figure 2.18?) Ionic bonds form between R groups containing amine and carboxyl groups. (Which amino acids have these?) Hydrophobic interactions occur between R groups which are non-polar, or hydrophobic.

Hydrogen bonds form between strongly polar groups – for example, –NH–, –CO– and –OH groups.
Disulfide bonds form between cysteine molecules. They are strong covalent bonds. They can be broken by reducing agents.

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

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

Quaternary structure
Many protein molecules are made up of two or more polypeptide chains. Haemoglobin is an example of this, having four polypeptide chains in each molecule (Figure 2.23). The association of different polypeptide chains is called the quaternary structure of the protein. The chains are held together by the same four types of bond as in the tertiary structure. More details of haemoglobin are given in the next section.

Each haemoglobin molecule contains four polypeptide chains. The two α chains are shown in purple and blue, and the two β chains in brown and orange.

Each polypeptide chain contains a haem group, shown in yellow and red.

The haem group contains an iron atom, which can bond reversibly with an oxygen molecule.

The complete haemoglobin molecule is nearly spherical.

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

Globular and fibrous proteins
A protein whose molecules curl up into a ‘ball’ shape, such as myoglobin or haemoglobin, is known as a globular protein. In a living organism, proteins may be found in cells and in other aqueous environments such as blood, tissue fluid and in phloem of plants. Globular proteins usually curl up so that their non-polar, hydrophobic R groups point into the centre of the molecule, away from their watery surroundings. Water molecules are excluded from the centre

of the folded protein molecule. The polar, hydrophilic R groups remain on the outside of the molecule. Globular proteins, therefore, are usually soluble, because water molecules cluster around their outwardpointing hydrophilic R groups (Figure 2.24).

Many globular proteins have roles in metabolic reactions. Their precise shape is the key to their functioning. Enzymes, for example, are globular proteins. Many other protein molecules do not curl up into a ball, but form long strands. These are known as fibrous proteins. Fibrous proteins are not usually soluble in water and most have structural roles. For example, keratin forms hair, nails and the outer layers of skin, making these structures waterproof. Another example of a fibrous protein is collagen (pages 44–45).

Haemoglobin – a globular protein

Haemoglobin is the oxygen-carrying pigment found in red blood cells, and is a globular protein. We have seen that it is made up of four polypeptide chains, so it has a quaternary structure. Each chain is itself a protein known as globin. Globin is related to myoglobin and so has a very similar tertiary structure (Figures 2.21 and 2.23). There are many types of globin – two types are used to make haemoglobin, and these are known as alpha globin (α-globin) and beta-globin (β-globin). Two of the haemoglobin chains, called α chains, are made from α-globin, and the other two chains, called β chains, are made from β-globin. The haemoglobin molecule is nearly spherical (Figure 2.23). The four polypeptide chains pack closely together, their hydrophobic R groups pointing in towards the centre of the molecule, and their hydrophilic ones pointing outwards. The interactions between the hydrophobic R groups inside the molecule are important in holding it in its correct three-dimensional shape. The outward-pointing hydrophilic R groups on the surface of the molecule are important in maintaining its solubility. In the genetic condition known as sickle cell anaemia, one amino acid which occurs in the surface of the β chain is replaced with a different amino acid. The correct amino acid is glutamic acid, which is polar. The substitute is valine, which is nonpolar. Having a non-polar R group on the outside of the molecule makes the haemoglobin much less soluble, and causes the unpleasant and dangerous symptoms associated with sickle cell anaemia in anyone whose haemoglobin is all of this ‘faulty’ type (Figure 2.25). Each polypeptide chain of haemoglobin contains a haem group, shown in Figure 2.23b. A group like this, which is an important, permanent, part of a protein molecule but is not made of amino acids, is called a prosthetic group. Each haem group contains an iron atom. One oxygen molecule, O2, can bind with each iron atom. So a complete haemoglobin molecule, with four haem groups, can carry four oxygen molecules (eight oxygen atoms) at a time. It is the haem group which is responsible for the colour of haemoglobin. This colour changes depending on whether or not the iron atoms are combined with oxygen. If they are, the molecule is known as oxyhaemoglobin, and is bright red. If not, the colour is purplish.

Background information

All proteins have peptide bonds, containing nitrogen atoms. These form a purple complex with copper(II) ions and this forms the basis of the biuret test. The reagent used for this test is called biuret reagent. You can use it as two separate solutions: a dilute solution of potassium hydroxide or sodium hydroxide, and a dilute solution of copper(II) sulfate. Alternatively, you can use a ready-made biuret reagent that contains both the copper(II) sulfate solution and the

hydroxide ready mixed. To stop the copper ions reacting with the hydroxide ions and forming a precipitate, this ready-mixed reagent also contains sodium potassium tartrate or sodium citrate.
Procedure
The biuret reagent is added to the solution to be tested. No heating is required. A purple colour indicates that protein is present. The colour develops slowly over several minutes

Collagen – a fibrous protein
Collagen is the most common protein found in animals, making up 25% of the total protein in mammals. It is an insoluble fibrous protein (Figure 2.26) found in skin (leather is preserved collagen), tendons, cartilage, bones, teeth and the walls of blood vessels. It is an important structural protein, not only in humans but in almost all animals, and is found in structures ranging from the body walls of sea anemones to the egg cases of dogfish. As shown in Figure 2.26b, a collagen molecule consists of three polypeptide chains, each in the shape of a helix. (This is not an α-helix – it is not as tightly wound.) These three helical polypeptides are wound around each other, forming a three-stranded 'rope' or 'triple helix'. The three strands are held together by hydrogen bonds and some covalent bonds. Almost every third amino acid in each polypeptide is glycine, the smallest amino acid. Glycine is found on the insides of the strands and its small size allows the three strands to lie close together and so form a tight coil. Any other amino acid would be too large. Each complete, three-stranded molecule of collagen interacts with other collagen molecules running parallel to it. Covalent bonds form between the R groups of amino acids lying next to each other. These cross-links hold many collagen molecules side by side, forming fibrils. The ends of the parallel molecules are staggered; if they were not, there would be a weak spot running right across the collagen fibril. Finally, many fibrils lie alongside each other, forming strong bundles called fibres. The advantage of collagen is that it is flexible but it has tremendous tensile strength, meaning it can withstand large pulling forces without stretching or breaking. The human Achilles tendon, which is almost pure collagen fibres, can withstand a pulling force of 300 N per mm2 of cross-sectional area, about one-quarter the tensile strength of mild steel. Collagen fibres line up according to the forces they must withstand. In tendons they line up in parallel bundles along the length of the tendon, the direction of tension. In skin, they may form layers, with the fibres running in different directions in the different layers, like cellulose in cell walls. In this way, they resist tensile (pulling) forces from many directions.

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

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

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

A scanning electron micrograph of collagen fibrils (×17000). Each fibril is made up of many triple helices lying parallel with one another. The banded appearance is caused by the regular way in which these helices are arranged, with the staggered gaps between the molecules (shown in c) appearing darker.

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

Water

Water is arguably the most important biochemical of all. Without water, life would not exist on this planet. It is important for two reasons. First, it is a major component of cells, typically forming between 70% and 95% of the mass of the cell. You are about 60% water. Second, it provides an environment for those organisms that live in water. Three-quarters of the planet is covered in water. Although it is a simple molecule, water has some surprising properties. For example, such a small molecule would exist as a gas at normal Earth temperatures were it not for its special property of hydrogen bonding to other water molecules (page 35). Also, because water is a liquid, it provides a medium for molecules and ions to mix in, and hence a medium in which life can evolve. The hydrogen bonding of water molecules makes the molecules more difficult to separate and affects the physical properties of water. For example, the energy needed to break the hydrogen bonds makes it more difficult to convert water from a liquid to a gas than to convert similar compounds which lack hydrogen bonds, such as hydrogen sulfide (H2 S), which is a gas at normal air temperatures.

Water as a solvent

Water is an excellent solvent for ions and polar molecules (molecules with an uneven charge distribution, such as sugars and glycerol) because the water molecules are attracted to the ions and polar molecules and therefore collect around and separate them (Figure 2.27). This is what happens when a chemical dissolves in water. Once a chemical is in solution, it is free to move about and react with other chemicals. Most processes in living organisms take place in solution in this way.

By contrast, non-polar molecules such as lipids are insoluble in water and, if surrounded by water, tend to be pushed together by the water, since the water molecules are attracted to each other. This is important, for example, in hydrophobic interactions in protein structure and in membrane structure (Chapter 4), and it increases the stability of these structures.

Water as a transport medium

Water is the transport medium in the blood, in the lymphatic, excretory and digestive systems of animals, and in the vascular tissues of plants. Here again its solvent properties are essential.

High specific heat capacity

The heat capacity of a substance is the amount of heat required to raise its temperature by a given amount. The specific heat capacity of water (or simply the specific heat) is the amount of

heat energy required to raise the temperature of 1 kg of water by 1 °C. Water has a relatively high heat capacity. In order for the temperature of a liquid to be raised, the molecules must gain energy and consequently move about more rapidly. The hydrogen bonds that tend to make water molecules stick to each other make it more difficult for the molecules to move about freely; the bonds must be broken to allow free movement. This explains why more energy is needed to raise the temperature of water than would be the case if there were no hydrogen bonds. Hydrogen bonding, in effect, allows water to store more energy than would otherwise be possible for a given temperature rise. The high heat capacity of water has important biological implications because it makes water more resistant to changes in temperature. This means that the temperature within cells and within the bodies of organisms (which have a high proportion of water) tends to be more constant than that of the air around them. Biochemical reactions therefore operate at relatively constant rates and are less likely to be adversely affected by extremes of temperature. It also means that large bodies of water such as lakes and oceans are slow to change temperature as environmental temperature changes. As a result they provide more stable habitats for aquatic organisms.

