



Cambridge International AS and A Level Biology Coursebook

Fourth Edition

OMPECH Cambridge Courses



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Cambridge International AS and A Level **Biology** Coursebook

Fourth Edition



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How to use this book

Each chapter begins with a short list of the facts and concepts that are explained in it.



This book does not contain detailed instructions for doing particular experiments, but you will find background information about the practical work you need to do in these boxes. There are also two chapters, P1 and P2, which provide detailed information about the practical skills you need to develop during your course.

The text and illustrations describe and explain all of the facts and concepts that you need to know. The chapters, and often the content within them as well, are arranged in the same sequence as in your syllabus.

Important equations and other facts are shown in highlight boxes.



There is a short context at the beginning of each chapter, containing an example of how the material covered in the chapter relates to the 'real world'.



Where biology meets psychology

We have five senses: touch, sight, hearing, taste an smell. It's a controversial view, but some people ve in extrasensory perception (ESP), telepathy and having premonitions as a 'sixth sense'. Recent research suggests that we detect subtle changes, which we cannot put into words, so imagine it is an condition where stimulation of, say, hearing also produces a visual response (Figure 15.1).

But we do have a genuine sixth sense, one which we take for granted. In his essay 'The Disembodied Lady', the neurologist Oliver Sacks relates the story of a woman who woke up one day to find she had lost from the receptors in her muscles and joints had stopped sending impulses. She had no feedback from her muscles and could not coordinate her movements The only way she could live without this sixth s was to train herself to rely entirely on her eyesight for coordinating her muscles. A man with the same

dition describes the efforts needed to do this as equivalent to running a marathon every day. Curiously, the night before Oliver Sacks's patient found she had iss of body awareness, she dreamt about it



Questions throughout the text give you a chance to check that you have understood the topic you have just read about. You can find the answers to these questions on the CD-ROM.

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 1.0 mol dm⁻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,

4.8 Two neighbo	uring plant cells	are shown in
Figure 4.16.	A	B
Figure 4.16	$\Psi = -250$ kPa	$\Psi = -400 \text{kPa}$

In which direction would there be net movement of water molecules?

- h Explain what is meant by net movement.
- Explain your answer to a.
- Explain what would happen if both cells were placed in i pure water
- ii a 1 mol dm⁻³ sucrose solution with a water potential of -3510 kPa.

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 and page 272). Such pumps are found in the



Figure 4.17 Changes in the shape of a carrier protein during active transport. Here, molecules or ions are being pumped into the cell against a concentration gradient. (Compare Figure 4.9.)

Definitions that are required by the

Wherever you need to know how to use a formula to carry out a calculation, there are worked example boxes to show you how to do this.



points at the end of each chapter. You might find this helpful when you are revising.

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 A = ¹/_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.

Questions at the end of each chapter begin with a few multiple choice questions, then move on to questions that will help you to organise and practise what you have learnt in that chapter. Finally, there are several more demanding exam-style questions, some of which may require use of knowledge from previous chapters. Answers to these questions can be found on the CD-ROM.



Introduction

This fourth edition of *Cambridge International AS and A Level Biology* provides everything that you need to do well in your Cambridge International Examinations AS and A level Biology (9700) courses. It provides full coverage of the syllabus for examinations from 2016 onwards.

The chapters are arranged in the same sequence as the material in your syllabus. Chapters 1 to P1 cover the AS material, and Chapters 12 to P2 cover the extra material you need for the full A level examinations. The various features that you will find in these chapters are explained on the next two pages.

In your examinations, you will be asked many questions that test deep understanding of the facts and concepts that you will learn during your course. It's therefore not enough just to learn words and diagrams that you can repeat in the examination; you need to ensure that you really understand each concept fully. Trying to answer the questions that you will find within each chapter, and at the end, should help you to do this. There are answers to all of these questions on the CD-ROM that comes with this book.

Although you will study your biology as a series of different topics, it's very important to appreciate that all of these topics link up with each other. Some of the questions in your examination will test your ability to make links between different areas of the syllabus. For example, in the AS examination you might be asked a question that involves bringing together knowledge about protein synthesis, infectious disease and transport in mammals. In particular, you will find that certain key concepts come up again and again. These include:

- cells as units of life
- biochemical processes
- DNA, the molecule of heredity
- natural selection
- organisms in their environment
- observation and experiment

As you work through your course, make sure that you keep on thinking about the work that you did earlier, and how it relates to the current topic that you are studying. On the CD-ROM, you will also find some suggestions for other sources of particularly interesting or useful information about the material covered in each chapter. Do try to track down and read some of these.

