

# Biology

Student Textbook  
Grade 11

Biology

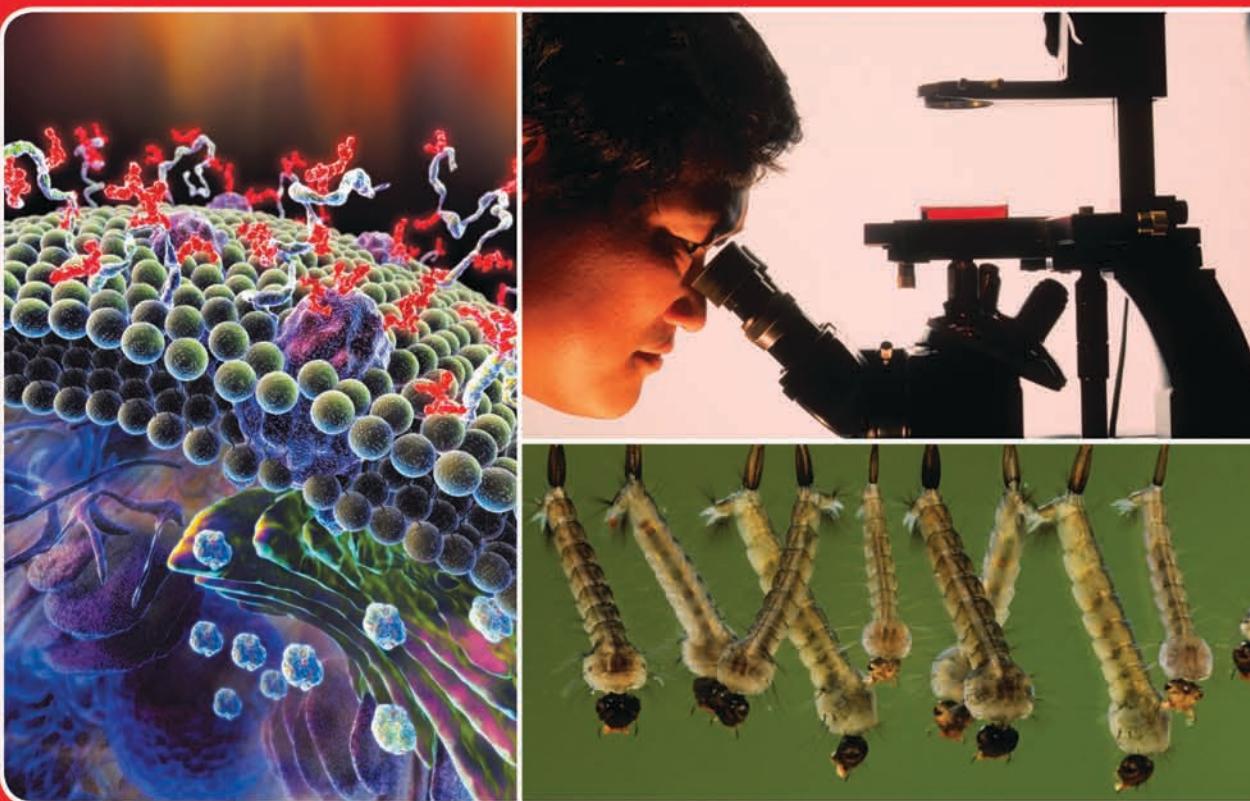
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Federal Democratic Republic of Ethiopia  
Ministry of Education

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## KEY WORDS

**active site** the part of an enzyme molecule that binds with its substrate so that the enzyme can catalyse the chemical reaction

**substrate** a substance upon which an enzyme acts in a biochemical reaction

**enzyme–substrate complex** the intermediate formed, temporarily, when an enzyme binds to its substrate

## 3.1 Nature of enzymes

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

- Define enzymes and explain the properties of enzymes.
- Explain how enzymes are named and then classify them according to their structure.
- Conduct an experiment to show the specificity of an enzyme.
- Appreciate the importance of enzymes in industries and local products.