High latent heat of vapourisation

The latent heat of vapourisation is a measure of the heat energy needed to vaporise a liquid (cause it to evaporate), changing it from a liquid to a gas. In the case of water, it involves the change from liquid water to water vapour. Water has a relatively high latent heat of vapourisation. This is a consequence of its high heat capacity. The fact that water molecules tend to stick to each other by hydrogen bonds means that relatively large amounts of energy are needed for vapourisation to occur, because hydrogen bonds have to be broken before molecules can escape as a gas. The energy transferred to water molecules during vapourisation results in a corresponding loss of energy from their surroundings, which therefore cool down. This is biologically important because it means that living organisms can use evaporation as a cooling mechanism, as in sweating or panting in mammals. A large amount of heat energy can be lost for relatively little loss of water, reducing the risk of dehydration. It can also be important in cooling leaves during transpiration. The reverse is true when water changes from liquid to solid ice. This time the water molecules must lose a relatively large amount of energy, making it less likely that the water will freeze. This is an advantage for aquatic organisms and makes it less likely that their bodies will freeze.

Density and freezing properties

Water is an unusual chemical because the solid form, ice, is less dense than its liquid form. Below 4 °C, the density of water starts to decrease. Ice therefore floats on liquid water and insulates the water under it. This reduces the tendency for large bodies of water to freeze completely, and increases the chances of life surviving in cold conditions. Changes in the density of water with temperature cause currents, which help to maintain the circulation of nutrients in the oceans.

High surface tension and cohesion

Water molecules have very high cohesion – in other words they tend to stick to each other. This explains why water can move in long, unbroken columns through the vascular tissue in plants

(Chapter 7), and is an important property in cells. High cohesion also results in high surface tension at the surface of water. This allows certain small organisms, such as pond skaters, to exploit the surface of water as a habitat, allowing them to settle on or skate over its surface (Figure 2.28).

Water as a reagent

Water takes part as a reagent in some chemical reactions inside cells. For example, it is used as a reagent in photosynthesis. During photosynthesis, energy from sunlight is used to separate hydrogen from the oxygen in water molecules. The hydrogen is then effectively used as a fuel to provide the energy needs of the plant – for example, by making glucose, an energy-rich molecule. The waste oxygen from photosynthesis is the source of the oxygen in the atmosphere which is needed by aerobic organisms for respiration. Water is also essential for all hydrolysis reactions. Hydrolysis is the mechanism by which large molecules are broken down to smaller molecules, as in digestion.

Summary

- The larger biological molecules are made from smaller molecules. Polysaccharides are made from monosaccharides, proteins from amino acids, nucleic acids from nucleotides, lipids from fatty acids and glycerol. Polysaccharides, proteins and nucleic acids are formed from repeating identical or similar subunits called monomers, and are therefore polymers. These build up into giant molecules called macromolecules.
- The smaller units are joined together by condensation reactions. Condensation involves removal of water. The reverse process, adding water, is called hydrolysis and is used to break the large molecules back down into smaller molecules.
- The linkages that join monosaccharides are called glycosidic bonds. Carbohydrates have the general formula Cx (H2 O)y and comprise monosaccharides, disaccharides and polysaccharides. Monosaccharides (e.g. glucose) and disaccharides (e.g. sucrose) are very water-soluble and together are known as sugars. They are important energy sources in cells and also important building blocks for larger molecules like polysaccharides.
- Monosaccharides may have straight-chain or ring structures and may exist in different isomeric forms such as α-glucose and β-glucose. Benedict's reagent can be used to test for reducing and non-reducing sugars. The test is semi-quantitative.
- Polysaccharides include starch, glycogen and cellulose. Starch is an energy storage compound in plants. 'Iodine solution' can be used to test for starch. Starch is made up of two types of molecule, amylose and amylopectin, both made from α-glucose. Amylose is an unbranching molecule, whereas amylopectin has a branching structure.
- Glycogen is an energy storage compound in animals, which is also made from α-glucose. Its structure is similar to that of amylopectin, but with more branching. Cellulose is a polymer of β-glucose molecules. The molecules are grouped together by hydrogen bonding to form mechanically strong fibres with high tensile strength that are found in plant cell walls.
- Lipids are a diverse group of chemicals, the most common of which are triglycerides (fats and oils). Triglycerides are made by condensation between three fatty acid

molecules and glycerol. They are hydrophobic and do not mix with water, acting as energy storage compounds in animals, as well as having other functions such as insulation and buoyancy in marine mammals. Phospholipids have a hydrophilic phosphate head and two hydrophobic fatty acid tails. This is important in the formation of membranes. The emulsion test can be used to test for lipids.

- Proteins are long chains of amino acids which fold into precise shapes. Biuret reagent can be used to test for proteins. The linkages that join amino acids are called peptide bonds. The sequence of amino acids in a protein, known as its primary structure, determines the way that it folds and hence determines its threedimensional shape and function.
- Many proteins contain areas where the amino acid chain is twisted into an α-helix; this is an example of secondary structure. The structure forms as a result of hydrogen bonding between the amino acids. Another secondary structure formed by hydrogen bonding is the β-pleated sheet. Further folding of proteins produces the tertiary structure. Often, a protein is made from more than one polypeptide chain. The association between the different chains is the quaternary structure of the protein. Tertiary and quaternary structures are very precise and are held in place by hydrogen bonds, disulfide bonds (which are covalent), ionic bonds and hydrophobic interactions.
- Proteins may be globular or fibrous. A molecule of a globular protein – for example haemoglobin – is roughly spherical. Most globular proteins are soluble and metabolically active. Haemoglobin contains a non-protein (prosthetic) group, the haem group, which contains iron. This combines with oxygen. A molecule of a fibrous protein – for example, collagen – is less folded and forms long strands. Fibrous proteins are insoluble. They often have a structural role. Collagen has high tensile strength and is the most common animal protein, being found in a wide range of tissues.
- Water is important within plants and animals, where it forms a large part of the mass of each cell. It is also an environment in which organisms can live. Extensive hydrogen bonding gives water unusual properties.
- Water is liquid at most temperatures on the Earth's surface. It has a high specific heat capacity, which makes liquid water relatively resistant to changes in temperature. Water acts as a solvent for ions and polar molecules, and causes non-polar molecules to group together. Water has a relatively high latent heat of vapourisation, meaning that evaporation has a strong cooling effect. It has high cohesion and surface tension which affects the way it moves through narrow tubes such as xylem and allows it to form a surface on which some organisms can live. Water acts as a reagent inside cells, as in hydrolysis reactions, and in photosynthesis as a source of hydrogen.

Enzymes

Mode of action of enzymes

Enzymes are protein molecules which can be defined as biological catalysts. A catalyst is a molecule which speeds up a chemical reaction but remains unchanged at the end of the reaction. Virtually every metabolic reaction which takes place within a living organism is

catalysed by an enzyme and enzymes are therefore essential for life to exist. Many enzyme names end in -ase – for example amylase and ATPase.

Intracellular and extracellular enzymes

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

Lock and key and induced fit hypotheses

Enzymes are globular proteins. Like all globular proteins, enzyme molecules are coiled into a precise threedimensional shape, with hydrophilic R groups (sidechains) on the outside of the molecule ensuring that they are soluble. Enzyme molecules also have a special feature in that they possess an active site (Figure 3.2). The active site of an enzyme is a region, usually a cleft or depression, to which another molecule or molecules can bind. This molecule is the substrate of the enzyme. The shape of the active site allows the substrate to fit perfectly. The idea that the enzyme has a particular shape into which the substrate fits exactly is known as the lock and key hypothesis. The substrate is the key whose shape fits the lock of the enzyme. The substrate is held in place by temporary bonds which form between the substrate and some of the R groups of the enzyme's amino acids. This combined structure is termed the enzyme–substrate complex.

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

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

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

Each type of enzyme will usually act on only one type of substrate molecule. This is because the shape of the active site will only allow one shape of molecule to fit. The enzyme is said to be specific for this substrate. In 1959 the lock and key hypothesis was modified in the light of evidence that enzyme molecules are more flexible than is suggested by a rigid lock and key. The modern hypothesis for enzyme action is known as the induced fit hypothesis. It is basically the same as the lock and key hypothesis, but adds the idea that the enzyme, and sometimes the substrate, can change shape slightly as the substrate molecule enters the enzyme, in order to ensure a perfect fit. This makes the catalysis even more efficient. An enzyme may catalyse a reaction in which the substrate molecule is split into two or more molecules, as shown in Figure

3.2. Alternatively, it may catalyse the joining together of two molecules, as when making a dipeptide. A simplified diagram is shown in Figure 3.3. This diagram also shows the enzyme–product complex which is briefly formed before release of the product. Interaction between the R groups of the enzyme and the atoms of the substrate can break, or encourage formation of, bonds in the substrate molecule, forming one, two or more products. When the reaction is complete, the product or products leave the active site. The enzyme is unchanged by this process, so it is now available to receive another substrate molecule. The rate at which substrate molecules can bind to the enzyme's active site, be formed into products and leave can be very rapid. The enzyme catalase, for example, can bind with hydrogen peroxide molecules, split them into water and oxygen, and release these products at a rate of 10 million molecules per second. The interaction between the substrate and the active site, including the slight change in shape of the enzyme (induced fit) which results from the binding of the substrate, is clearly shown by the enzyme lysozyme. Lysozyme is a natural defence against bacteria that is found in tears, saliva and other secretions. It breaks the polysaccharide chains that form the cell walls of bacteria. The tertiary structure of the enzyme has already been shown in Figure 2.20 (page 41). Figure 3.4 shows how part of the polysaccharide substrate is broken down in the active site.