Practical skills are an important part of your biology course. You will develop these skills as you do experiments and other practical work related to the topic you are studying. Chapters P1 (for AS) and P2 (for A level) explain what these skills are, and what you need to be able to do to succeed in the examination papers that test these skills.

Chapter 1: Cell structure

Learning outcomes

You should be able to:

- describe and compare the structure of animal, plant and bacterial cells, and discuss the noncellular nature of viruses
- describe the use of light microscopes and electron microscopes to study cells
- draw and measure cell structures

- discuss the variety of cell structures and their functions
- describe the organisation of cells into tissues and organs
- outline the role of ATP in cells



Thinking outside the box

Progress in science often depends on people thinking 'outside the box' - original thinkers who are often ignored or even ridiculed when they first put forward their radical new ideas. One such individual, who battled constantly throughout her career to get her ideas accepted, was the American biologist Lynn Margulis (born 1938, died 2011: Figure 1.1). Her greatest achievement was to use evidence from microbiology to help firmly establish an idea that had been around since the mid-19th century - that new organisms can be created from combinations of existing organisms which are not necessarily closely related. The organisms form a symbiotic partnership, typically by one engulfing the other - a process known as endosymbiosis. Dramatic evolutionary changes result.

The classic examples, now confirmed by later work, were the suggestions that mitochondria and chloroplasts were originally free-living bacteria (prokaryotes) which invaded the ancestors of modern eukaryotic cells (cells with nuclei). Margulis saw such symbiotic unions as a major driving cause of

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. evolutionary change. She continued to challenge the Darwinian view that evolution occurs mainly as a result of competition between species.



Figure 1.1 Lynn Margulis: 'My work more than didn't fit in. It crossed the boundaries that people had spent their lives building up. It hits some 30 sub-fields of biology, even geology.'



Figure 1.2 Drawing of cork cells published by Robert Hooke in 1665.

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.



Figure 1.3 How the light microscope works.



Figure 1.4 Structure of a generalised animal cell (diameter about 20µm) as seen with a very high quality light microscope.



Figure 1.5 Structure of a generalised plant cell (diameter about 40 µm) as seen with a very high quality light microscope.



Figure 1.6 Cells from the lining of the human cheek (×400), each showing a centrally placed nucleus, which is a typical animal cell characteristic. The cells are part of a tissue known as squamous (flattened) epithelium.

QUESTION

1.1 Using Figures 1.4 and 1.5, name the structures that animal and plant cells have in common, those found in only plant cells, and those found only in animal cells.



Figure 1.7 Photomicrograph of a cells in a moss leaf (×400).

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, 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 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 μ m in diameter. Your body contains about 60 million million cells, varying in size from about 5 μ m to 40 μ m. Try to imagine structures like mitochondria, which have an average diameter of 1 μ m. 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 =
$$\frac{\text{observed size of the image}}{\text{actual size}}$$

or $M = \frac{I}{A}$

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: $A = \frac{1}{M}$. If you write the formula in a triangle

Fraction of a metre	Unit	Symbol
one thousandth = $0.001 = 1/1000 = 10^{-3}$	millimetre	mm
one millionth = $0.000001 = 1/1000000 = 10^{-6}$	micrometre	μm
one thousand millionth = $0.000000001 = 1/1000000000 = 10^{-9}$	nanometre	nm

Table 1.1 Units of measurement relevant to cell studies: μ is the Greek letter mu; 1 micrometre is a thousandth of a millimetre; 1 nanometre is a thousandth of a micrometre.

WORKED EXAMPLE 1

Measuring cells

Cells and organelles can be measured with a microscope by means of an **eyepiece graticule**. This is a transparent scale. It usually has 100 divisions (see Figure 1.8a). The eyepiece graticule is placed in the microscope eyepiece so that it can be seen at the same time as the object to be measured, as shown in Figure 1.8b. Figure 1.8b shows the scale over a human cheek epithelial cell. The cell lies between 40 and 60 on the scale. We therefore say it measures 20 eyepiece units in diameter (the difference between 60 and 40). We will not know the actual size of the eyepiece units until the eyepiece graticule scale is calibrated.