Enzymes reduce activation energy

As catalysts, enzymes increase the rate at which chemical reactions occur. Most of the reactions which occur in living cells would occur so slowly without enzymes that they would virtually not happen at all. In many chemical reactions, the substrate will not be converted to a product unless it is temporarily given some extra energy. This energy is called activation energy (Figure 3.5a). One way of increasing the rate of many chemical reactions is to increase the energy of the reactants by heating them. You have probably done this by heating substances which you want to react together. In the Benedict's test for reducing sugar, for example, you need to heat the Benedict's reagent and sugar solution together before they will react (page 32).

Mammals such as humans also use this method of speeding up their metabolic reactions. Our body temperature is maintained at 37 °C, which is usually much warmer than the temperature of the air around us. But even raising the temperature of cells to 37 °C is not enough to give most substrates the activation energy which they need to change into products. Enzymes avoid this problem because they decrease the activation energy of the reaction which they catalyse (Figure 3.5b). They do this by holding the substrate or substrates in such a way that their molecules can react more easily. Reactions catalysed by enzymes will take place rapidly at a much lower temperature than they otherwise would.

The course of a reaction

You may be able to carry out an investigation into the rate at which substrate is converted into product during an enzyme-controlled reaction. Figure 3.6 shows the results of such an investigation using the enzyme catalase. This enzyme is found in the tissues of most living things and catalyses the breakdown of hydrogen peroxide into water and oxygen. (Hydrogen peroxide is a toxic product of several different metabolic reactions, and so it must be got rid of quickly.) It is an easy reaction to follow, as the oxygen that is released can be collected and

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

The explanation for the course of the reaction is quite straightforward. When the enzyme and substrate are first mixed, there are a large number of substrate molecules. At any moment, virtually every enzyme molecule has a substrate molecule in its active site. The rate at which the reaction occurs depends only on how many enzyme molecules there are and the speed at which the enzyme can convert the substrate into product, release it, and then bind with another substrate molecule. However, as more and more substrate is converted into product, there are fewer and fewer substrate molecules to bind with enzymes. Enzyme molecules may be 'waiting' for substrate molecules to hit their active sites. As fewer substrate molecules are left, the reaction gets slower and slower, until it eventually stops. The curve of a graph such as the one in Figure 3.6 is therefore steepest at the beginning of the reaction: the rate of an enzyme-controlled reaction is always fastest at the beginning. This rate is called the initial rate of reaction. You can measure the initial rate of the reaction by calculating the slope of a tangent to the curve, as close to time 0 as possible (see Figure P1.15, page 260, for advice on how to do this). An easier way of doing this is simply to read off the graph the amount of oxygen given off in the first 30 seconds. In this case, the rate of oxygen production in the first 30 seconds is 2.7 cm3 of oxygen per 30 seconds, or 5.4 cm3 per minute.

Factors that affect enzyme action

The effect of enzyme concentration

Figure 3.7a shows the results of an investigation in which different concentrations of catalase solution (from celery extract) were added to the same volumes of hydrogen peroxide solution. Concentration was varied by varying the initial volume of extract and then making up to a standard volume. You can see that the shape of all five curves is similar. In each case, the reaction begins very quickly (steep curve) and then gradually slows down (curve levels off). Because the quantity of hydrogen peroxide is the same in all five reactions, the total amount of oxygen eventually produced will be the same; so, if the investigation goes on long enough, all the curves will meet. To compare the rates of these five reactions, in order to look at the effect of enzyme concentration on reaction rate, it is fairest to look at the rate right at the beginning of the reaction. This is because, once the reaction is under way, the amount of substrate in each reaction begins to vary, because substrate is converted to product at different rates in each of the five reactions. It is only at the very beginning of the reaction that we can be sure that differences in reaction rate are caused only by differences in enzyme concentration. To work out the initial rate for each enzyme concentration, we can calculate the slope of the curve 30 seconds after the beginning of the reaction, as explained earlier. Ideally, we should do this for an even earlier stage of the reaction, but in practice this is impossible. We can then plot a second graph, Figure 3.7b, showing the initial rate of reaction against enzyme concentration.

This graph shows that the initial rate of reaction increases linearly. In these conditions, reaction rate is directly proportional to the enzyme concentration. This is just what common sense says

should happen. The more enzyme present, the more active sites will be available for the substrate to slot into. As long as there is plenty of substrate available, the initial rate of a reaction increases linearly with enzyme concentration.

Measuring reaction rate

It is easy to measure the rate of the catalase–hydrogen peroxide reaction, because one of the products is a gas, which is released and can be collected. Unfortunately, it is not always so easy to measure the rate of a reaction. If, for example, you wanted to investigate the rate at which amylase breaks down starch, it would be very difficult to observe the course of the reaction because the substrate (starch) and the product (maltose) remain as colourless substances in the reaction mixture. The easiest way to measure the rate of this reaction is to measure the rate at which starch disappears from the reaction mixture. This can be done by taking samples from the mixture at known times, and adding each sample to some iodine in potassium iodide solution. Starch forms a blue-black colour with this solution. Using a colorimeter, you can measure the intensity of the blue-black colour obtained, and use this as a measure of the amount of starch still remaining. If you do this over a period of time, you can plot a curve of 'amount of starch remaining' against 'time'. You can then calculate the initial reaction rate in the same way as for the catalase–hydrogen peroxide reaction. It is even easier to observe the course of this reaction if you mix starch, iodine in potassium iodide solution and amylase in a tube, and take regular readings of the colour of the mixture in this one tube in a colorimeter. However, this is not ideal, because the iodine interferes with the rate of the reaction and slows it down.

The effect of substrate concentration

Figure 3.8 shows the results of an investigation in which the amount of catalase was kept constant and the amount of hydrogen peroxide was varied. Once again, curves of oxygen released against time were plotted for each reaction, and the initial rate of reaction calculated for the first 30 seconds. These initial rates of reaction were then plotted against substrate concentration. As substrate concentration increases, the initial rate of reaction also increases. Again, this is only what we would expect: the more substrate molecules there are around, the more often an enzyme's active site can bind with one. However, if we go on increasing substrate concentration, keeping the enzyme concentration constant, there comes a point where every enzyme active site is working continuously. If more substrate is added, the enzyme simply cannot work faster; substrate molecules are effectively 'queuing up' for an active site to become vacant. The enzyme is working at its maximum possible rate, known as Vmax. V stands for velocity.

Temperature and enzyme activity

Figure 3.9 shows how the rate of a typical enzyme-catalysed reaction varies with temperature. At low temperatures, the reaction takes place only very slowly. This is because molecules are moving relatively slowly. Substrate molecules will not often collide with the active site, and so binding between substrate and enzyme is a rare event. As temperature rises, the enzyme and substrate molecules move faster. Collisions happen more frequently, so that substrate molecules enter the active site more often. Moreover, when they do collide, they do so with

more energy. This makes it easier for bonds to be formed or broken so that the reaction can occur. As temperature continues to increase, the speed of movement of the substrate and enzyme molecules also continues to increase. However, above a certain temperature, the structure of the enzyme molecule vibrates so energetically that some of the bonds holding the enzyme molecule in its precise shape begin to break. This is especially true of hydrogen bonds. The enzyme molecule begins to lose its shape and activity, and is said to be denatured. This is often irreversible. At first, the substrate molecule fits less well into the active site of the enzyme, so the rate of the reaction begins to slow down. Eventually the substrate no longer fits at all, or can no longer be held in the correct position for the reaction to occur. The temperature at which an enzyme catalyses a reaction at the maximum rate is called the optimum temperature. Most human enzymes have an optimum temperature of around 40 °C. By keeping our body temperatures at about 37 °C, we ensure that enzyme-catalysed reactions occur at close to their maximum rate.

It would be dangerous to maintain a body temperature of 40 °C, as even a slight rise above this would begin to denature enzymes. Enzymes from other organisms may have different optimum temperatures. Some enzymes, such as those found in bacteria which live in hot springs (Figure 3.10), have much higher optimum temperatures. Some plant enzymes have lower optimum temperatures, depending on their habitat.

pH and enzyme activity

Figure 3.11 shows how the activity of an enzyme is affected by pH. Most enzymes work fastest at a pH of somewhere around 7 – that is, in fairly neutral conditions. Some, however, such as the protease pepsin, which is found in the acidic conditions of the stomach, have a different optimum pH. pH is a measure of the concentration of hydrogen ions in a solution. The lower the pH, the higher the hydrogen ion concentration. Hydrogen ions can interact with the R groups of amino acids – for example, by affecting ionisation (the negative or positive charges) of the groups. This affects the ionic bonding between the groups (page 42), which in turn affects the three-dimensional arrangement of the enzyme molecule. The shape of the active site may change and therefore reduce the chances of the substrate molecule fitting into it. A pH which is very different from the optimum pH can cause denaturation of an enzyme. When investigating pH, you can use buffer solutions (Chapter P1). Buffer solutions each have a particular pH and maintain it even if the reaction taking place would otherwise cause pH to change. You add a measured volume of the buffer to your reacting mixture.

Enzyme inhibitors

Competitive, reversible inhibition

As we have seen, the active site of an enzyme fits one particular substrate perfectly. It is possible, however, for some other molecule to bind to an enzyme's active site if it is very similar in shape to the enzyme's substrate. This would then inhibit the enzyme's function. If an inhibitor molecule binds only briefly to the site, there is competition between it and the substrate for the site. If there is much more of the substrate present than the inhibitor, substrate molecules can easily bind to the active site in the usual way, and so the enzyme's function is unaffected. However, if the concentration of the inhibitor rises, or that of the substrate falls, it becomes less

and less likely that the substrate will collide with an empty site. The enzyme's function is then inhibited. This is therefore known as competitive inhibition (Figure 3.12a). It is said to be reversible (not permanent) because it can be reversed by increasing the concentration of the substrate. An example of competitive inhibition occurs in the treatment of a person who has drunk ethylene glycol. Ethylene glycol is used as antifreeze, and is sometimes drunk accidentally. Ethylene glycol is rapidly converted in the body to oxalic acid, which can cause irreversible kidney damage. However, the active site of the enzyme which converts ethylene glycol to oxalic acid will also accept ethanol. If the poisoned person is given a large dose of ethanol, the ethanol acts as a competitive inhibitor, slowing down the action of the enzyme on ethylene glycol for long enough to allow the ethylene glycol to be excreted.

Non-competitive, reversible inhibition

A different kind of reversible inhibition takes place if a molecule can bind to another part of the enzyme rather than the active site. While the inhibitor is bound to the enzyme it can seriously disrupt the normal arrangement of hydrogen bonds and hydrophobic interactions holding the enzyme molecule in its three-dimensional shape (Chapter 2). The resulting distortion ripples across the molecule to the active site, making the enzyme unsuitable for the substrate. While the inhibitor is attached to the enzyme, the enzyme's function is blocked no matter how much substrate is present, so this is an example of noncompetitive inhibition (Figure 3.12b). Inhibition of enzyme function can be lethal, but in many situations inhibition is essential. For example, metabolic reactions must be very finely controlled and balanced, so no single enzyme can be allowed to 'run wild', constantly churning out more and more product. One way of controlling metabolic reactions is to use the end-product of a chain of reactions as a non-competitive, reversible inhibitor (Figure 3.13). As the enzyme converts substrate to product, it is slowed down because the end product binds to another part of the enzyme and prevents more substrate binding. However, the end-product can lose its attachment to the enzyme and go on to be used elsewhere, allowing the enzyme to reform into its active state. As product levels fall, the enzyme is able to top them up again. This is termed end-product inhibition.

Comparing enzyme affinities

There is enormous variation in the speed at which different enzymes work. A typical enzyme molecule can convert around one thousand substrate molecules into product per second. This is known as the turnover rate. The enzyme carbonic anhydrase (Chapter 12) is one of the fastest enzymes known. It can remove 600,000 molecules of carbon dioxide from respiring tissue per second, roughly 107 times as fast as the reaction would occur in the absence of the enzyme. It has presumably evolved such efficiency because a build-up of carbon dioxide in tissues would quickly become lethal. Speeds such as these are only possible because molecules within cells move about very quickly by diffusion over short distances, with tens or hundreds of thousands of collisions per second occurring between enzyme and substrate molecules. Simple measurements of the rate of activity of enzymes can be carried out. See for example, Box 3.1, Figures 3.6 and 3.7 and Questions 3.4 and 3.6. More precise measurements of the rate at which enzymes work are difficult and complex to make, but are important for our understanding of how enzymes work together to control cell metabolism. One of the key steps towards understanding how well an enzyme performs is to measure the theoretical maximum rate

(velocity), Vmax, of the reaction it catalyses. At Vmax all the enzyme molecules are bound to substrate molecules – the enzyme is saturated with substrate. The principle of how Vmax is measured is described on page 58. To summarise, the reaction rate is measured at different substrate concentrations while keeping the enzyme concentration constant. As substrate concentration is increased, reaction rate rises until the reaction reaches its maximum rate, Vmax

The initial rate for each substrate concentration is plotted against substrate concentration, producing a curve like that shown in Figure 3.8. This type of curve is described as asymptotic and such curves have certain mathematical properties. In particular, the curve never completely flattens out in practice. In theory, it does so at infinite substrate concentration, but this is obviously impossible to measure. This makes it impossible to accurately read off the value for Vmax from the graph. There is, however, a way round this problem. Instead of plotting substrate concentration, [S], on the x-axis and velocity (rate) on the y-axis, we can plot 1/[S] (the inverse of substrate concentration) and 1/velocity (the inverse of velocity) respectively. Such a plot is called a double reciprocal plot. (Remember, the word ‘reciprocal’ means ‘inverse’.) One advantage of doing this is that while it is impossible to plot infinite substrate concentration, 1/infinity is zero, which can be plotted, so Vmax can be found accurately. Also, the resulting graph is a straight line. It is easier to understand this if you use some specimen results to plot the two types of graph. The table in Question 3.8 gives you some results and it is worth spending some time answering the question before proceeding. Figure 3.14a shows a double-reciprocal plot. Note that it is a straight line. Using this graph, we can find Vmax in the following way. First, we find 1/Vmax. This is the point where the line crosses (intersects) the y-axis because this is where 1/[S] is zero (and therefore [S] is infinite). Once we know 1/Vmax we can calculate Vmax. Another useful value can be obtained from the double-reciprocal plot, namely the Michaelis–Menten constant, Km. The Michaelis–Menten constant is the substrate concentration at which an enzyme works at half its maximum rate (½Vmax). At this point, half the active sites of the enzyme are occupied by the substrate. The higher the affinity of the enzyme for the substrate, the lower the substrate concentration needed for this to happen. Thus the Michaelis–Menten constant is a measure of the affinity of the enzyme for its substrate. The higher the affinity, the lower the Michaelis–Menten constant and the quicker the reaction will proceed to its maximum rate, although the maximum rate itself is not affected by the Michaelis–Menten constant. Vmax and Km therefore provide two different ways of comparing the efficiency of different enzymes. Vmax gives information about the maximum rate of reaction that is possible (though not necessarily the rate under cell conditions) while Km measures the affinity of the enzyme for the substrate. The higher the affinity, the more likely the product will be formed when a substrate molecule enters the active site, rather than the substrate simply leaving the active site again before a reaction takes place. These two aspects of efficiency are rather like using the maximum speed and acceleration to measure the efficiency of a car. How can we find Km from a double-reciprocal plot? The answer is that the point where the line of the graph intersects the x-axis is −1/Km (note that it is in the negative region of the x-axis). Figure 3.14a shows this point. From the value for −1/Km, we can calculate Km. Figure 3.14b shows the normal plot (as in Figure 3.7 and your first graph in Question 3.8). The relationship between ½Vmax and Km is shown in Figure 3.14b. The value of Km for a particular enzyme can vary, depending on a number of factors. These include the identity of the substrate, temperature, pH,

presence of particular ions, overall ion concentration, and the presence of poisons, pollutants or inhibitors. Turnover numbers, which are related to Vmax and Km values, for four enzymes are shown in Table 3.2. This shows the great variation in efficiency that is possible between enzymes, and the fact that Vmax and Km are independent of each other.

The significance of Vmax and Km values

Knowing the values of Vmax and Km has a number of applications.

- It enables scientists to make computerised models of biochemical pathways or even the behaviour of whole cells because it helps to predict how each reaction in a proposed pathway will proceed and therefore how the enzymes will interact. The consequences of changing conditions such as temperature, pH or the presence of inhibitors can be built into the models.
- An enzyme's preference for different substrates can be compared quantitatively.
- By understanding what affects enzyme efficiency, scientists may in future be able to design better catalysts, linking this to genetic engineering.
- For a commercially important enzyme, the performance of the same enzyme from different organisms can be compared.
- The calculations involved can be applied to other fields of biochemistry, such as antibody–antigen binding.
- Knowing Km means the proportion of active sites occupied by substrate molecules can be calculated for any substrate concentration.

Immobilising enzymes

Enzymes have an enormous range of commercial applications – for example, in medicine, food technology and industrial processing. Enzymes are expensive. No company wants to have to keep buying them over and over again if it can recycle them in some way. One of the best ways of keeping costs down is to use immobilised enzymes. The enzyme lactase can be immobilised using alginate beads (Box 3.2). Milk is then allowed to run through the column of lactase-containing beads. The lactase hydrolyses the lactose in the milk to glucose and galactose. The milk is therefore lactose-free, and can be used to make lactose-free dairy products for people who cannot digest lactose. You can see that enzyme immobilisation has several obvious advantages compared with just mixing up the enzyme with its substrate. If you just mixed lactase with milk, you would have a very difficult task to get the lactase back again. Not only would you lose the lactase, but also you would have milk contaminated with the enzyme. Using immobilised enzymes means that you can keep and re-use the enzymes, and that the product is enzyme-free. Another advantage of this process is that the immobilised enzymes are more tolerant of temperature changes and pH changes than enzymes in solution. This may be partly because their molecules are held firmly in shape by the alginate in which they are embedded, and so do not denature as easily. It may also be because the parts of the molecules that are embedded in the beads are not fully exposed to the temperature or pH changes.

Immobilised enzymes

Figure 3.15 shows one way in which enzymes can be immobilised. The enzyme is mixed with a solution of sodium alginate. Little droplets of this mixture are then added to a solution of calcium chloride. The sodium alginate and calcium chloride instantly react to form jelly, which turns each droplet into a little bead. The jelly bead contains the enzyme. The enzyme is held in the bead, or immobilised. These beads can be packed gently into a column. A liquid containing the enzyme's substrate can be allowed to trickle steadily over them (Figure 3.16). As the substrate runs over the surface of the beads, the enzymes in the beads catalyse a reaction that converts the substrate into product. The product continues to trickle down the column, emerging from the bottom, where it can be collected and purified.