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.

To calibrate the eyepiece graticule scale, a miniature transparent ruler called a **stage micrometer** scale is placed on the microscope stage and is brought into focus. This scale may be etched onto a glass slide or printed on a transparent film. It commonly has subdivisions of 0.1 and 0.01 mm. The images of the two scales can then be superimposed as shown in Figure 1.8c.

In the eyepiece graticule shown in the figure, 100 units measure 0.25 mm. Hence, the value of each eyepiece unit is:

$$\frac{0.25}{100} = 0.0025 \,\mathrm{mm}$$

Or, converting mm to μ m:

$$\frac{0.25 \times 1000}{100} = 2.5 \,\mu\text{m}$$

The diameter of the cell shown superimposed on the scale in Figure 1.8b measures 20 eyepiece units and so its actual diameter is:

$$20 \times 2.5 \,\mu m = 50 \,\mu m$$

This diameter is greater than that of many human cells because the cell is a flattened epithelial cell.

Figure 1.8 Microscopical measurement. Three fields of view seen using a high-power (×40) objective lens. **a** An eyepiece graticule scale. **b** Superimposed images of human cheek epithelial cells and the eyepiece graticule scale. **c** Superimposed images of the eyepiece graticule scale and the stage micrometer scale.



WORKED EXAMPLE 2

Calculating the magnification of a photograph or image

To calculate *M*, the magnification of a photograph or an object, we can use the following method.

Figure 1.9 shows two photographs of a section through the same plant cells. The magnifications of the two photographs are the same. Suppose we want to know the magnification of the plant cell labelled P in Figure 1.9b. If we know its actual (real) length we can calculate its magnification using the formula

$$M = \frac{1}{4}$$

The real length of the cell is $80\,\mu m$.

Step 1 Measure the length in mm of the cell in the photograph using a ruler. You should find that it is about 60 mm.

Step 2 Convert mm to μ m. (It is easier if we first convert all measurements to the same units – in this case micrometres, μ m.)

	$1mm = 1000\mu m$
50	$60\text{mm} = 60 \times 1000\mu\text{m}$
	$=60000 \mu m$

Step 3 Use the equation to calculate the magnification.

magnification, $M = \frac{\text{image size, } I}{\text{actual size, } A}$ = $\frac{60\,000\,\mu\text{m}}{80\,\mu\text{m}}$ = $\times 750$

The multiplication sign in front of the number 750 means 'times'. We say that the magnification is 'times 750'.



Figure 1.9 Photographs of the same types of plant cells seen **a** with a light microscope, **b** with an electron microscope, both shown at a magnification of about ×750.

QUESTION

- **1.2 a** Calculate the magnification of the drawing of the animal cell in Figure 1.4 on page 3.
 - **b** Calculate the actual (real) length of the chloroplast labelled **X** in Figure 1.29 on page 21.

WORKED EXAMPLE 3

Calculating magnification from a scale bar Figure 1.10 shows a lymphocyte.

βμm

Figure 1.10 A lymphocyte.

We can calculate the magnification of the lymphocyte by simply using the scale bar. All you need to do is measure the length of the scale bar and then substitute this and the length it represents into the equation.

Step 1 Measure the scale bar. Here, it is 36 mm.

Step 2 Convert mm to μm :

 $36\,\text{mm} = 36 \times 1000\,\mu\text{m} = 36\,000\,\mu\text{m}$

Step 3 Use the equation to calculate the magnification:

magnification, $M = \frac{\text{image size, } I}{\text{actual size, } A}$ = $\frac{36\,000\,\mu\text{m}}{6\,\mu\text{m}}$ = ×6000

WORKED EXAMPLE 4

Calculating the real size of an object from its magnification

To calculate A, the real or actual size of an object, we can use the following method.

Figure 1.27 on page 19 shows parts of three plant cells magnified × 5600. One of the chloroplasts is labelled 'chloroplast' in the figure. Suppose we want to know the actual length of this chloroplast.

Step 1 Measure the observed length of the image of the chloroplast (I), in mm, using a ruler. The maximum length is 40 mm.

Step 2 Convert mm to μ m:

 $40 \,\mathrm{mm} = 40 \times 1000 \,\mathrm{\mu m} = 40\,000 \,\mathrm{\mu m}$

Step 3 Use the equation to calculate the actual length:

actual size,
$$A = \frac{\text{image size, } I}{\text{magnification, } M}$$

= $\frac{40\,000\,\mu\text{m}}{5600}$
= 7.1 μm (to one decimal place)

BOX 1.1: Making temporary slides

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 blueblack 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 200 nm. 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 200 nm, 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.



Figure 1.11 Diagram of the electromagnetic spectrum (the waves are not drawn to scale). The numbers indicate the wavelengths of the different types of electromagnetic radiation. Visible light is a form of electromagnetic radiation. The arrow labelled uv is ultraviolet light.



Figure 1.12 A mitochondrion and some ribosomes in the path of light waves of 400 nm length.

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.

QUESTION

1.3 Explain why ribosomes are not visible using a light microscope.

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.



Figure 1.13 False-colour scanning electron micrograph of the head of a cat flea (\times 100).

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.



Figure 1.15 A transmission electron microscope (TEM) in use.

Figure 1.14 How an electron microscope (EM) works.

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.



Figure 1.16 Representative animal cells as seen with a TEM. The cells are liver cells from a rat (×9600). The nucleus is clearly visible in one of the cells.



Figure 1.17 Ultrastructure of a typical animal cell as seen with an electron microscope. In reality, the ER is more extensive than shown, and free ribosomes may be more extensive. Glycogen granules are sometimes present in the cytoplasm.

QUESTION

1.4 Compare Figure 1.17 with Figure 1.4 on page 3. Name the structures in an animal cell which can be seen with the electron microscope but not with the light microscope.

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).



Figure 1.18 Transmission electron micrograph of the nucleus of a cell from the pancreas of a bat (×7500). The circular nucleus is surrounded by a double-layered nuclear envelope containing nuclear pores. The nucleolus is more darkly stained. Rough ER (page 15) is visible in the surrounding cytoplasm. 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



Figure 1.19 Transmission electron micrograph of rough ER covered with ribosomes (black dots) (×17000). Some free ribosomes can also be seen in the cytoplasm on the left.

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 which 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.



Figure 1.20 Transmission electron micrograph of a Golgi body. A central stack of saucer-shaped sacs can be seen budding off small Golgi vesicles (green). These may form secretory vesicles whose contents can be released at the cell surface by exocytosis (page 87).

Lysosomes

Lysosomes (Figure 1.21) are spherical sacs, surrounded by a single membrane and having no internal structure. They are commonly $0.1-0.5 \,\mu$ m 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 (breast feeding). 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).



Figure 1.21 Lysosomes (orange) in a mouse kidney cell (×55000). They contain cell structures in the process of digestion, and vesicles (green). Cytoplasm is coloured blue here.

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 μ m 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



Figure 1.22 Mitochondrion (orange) with its double membrane (envelope); the inner membrane is folded to form cristae (×20000). Mitochondria are the sites of aerobic cell respiration. Note also the rough ER.

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 \times 100 000, it can be seen to have three layers, described as a **trilaminar 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.



Figure 1.23 Cell surface membrane (×250000). At this magnification the membrane appears as two dark lines at the edge of the cell.

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 25 nm 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. Membranebound 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



The dimers have a helical arrangement.

The dimers form 13 protofilaments around a hollow core.

Figure 1.24 a The structure of a microtubule and **b** the arrangement of microtubules in two cells. The microtubules are coloured yellow.

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). They 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).

triplet of microtubules (one complete microtubule and two partial microtubules)



200 nm

Figure 1.25 The structure of a centriole. It consists of nine groups of microtubules arranged in triplets.



Figure 1.26 Centrioles in transverse and longitudinal section (TS and LS) (×86000). The one on the left is seen in TS and clearly shows the nine triplets of microtubules which make up the structure.

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 μ m (compare 1 μ m 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



Figure 1.27 A representative plant cell as seen with a TEM. The cell is a palisade cell from a soya bean leaf (×5600).

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.

QUESTION

1.5 Compare Figure 1.28 with Figure 1.5 on page 4. Name the structures in a plant cell which can be seen with the electron microscope but not with the light microscope.



Figure 1.28 Ultrastructure of a typical plant cell as seen with the electron microscope. In reality, the ER is more extensive than shown. Free ribosomes may also be more extensive.