Summary

- Enzymes are globular proteins which catalyse metabolic reactions. Each enzyme has an active site with a flexible structure which can change shape slightly to fit precisely the substrate molecule. This is called the induced fit hypothesis. When the substrate enters the active site, an enzyme–substrate complex is temporarily formed in which the R groups of the amino acids in the enzyme hold the substrate in place.
- Enzymes may be involved in reactions which break down molecules or join molecules together. They work by lowering the activation energy of the reactions they catalyse.
- The course of an enzyme reaction can be followed by measuring the rate at which a product is formed or the rate at which a substrate disappears. A progress curve, with time on the x-axis, can be plotted. The curve is steepest at the beginning of the reaction, when substrate concentration is at its highest. This rate is called the initial rate of reaction.
- Various factors affect the rate of activity of enzymes. Four important factors are enzyme concentration, substrate concentration, temperature and pH. The greater the concentration of the enzyme, the faster the rate of reaction, provided there are enough substrate molecules present. The greater the concentration of the substrate, the faster the rate of reaction, provided enough enzyme molecules are present. During enzyme reactions, rates slow down as substrate molecules are used up.
- Each enzyme has an optimum temperature at which it works fastest. As temperature increases above the optimum temperature, the enzyme gradually denatures (loses its precise tertiary structure). When an enzyme is completely denatured, it ceases to function, but denaturation is sometimes reversible.
- Each enzyme has an optimum pH. Some enzymes operate within a narrow pH range; some have a broad pH range.
- Enzymes are also affected by the presence of inhibitors, which slow down their rate of reaction or stop it completely. Competitive inhibitors are molecules which are similar in shape to the normal substrate molecules. They compete with the substrate for the active site of the enzyme. Competitive inhibition is reversible because the inhibitor can enter and leave the active site.
- Non-competitive inhibitors either bind permanently to the active site or bind at a site elsewhere on the enzyme, causing a change in shape of the active site. Binding of non-competitive inhibitors may or may not be reversible.

- The efficiency of an enzyme can be measured by finding the value known as the Michaelis–Menten constant, Km. To do this the maximum rate of reaction, Vmax, must first be determined. Determination of Vmax involves finding the initial rates of reactions at different substrate concentrations while ensuring that enzyme concentration remains constant.
- Enzymes can be immobilised – for example by trapping them in jelly (alginate) beads. This is commercially useful because the enzyme can be re-used and the product is separate from (uncontaminated by) the enzyme. Immobilisation oft en makes enzymes more stable.

Cell membranes and transport

In Chapter 1, you saw that all living cells are surrounded by a very thin membrane, the cell surface membrane. This controls the exchange of materials such as nutrients and waste products between the cell and its environment. Inside cells, regulation of transport across the membranes of organelles is also vital. Membranes also have other important functions. For example, they enable cells to receive hormone messages. It is important to study the structure of membranes if we are to understand how these functions are achieved.

Phospholipids

An understanding of the structure of membranes depends on an understanding of the structure of phospholipids (page 38). From phospholipids, little bags can be formed inside which chemicals can be isolated from the external environment. These bags are the membrane-bound compartments that we know as cells and organelles.

Figure 4.2a shows what happens if phospholipid molecules are spread over the surface of water. They form a single layer with their heads in the water, because these are polar (hydrophilic), and their tails projecting out of the water, because these are non-polar (hydrophobic). The term ‘polar’ refers to the uneven distribution of charge which occurs in some molecules. The significance of this is explained on pages 35–36. If the phospholipids are shaken up with water, they can form stable ball-like structures in the water called micelles (Figure 4.2b). Here all the hydrophilic heads face outwards into the water, shielding the hydrophobic tails, which point in towards each other. Alternatively, two-layered structures, called bilayers, can form in sheets (Figure 4.2c). It is now known that this phospholipid bilayer is the basic structure of membranes (Figure 4.2d).

Structure of membranes

The phospholipid bilayer is visible using the electron microscope at very high magnifications of at least ×100 000 (Figure 1.23 on page 17). The double black line visible using the electron microscope is thought to show the hydrophilic heads of the two phospholipid layers; the pale zone between is the hydrophobic interior of the membrane. The bilayer (membrane) is about 7 nm wide. Membranes also contain proteins. These can be seen in certain electron micrographs, such as Figure 4.3. In 1972, two scientists, Singer and Nicolson, used all the available evidence to put forward a hypothesis for membrane structure. They called their model the fluid mosaic model. It is described as ‘fluid’ because both the phospholipids and the proteins can move about

by diffusion. The phospholipid bilayer has the sort of fluidity we associate with olive oil. The phospholipids move sideways, mainly in their own layers. Some of the protein molecules also move about within the phospholipid bilayer, like icebergs in the sea. Others remain fixed to structures inside or outside the cell. The word ‘mosaic’ describes the pattern produced by the scattered protein molecules when the surface of the membrane is viewed from above. Figures 4.4 and 4.5 are diagrams of what we imagine a membrane might look like if we could see the individual molecules.

Features of the fluid mosaic model

The membrane is a double layer (bilayer) of phospholipid molecules. The individual phospholipid molecules move about by diffusion within their own monolayers. The phospholipid tails point inwards, facing each other and forming a non-polar hydrophobic interior. The phospholipid heads face the aqueous (water-containing) medium that surrounds the membranes. Some of the phospholipid tails are saturated and some are unsaturated. The more unsaturated they are, the more fluid the membrane. This is because the unsaturated fatty acid tails are bent (Figure 2.11, page 36) and therefore fit together more loosely. Fluidity is also affected by tail length: the longer the tail, the less fluid the membrane. As temperature decreases, membranes become less fluid, but some organisms which cannot regulate their own temperature, such as bacteria and yeasts, respond by increasing the proportion of unsaturated fatty acids in their membranes. Two types of protein are recognised, according to their position in the membrane.

Proteins that are found embedded within the membrane, such as those in Figure 4.5, are called intrinsic proteins (or integral proteins). Intrinsic proteins may be found in the inner layer, the outer layer or, most commonly, spanning the whole membrane, in which case they are known as transmembrane proteins. In transmembrane proteins, the hydrophobic regions which cross the membrane are often made up of one or more α-helical chains.

Intrinsic proteins have hydrophobic and hydrophilic regions. They stay in the membrane because the hydrophobic regions, made from hydrophobic amino acids, are next to the hydrophobic fatty acid tails and are repelled by the watery environment either side of the membrane. The hydrophilic regions, made from hydrophilic amino acids, are repelled by the hydrophobic interior of the membrane and therefore face into the aqueous environment inside or outside the cell, or line hydrophilic pores which pass through the membrane. Most of the intrinsic protein molecules float like mobile icebergs in the phospholipid layers, although some are fixed like islands to structures inside or outside the cell and do not move about. A second type of protein molecule is the extrinsic protein (or peripheral protein). These are found on the inner or outer surface of the membrane. Many are bound to intrinsic proteins. Some are held in other ways – for example, by binding to molecules inside or outside the cell, or to the phospholipids. All the proteins referred to from now on in this chapter are intrinsic proteins. Many proteins and lipids have short, branching carbohydrate chains attached to that side of the molecule which faces the outside of the membrane, thus forming glycoproteins and glycolipids, respectively. The total thickness of the membrane is about 7 nm on average. Molecules of cholesterol are also found in the membrane.

Roles of the components of cell membranes

We have seen that cell membranes contain several different types of molecule. There are three types of lipid, namely phospholipids, cholesterol and glycolipids. There are also proteins and glycoproteins. Each of these has a particular role to play in the overall structure and function of the membrane.

Phospholipids

As explained on pages 73–76, phospholipids form the bilayer, which is the basic structure of the membrane. Because the tails of phospholipids are non-polar, it is difficult for polar molecules, or ions, to pass through membranes, so they act as a barrier to most water-soluble substances. For example, water-soluble molecules such as sugars, amino acids and proteins cannot leak out of the cell, and unwanted water-soluble molecules cannot enter the cell.

Some phospholipids can be modified chemically to act as signalling molecules. They may move about in the phospholipid bilayer, activating other molecules such as enzymes. Alternatively, they may be hydrolysed to release small, water-soluble, glycerol-related molecules. These diffuse through the cytoplasm and bind to specific receptors (page 78). One such system results in the release of calcium ions from storage in the ER, which in turn brings about exocytosis of digestive enzymes from pancreatic cells as described on page 87.

Cholesterol

Cholesterol is a relatively small molecule. Like phospholipids, cholesterol molecules have hydrophilic heads and hydrophobic tails, so they fit neatly between the phospholipid molecules with their heads at the membrane surface. Cell surface membranes in animal cells contain almost as much cholesterol as phospholipid. Cholesterol is much less common in plant cell membranes and absent from prokaryotes. In these organisms, compounds very similar to cholesterol serve the same function. At low temperatures, cholesterol increases the fluidity of the membrane, preventing it from becoming too rigid. This is because it prevents close packing of the phospholipid tails. The increased fluidity means cells can survive colder temperatures. The interaction of the phospholipid tails with the cholesterol molecules also helps to stabilise cells at higher temperatures when the membrane could otherwise become too fluid. Cholesterol is also important for the mechanical stability of membranes, as without it membranes quickly break and cells burst open. The hydrophobic regions of cholesterol molecules help to prevent ions or polar molecules from passing through the membrane. This is particularly important in the myelin sheath (made up of many layers of cell surface membrane) around nerve cells, where leakage of ions would slow down nerve impulses.

Glycolipids, glycoproteins and proteins

Many of the lipid molecules on the outer surfaces of cell surface membranes, and probably all of the protein molecules, have short carbohydrate chains attached to them. These ‘combination’ molecules are known as glycolipids and glycoproteins, respectively. The carbohydrate chains project like antennae into the watery fluids surrounding the cell, where they form hydrogen bonds with the water molecules and so help to stabilise the membrane structure. The

carbohydrate chains form a sugary coating to the cell, known as the glycocalyx. In animal cells, the glycocalyx is formed mainly from glycoproteins; in plant cells it mainly comprises glycolipid.

The carbohydrate chains help the glycoproteins and glycolipids to act as receptor molecules, which bind with particular substances at the cell surface. Different cells have different receptors, depending on their function. There are three major groups of receptor. One group of receptors can be called ‘signalling receptors’, because they are part of a signalling system that coordinates the activities of cells. The receptors recognise messenger molecules like hormones and neurotransmitters. (Neurotransmitters are the chemicals that cross synapses, allowing nerve impulses to pass from one cell to another, and are discussed in Chapter 15.) When the messenger molecule binds to the receptor, a series of chemical reactions is triggered inside the cell. An example of a signalling receptor is the glucagon receptor in liver cells (Figure 14.24, page 317). Cells that do not have glucagon receptors are not affected by glucagon. Signalling is discussed in the next section. A second group of receptors are involved in endocytosis (page 87). They bind to molecules that are parts of the structures to be engulfed by the cell surface membrane. A third group of receptors is involved in binding cells to other cells (cell adhesion) in tissues and organs of animals. Some glycolipids and glycoproteins act as cell markers or antigens, allowing cell–cell recognition. Each type of cell has its own type of antigen, rather like countries with different flags. For example, the ABO blood group antigens are glycolipids and glycoproteins which have small differences in their carbohydrate portions. Many proteins act as transport proteins. These provide hydrophilic channels or passageways for ions and polar molecules to pass through the membrane. There are two types of transport protein: channel proteins and carrier proteins. Their roles are described on pages 82 and 86. Each transport protein is specific for a particular kind of ion or molecule. Therefore the types of substances that enter or leave the cell can be controlled. Other membrane proteins may be enzymes – for example, the digestive enzymes found in the cell surface membranes of the cells lining the small intestine. These catalyse the hydrolysis of molecules such as disaccharides. Some proteins on the inside of the cell surface membrane are attached to a system of protein filaments inside the cell, known as the cytoskeleton. These proteins help to maintain and decide the shape of the cell. They may also be involved in changes of shape when cells move. Proteins also play important roles in the membranes of organelles. For example, in the membranes of mitochondria and chloroplasts they are involved in the processes of respiration and photosynthesis. (You will find out much more about this if you continue your biology course to A Level.)

Cell signalling

Cell signalling is an important, rapidly expanding area of research in modern biology, with wide applications. It is important because it helps to explain how living organisms control and coordinate their bodies. In this chapter, we concentrate on a few basic principles of signalling, highlighting the importance of membranes. As with other areas of biology, such as biochemistry, many of the fundamental principles and mechanisms are shared between all living organisms – plants, animals, fungi, protoctists and bacteria. What is signalling? Basically, signalling is getting a message from one place to another. Why do living organisms need signalling? All cells and organisms must be able to respond appropriately to their environments. This is made possible

by means of a complex range of signalling pathways which coordinate the activities of cells, even if they are large distances apart in the same body. The basic idea of a signalling pathway can be summarised in a simple diagram (Figure 4.6). You will meet examples of cell signalling throughout this book and this diagram is a useful starting point for analysing the various pathways. As Figure 4.6 shows, a signalling pathway includes receiving a stimulus or signal, transmitting the message and making an appropriate response. Conversion of the original signal to a message that is then transmitted is called transduction. Transmitting the message involves crossing barriers such as cell surface membranes. Signalling molecules are usually very small for easy transport.

Distances travelled may be short, as with diffusion within one cell, or long, as with long-distance transport in blood (animals) or phloem (plants). There are usually many components and different mechanisms along the route. Signalling includes both electrical and chemical events and their interactions with each other – for example, the events associated with the nervous and hormonal systems in animals. These events involve a wide range of molecules produced by cells within the body (e.g. hormones and neurotransmitters) as well as outside stimuli (e.g. light, drugs, pheromones and odours). The cell surface membrane is a critical component of most signalling pathways because it is a barrier to the movement of molecules, controlling what moves between the external and internal environments of the cell. In a typical signalling pathway, molecules must cross or interact with cell surface membranes. Signalling molecules are very diverse. If they are hydrophobic, such as the steroid hormones (e.g. oestrogen), they can diffuse directly across the cell surface membrane and bind to receptors in the cytoplasm or nucleus.

More commonly, the signalling molecule is water soluble. In this case, a typical signalling pathway starts with the signal arriving at a protein receptor in a cell surface membrane. The receptor is a specific shape which recognises the signal. Only cells with this receptor can recognise the signal. The signal brings about a change in the shape of the receptor, and since this spans the membrane, the message is in effect passed to the inside of the cell (signal transduction). Changing the shape of the receptor allows it to interact with the next component of the pathway, so the message gets transmitted. This next component is often a ‘G protein’, which acts as a switch to bring about the release of a ‘second messenger’, a small molecule which diffuses through the cell relaying the message. (G proteins are so-called because the switch mechanism involves binding to GTP molecules. GTP is similar to ATP, but with guanine in place of adenine.) Many second messenger molecules can be made in response to one receptor molecule being stimulated. This represents an amplification (magnification) of the original signal, a key feature of signalling. The second messenger typically activates an enzyme, which in turn activates further enzymes, increasing the amplification at each stage. Finally, an enzyme is produced which brings about the required change in cell metabolism. The sequence of events triggered by the G protein is called a signalling cascade. Figure 4.7 is a diagram of a simplified cell signalling pathway involving a second messenger. Examples of such a pathway involving the hormones adrenaline and glucagon are discussed in Chapter 14.

Besides examples involving second messengers, there are three other basic ways in which a receptor can alter the activity of a cell:

- opening an ion channel, resulting in a change of membrane potential (e.g. nicotine-accepting acetylcholine receptors, Chapter 15)
- acting directly as a membrane-bound enzyme (e.g. insulin receptor)
- acting as an intracellular receptor when the initial signal passes straight through the cell surface membrane. For example, the oestrogen receptor is in the nucleus and directly controls gene expression when combined with oestrogen.

Figure 4.8 summarises some typical signalling systems. Note that apart from the secretion of chemical signals, direct cell-cell contact is another mechanism of signalling. This occurs, for example, during embryonic development and when lymphocytes detect foreign antigens on other cells.

Movement of substances into and out of cells

We have seen that a phospholipid bilayer around cells makes a very effective barrier, particularly against the movement of water-soluble molecules and ions. The aqueous contents of the cell are therefore prevented from escaping. However, some exchange between the cell and its environment is essential. There are five basic mechanisms by which this exchange is achieved: diffusion, facilitated diffusion, osmosis, active transport and bulk transport

Diffusion

If you open a bottle of perfume in a room, it is not long before molecules of scent spread to all parts of the room (and are detected when they fit into membrane receptors in your nose). This will happen, even in still air, by the process of diffusion. Diffusion can be defined as the net movement, as a result of random motion of its molecules or ions, of a substance from a region of its higher concentration to a region of its lower concentration. The molecules or ions move down a concentration gradient. The random movement is caused by the natural kinetic energy (energy of movement) of the molecules or ions. As a result of diffusion, molecules or ions tend to reach an equilibrium situation, where they are evenly spread within a given volume of space. The phenomenon of diffusion can be demonstrated easily using non-living materials such as glucose and Visking tubing (Box 4.1) or plant tissue (Box 4.2).

Some molecules or ions are able to pass through living cell membranes by diffusion. Temporary staining of plant cells, e.g. adding iodine solution to epidermal cells, shows that this is possible. The rate at which a substance diffuses across a membrane depends on a number of factors, including the following.

- The ‘steepness’ of the concentration gradient – that is, the difference in the concentration of the substance on the two sides of the surface. If there are, for example, many more molecules on one side of a membrane than on the other, then at any one moment more molecules will be moving (entirely randomly) from this side than from the other. The greater the difference in concentration, the greater the difference in the number of molecules passing in the two directions, and hence the faster the rate of diffusion.

- Temperature. At high temperatures, molecules and ions have much more kinetic energy than at low temperatures. They move around faster, and thus diffusion takes place faster.
- The surface area across which diffusion is taking place. The greater the surface area, the more molecules or ions can cross it at any one moment, and therefore the faster diffusion can occur. The surface area of cell membranes can be increased by folding, as in microvilli in the intestine and kidneys or the cristae inside mitochondria. The larger the cell, the smaller its surface area in relation to its volume. This can easily be demonstrated by studying the diagram in Question 4.3. To make the calculations easier, cells are shown as cubes, but the principle remains the same – volume increases much more rapidly than surface area as size increases. (See also Box 4.3.) Cells rely on diffusion for internal transport of molecules. This results in a limit on the size of cells, because once inside a cell, the time it takes a molecule to reach a certain destination by diffusion increases rapidly with distance travelled. In fact, the rate falls in proportion to the square of the distance. Diffusion is therefore only effective over very short distances, such as the 7 nm across a membrane. An amino acid molecule, for example, can travel a few micrometres in several seconds, but would take several hours to diffuse a centimetre. An aerobic cell would quickly run out of oxygen and die if it were too large. Most cells are no larger than about 50 μm in diameter. The surface area: volume ratio decreases as the size of any three-dimensional object increases.
- The nature of the molecules or ions. Large molecules require more energy to get them moving than small ones do, so large molecules tend to diffuse more slowly than small molecules. Non-polar molecules, such as glycerol, alcohol and steroid hormones, diffuse much more easily through cell membranes than polar ones, because they are soluble in the non-polar phospholipid tails. The respiratory gases – oxygen and carbon dioxide – cross membranes by diffusion. They are uncharged and non-polar, and so can cross through the phospholipid bilayer directly between the phospholipid molecules. Water molecules, despite being very polar, can diffuse rapidly across the phospholipid bilayer because they are small enough.

Diffusion is the net movement of molecules or ions from a region of higher concentration to a region of lower concentration down a gradient, as a result of the random movements of particles.

Demonstrating diffusion using Visking tubing

Visking tubing (also known as dialysis tubing) is a partially permeable, non-living membrane made from cellulose. It possesses molecular-sized pores which are small enough to prevent the passage of large molecules, such as starch and sucrose, but will allow the passage of smaller molecules by diffusion, such as glucose. This can be demonstrated by filling a length of Visking tubing (about 15cm) with a mixture of starch and glucose solutions. If the tubing is suspended in a boiling tube of water for a period of time, the presence of starch and glucose outside the tubing can be tested for at intervals to monitor whether diffusion out of the tubing has occurred. The results should indicate that glucose, but not starch, diffuses out of the tubing. This experiment can be made more quantitative. It would be interesting, for example, to try to

estimate the concentration of glucose at each time interval by setting up separate tubes, one for each planned time interval, and using a semi-quantitative Benedict's test each time. A colorimeter would be useful for this. Alternatively, a set of colour standards could be prepared. A graph could be drawn showing how the rate of diffusion changes with the concentration gradient between the inside and outside of the tubing. Further experiments could be designed if sucrose and an enzyme that breaks down sucrose (sucrase) are added to the Visking tubing. Experiments involving amylase, which breaks down starch, could also be designed.

Demonstrating diffusion using Visking tubing

The effect of size on diffusion can be investigated by timing the diffusion of ions through blocks of agar of different sizes. Solid agar is prepared in suitable containers such as ice cube trays. If the agar is made up with very dilute sodium hydroxide solution and Universal Indicator, it will be coloured purple. Cubes of the required dimensions (for example, sides of 2cm × 2 cm, 1 cm × 1 cm, 0.5 cm × 0.5 cm) can be cut from the agar, placed in a container and covered with a diffusion solution such as dilute hydrochloric acid. (The acid should have a higher molarity than the sodium hydroxide so that its diffusion can be monitored by a change in colour of the indicator. Alternatively, the agar can be made up with Universal Indicator only, although its colour will be affected by the pH of the water used.) Either the time taken for the acid to completely change the colour of the indicator in the agar blocks, or the distance travelled into the block by the acid in a given time (e.g. 5 minutes), can be measured. The times can be converted to rates. Finally, the rate of diffusion (rate of colour change) can be plotted against the surface area: volume ratio. Using the same techniques, you may be able to design further experiments. For example, you could investigate the effect on the rate of diffusion of the steepness of the concentration gradient.

Demonstrating diffusion using plant tissue

An experiment showing how the permeability of membranes is affected by environmental factors such as chemicals and temperature can be performed with beetroot. Pieces of beetroot can be placed into water at different temperatures or into different alcohol concentrations. Any damage to the cell membranes results in the red pigment, which is normally contained within the large central vacuole, leaking out of the cells by diffusion. Changes in the colour of the surrounding solution can be monitored qualitatively or quantitatively. As in the experiment in Box 4.1, a colorimeter or a set of colour standards could be used. Alternatively, you could simply put the tubes in order and make up a colour scale (e.g. from 0 to 10), using water as 0 and the darkest solution as 10. There is an opportunity to design your own experiment. What is being observed is diffusion of the red dye from a region of high concentration in the vacuoles to a region of low concentration in the solution outside the pieces of beetroot. Diffusion is normally prevented by the partially permeable nature of the cell membranes. After reading how molecules cross membranes, you may like to think about how the dye gets into the vacuoles in the first place.

Facilitated diffusion

Large polar molecules, such as glucose and amino acids, cannot diffuse through the phospholipid bilayer. Nor can ions such as sodium (Na^+) or chloride (Cl^-). These can only cross the membrane with the help of certain protein molecules. Diffusion that takes place in this

way is called facilitated diffusion. ‘Facilitated’ means made easy or made possible, and this is what the proteins do. There are two types of protein involved, namely channel proteins and carrier proteins. Each is highly specific, allowing only one type of molecule or ion to pass through it. Channel proteins are water-filled pores. They allow charged substances, usually ions, to diffuse through the membrane. Most channel proteins are ‘gated’. This means that part of the protein molecule on the inside surface of the membrane can move to close or open the pore, like a gate. This allows control of ion exchange. Two examples are the gated proteins found in nerve cell surface membranes. One type allows entry of sodium ions, which happens during the production of an action potential (page 335). Another allows exit of potassium ions (K^+) during the recovery phase, known as repolarisation. Some channels occur in a single protein; others are formed by several proteins combined. Whereas channel proteins have a fixed shape, carrier proteins can flip between two shapes (Figure 4.9). As a result, the binding site is alternately open to one side of the membrane, then the other. If the molecules are diffusing across the membrane, then the direction of movement will normally depend on their relative concentration on each side of the membrane. They will move down a concentration gradient from a higher to a lower concentration. However, the rate at which this diffusion takes place is affected by how many channel or carrier protein molecules there are in the membrane, and, in the case of channel proteins, on whether they are open or not. For example, the disease cystic fibrosis is caused by a defect in a channel protein that should be present in the cell surface membranes of certain cells, including those lining the lungs. This protein normally allows chloride ions to move out of the cells. If the channel protein is not correctly positioned in the membrane, or if it does not open the chloride channel as and when it should, then the chloride ions cannot move out.

Facilitated diffusion is the diffusion of a substance through transport proteins in a cell membrane; the proteins provide hydrophilic areas that allow the molecules or ions to pass through the membrane which would otherwise be less permeable to them.

Osmosis

Osmosis is a special type of diffusion involving water molecules only. In the explanations that follow, remember that:

solute + solvent = solution

In a sugar solution, for example, the solute is sugar and the solvent is water. In Figure 4.10 there are two solutions separated by a partially permeable membrane. This is a membrane that allows only certain molecules through, just like membranes in living cells. In the situation shown in Figure 4.10a, solution B has a higher concentration of solute molecules than solution A. Solution B is described as more concentrated than solution A, and solution A as more dilute than solution B. First, imagine what would happen if the membrane was not present. Both solute molecules and water molecules are free to move anywhere within the solutions. As they move randomly, both water molecules and solute molecules will tend to spread themselves evenly throughout the space available, by diffusion. Now consider the situation where a partially permeable membrane is present, as shown in Figure 4.10. The solute molecules are too large to get through the membrane. Only water molecules can pass through. The solute molecules move about randomly, but as they hit the membrane they simply bounce back. The numbers of solute molecules each side of the membrane stay the same. The water molecules also move

about randomly, but they are able to move both from A to B and from B to A. Over time, the water molecules will tend to spread themselves out more evenly between A and B. This means that A will end up with fewer water molecules, so that the solution becomes more concentrated with solute. B will end up with more water molecules, so that it becomes more dilute. We will also find that the volume of liquid in B will increase, because it now contains the same number of solute molecules, but more water molecules. This movement of water molecules from a dilute solution to a concentrated solution, through a partially permeable membrane, is called osmosis.

Water potential

The term water potential is very useful when considering osmosis. The Greek letter psi, ψ, can be used to mean water potential. You can think of water potential as being the tendency of water to move out of a solution. This depends on two factors:

- how much water the solution contains in relation to solutes, and
- how much pressure is being applied to it.

Water always moves from a region of high water potential to a region of low water potential. We say water always moves down a water potential gradient. This will happen until the water potential is the same throughout the system, at which point we can say that equilibrium has been reached. For example, a solution containing a lot of water (a dilute solution) has a higher water potential than a solution containing only a little water (a concentrated solution). In Figure 4.10a, solution A has a higher water potential than solution B, because solution A is more dilute than solution B. This is why the net movement of water is from A to B. Now look again at Figure 4.10b. What would happen if we could press down very hard on side B (Figure 4.11)?

It would be possible to 'squeeze' some of the water back into A. By increasing the pressure on the liquid in B, we are increasing the tendency for water to move out of it – that is, we are increasing its water potential, until it is higher than the water potential in A. Pressure on a liquid increases water potential. By definition, the water potential of pure water at atmospheric pressure is 0. This means that a solution (water with a solute or solutes dissolved in it) must have a water potential less than 0 – that is, a negative value.

Osmosis is the net movement of water molecules from a region of higher water potential to a region of lower water potential, through a partially permeable membrane, as a result of their random motion (diffusion).

Solute potential and pressure potential

We have seen that there are two factors that determine the water potential of a solution – the concentration of the solution, and the pressure applied to it. The contribution of the concentration of the solution to water potential is called solute potential. We can think of solute potential as being the extent to which the solute molecules decrease the water potential of the solution. The more solute there is, the lower the tendency for water to move out of the solution. Just like water potential, solute potential is 0 for pure water, and has a negative value for a solution. Adding more solute to a solution decreases its water potential. So the greater the concentration of the solute, the more negative the value of the solute potential. The psi symbol

can be used to show the solute potential, but this time with the subscript s – ψs. The contribution of pressure to the water potential of a solution is called pressure potential. We can see in Figure 4.11 that increasing the pressure on B increases the tendency of water to move out of it – that is, it increases its water potential. Pressure potential can be shown using the symbol ψp

Osmosis in animal cells

Figure 4.12 shows the effect of osmosis on an animal cell. A convenient type of animal cell to study in practical work is the red blood cell. A slide of fresh blood viewed with a microscope will show large numbers of red blood cells. Different samples of blood can be mixed with solutions of different water potential. Figure 4.12a shows that if the water potential of the solution surrounding the cell is too high, the cell swells and bursts. If it is too low, the cell shrinks (Figure 4.12c). This shows one reason why it is important to maintain a constant water potential inside the bodies of animals.

Osmosis in plant cells

Unlike animal cells, plant cells are surrounded by cell walls, which are very strong and rigid (page 5). Imagine a plant cell being placed in pure water or a dilute solution (Figure 4.13a). The water or solution has a higher water potential than the plant cell, and water therefore enters the cell through its partially permeable cell surface membrane by osmosis. Just like in the animal cell, the volume of the cell increases, but in the plant cell the the cell wall pushes back against the expanding protoplast (the living part of the cell inside the cell wall), and pressure starts to build up rapidly. This is the pressure potential, and it increases the water potential of the cell until the water potential inside the cell equals the water potential outside the cell, and equilibrium is reached (Figure 4.13b). The cell wall is so inelastic that it takes very little water to enter the cell to achieve this. The cell wall prevents the cell from bursting, unlike the situation when an animal cell is placed in pure water or a dilute solution. When a plant cell is fully inflated with water it is described as fully turgid. For plant cells, then, water potential is a combination of solute potential and pressure potential. This can be expressed in the following equation: ψ = ψs + ψp Figure 4.13c shows the situation where a plant cell is placed in a solution of lower water potential. An example of the latter would be a concentrated sucrose solution. In such a solution, water will leave the cell by osmosis. As it does so, the protoplast gradually shrinks until it is exerting no pressure at all on the cell wall. At this point the pressure potential is zero, so the water potential of the cell is equal to its solute potential (see the equation above). Both the solute molecules and the water molecules of the external solution can pass through the freely permeable cell wall, and so the external solution remains in contact with the shrinking protoplast. As the protoplast continues to shrink, it begins to pull away from the cell wall (Figure 4.14). This process is called plasmolysis, and a cell in which it has happened is said to be plasmolysed (Figures 4.13c and 4.14). The point at which pressure potential has just reached zero and plasmolysis is about to occur is referred to as incipient plasmolysis. Eventually, as with the animal cell, an equilibrium is reached when the water potential of the cell has decreased until it equals that of the external solution. The changes described can easily be observed with a light microscope using strips of epidermis peeled from rhubarb petioles or from the swollen

storage leaves of onion bulbs and placed in a range of sucrose solutions of different concentration (Figure 4.15).

Investigating osmosis in plant cells

1 Observing osmosis in plant cells

Epidermal strips are useful material for observing plasmolysis. Coloured sap makes observation easier. Suitable sources are the inner surfaces of the fleshy storage leaves of red onion bulbs, rhubarb petioles and red cabbage. The strips of epidermis may be placed in a range of molarities of sucrose solution (up to 1.0moldm−3) or sodium chloride solutions of up to 3%. Small pieces of the strips can then be placed on glass slides, mounted in the relevant solution, and observed with a microscope. Plasmolysis may take several minutes, if it occurs.

2 Determining the water potential of a plant tissue

The principle in this experiment is to find a solution of known water potential which will cause neither a gain nor a loss in water of the plant tissue being examined. Samples of the tissue – for example, potato – are allowed to come into equilibrium with a range of solutions (for example, sucrose solutions) of different water potentials, and changes in either mass or volume are recorded. Plotting a graph of the results allows the solution that causes no change in mass or volume to be determined. This solution will have the same water potential as the plant tissue.

Active transport

If the concentration of particular ions, such as potassium and chloride, inside cells is measured, it is often found that they are 10–20 times more concentrated inside than outside. In other words, a concentration gradient exists, with a lower concentration outside and a higher concentration inside the cell. The ions inside the cell originally came from the external solution, therefore diffusion cannot be responsible for this gradient because, as we have seen, ions diffuse from high concentration to low concentration. The ions must therefore accumulate against a concentration gradient. The process responsible is called active transport. It is achieved by carrier proteins, each of which is specific for a particular type of molecule or ion. However, unlike facilitated diffusion, active transport requires energy, because movement occurs up a concentration gradient. The energy is supplied by the molecule ATP (adenosine triphosphate) which is produced during respiration inside the cell. The energy is used to make the carrier protein change its shape, transferring the molecules or ions across the membrane in the process (Figure 4.17). An example of a carrier protein used for active transport is the sodium–potassium ($Na^+ – K^+$) pump (Figure 4.18). Such pumps are found in the cell surface membranes of all animal cells. In most cells, they run all the time, and it is estimated that on average they use 30% of a cell's energy (70% in nerve cells). The role of the $Na^+ – K^+$ pump is to pump three sodium ions out of the cell at the same time as allowing two potassium ions into the cell for each ATP molecule used. The ions are both positively charged, so the net result is that the inside of the cell becomes more negative than the outside – a potential difference (p.d.) is created across the membrane. The significance of this in nerve cells is discussed in Chapter 15 (pages 333–334).

In Figure 4.18, you can see that the pump has a receptor site for ATP on its inner surface. It acts as an ATPase enzyme in bringing about the hydrolysis of ATP to ADP (adenosine diphosphate) and phosphate to release energy. Active transport can therefore be defined as the energy-consuming transport of molecules or ions across a membrane against a concentration gradient (from a lower to a higher concentration). The energy is provided by ATP from cell respiration. Active transport can occur either into or out of the cell. Active transport is important in reabsorption in the kidneys, where certain useful molecules and ions have to be reabsorbed into the blood after filtration into the kidney tubules. It is also involved in the absorption of some products of digestion from the gut. In plants, active transport is used to load sugar from the photosynthesising cells of leaves into the phloem tissue for transport around the plant (Chapter 7), and to load inorganic ions from the soil into root hairs.

Bulk transport

So far we have been looking at ways in which individual molecules or ions cross membranes. Mechanisms also exist for the bulk transport of large quantities of materials into cells (endocytosis) or out of cells (exocytosis). Large molecules such as proteins or polysaccharides, parts of cells or even whole cells may be transported across the membrane. This requires energy, so it is a form of active transport. Endocytosis involves the engulfing of the material by the cell surface membrane to form a small sac, or 'endocytic vacuole'. It takes two forms.

- Phagocytosis or 'cell eating' – this is the bulk uptake of solid material. Cells specialising in this are called phagocytes. The process is called phagocytosis and the vacuoles are phagocytic vacuoles. An example is the engulfing of bacteria by certain white blood cells (Figure 4.19).
- Pinocytosis or 'cell drinking' – this is the bulk uptake of liquid. The vacuoles (vesicles) formed are often extremely small, in which case the process is called micropinocytosis.

Exocytosis is the reverse of endocytosis and is the process by which materials are removed from cells (Figure 4.20). It happens, for example, in the secretion of digestive enzymes from cells of the pancreas (Figure 4.21). Secretory vesicles from the Golgi body carry the enzymes to the cell surface and release their contents. Plant cells use exocytosis to get their cell wall building materials to the outside of the cell surface membrane.

Active transport is the movement of molecules or ions through transport proteins across a cell membrane, against their concentration gradient, using energy from ATP.

Endocytosis is the bulk movement of liquids (pinocytosis) or solids (phagocytosis) into a cell, by the infolding of the cell surface membrane to form vesicles containing the substance; endocytosis is an active process requiring ATP

Exocytosis is the bulk movement of liquids or solids out of a cell, by the fusion of vesicles containing the substance with the cell surface membrane; exocytosis is an active process requiring ATP.

Summary

- The basic structure of a membrane is a 7 nm thick phospholipid bilayer with protein molecules spanning the bilayer or within one or other layer. Phospholipids and some proteins move within the layers. Hence the structure is described as a fluid mosaic – the scattered protein molecules resemble pieces of a mosaic. Phospholipid bilayers are a barrier to most water-soluble substances because the interior of the membrane is hydrophobic. Cholesterol is needed for membrane fluidity and stability.
- Some proteins are transport proteins, transporting molecules or ions across the membrane. They may be either channel proteins or carrier proteins. Channel proteins have a fixed shape; carrier proteins change shape. Some proteins act as enzymes – for example, in the cell surface membranes of microvilli in the gut.
- Glycolipids and glycoproteins form receptors – for example, for hormones or neurotransmitters. Glycolipids and glycoproteins also form antigens, which are cell recognition markers. Membranes play an important role in cell signalling, the means by which cells communicate with each other.
- The cell surface membrane controls exchange between the cell and its environment. Some chemical reactions take place on membranes inside cell organelles, as in photosynthesis and respiration.
- Diffusion is the net movement of molecules or ions from a region of their higher concentration to one of lower concentration. Oxygen, carbon dioxide and water cross membranes by diffusion through the phospholipid bilayer. Diffusion of ions and larger polar molecules through membranes is allowed by transport proteins. This process is called facilitated diffusion.
- Water moves from regions of higher water potential to regions of lower water potential. When water moves from regions of higher water potential to regions of lower water potential through a partially permeable membrane, such as the cell surface membrane, this diffusion is called osmosis.
- Pure water has a water potential (Ψ) of zero. Adding solute reduces the water potential by an amount known as the solute potential (Ψs), which has a negative value. Adding pressure to a solution increases the water potential by an amount known as the pressure potential (Ψp), which has a positive value. The following equation is used: $\Psi = \Psi s + \Psi p$
- In dilute solutions, animal cells burst as water moves into the cytoplasm from the solution. In dilute solutions, a plant cell does not burst, because the cell wall provides resistance to prevent it from expanding. The pressure that builds up as water diffuses into a plant cell by osmosis is the pressure potential. A plant cell in this state is turgid. In concentrated solutions, animal cells shrink, while in plant cells the protoplast shrinks away from the cell wall in a process known as plasmolysis.
- Some ions and molecules move across membranes by active transport, against the concentration gradient. This needs a carrier protein and ATP to provide energy. Exocytosis and endocytosis involve the formation of vacuoles to move larger quantities of materials respectively out of, or into, cells by bulk transport. There are two types of endocytosis, namely phagocytosis (cell eating) and pinocytosis (cell drinking).