## What are enzyme molecules like?

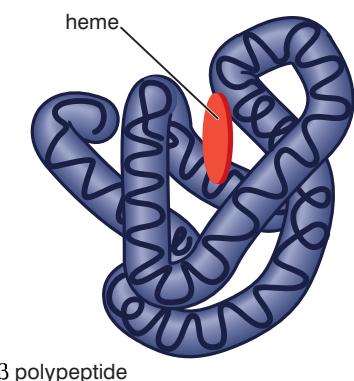


Figure 3.1 The human lipase enzyme

First, all enzymes are globular proteins. We learned in unit 2 that globular proteins all have a unique tertiary structure, which gives them a unique shape. Figure 3.1 shows a model of the tertiary structure of the human lipase enzyme that hydrolyses lipids into fatty acids and glycerol.

You should be able to identify regions where there is:

- an  $\alpha$ -helix
- a  $\beta$ -pleated sheet
- no folding into a secondary structure.

Second, within that very complex structure is a region called the **active site**. This is the part of the enzyme molecule that binds with its **substrate** so that the enzyme can catalyse the chemical reaction. The active site of an enzyme is shaped to allow:

- binding with a particular substrate and that substrate only, and
- binding in such a way that the reaction can take place requiring less energy than if the enzyme was not present.

We can use the example of the enzyme sucrase catalysing the hydrolysis of sucrose into glucose and fructose to illustrate this. The substrate for the enzyme is the molecule of sucrose. This binds with the active site to form an **enzyme–substrate complex**.

A molecule of water then reacts with the sucrose to hydrolyse the molecule into glucose and fructose. These are then released from the active site, which can then accept another molecule of sucrose. It is important to note that the enzyme is unaltered by the reaction.

This is shown in figure 3.2.

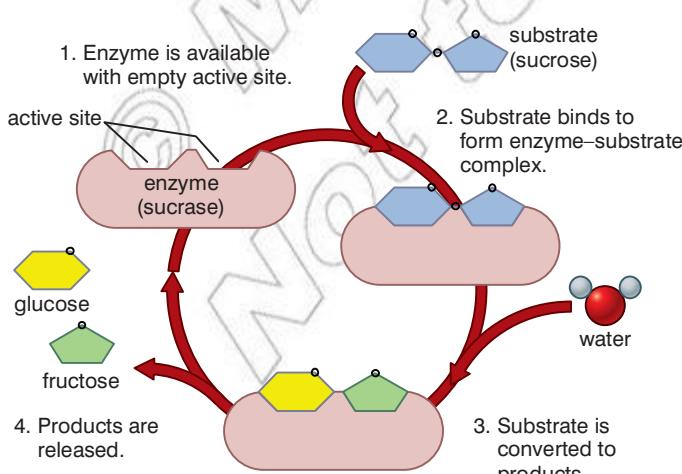


Figure 3.2 The hydrolysis of sucrose by sucrase

We are now in a position where we can define more precisely what we mean when we are talking about an enzyme:

*An enzyme is a globular protein with a uniquely shaped active site; it acts as a biological catalyst for a specific reaction, but remains unaltered by the reaction.*

### KEY WORD

**catalyst** *a substance that speeds up a chemical reaction and remains unchanged at the end of the reaction*

## What are the properties of enzymes?

- They are all proteins.
- They are biological catalysts: they speed up a reaction without being used up, so they can be used over and over again.
- They are specific: they catalyse one reaction only.
- A small amount of enzyme can bring about a change in a large amount of its substrate.
- Enzymes are affected by pH and temperature. They can be destroyed by excessive heat. They are also affected by the concentration of their substrate and the presence of certain substances that act as inhibitors.

## What are catalysts?

A **catalyst** is a substance that speeds up a reaction; the reaction itself is unaltered. There is no overall change to:

- the nature of the products
- the energy change that takes place during the reaction
- the catalyst itself

Enzymes allow biochemical reactions inside cells to take place quickly, at a temperature that will not damage the structure of the cell.

## Why are enzymes specific?

This is also a function of the active site. Because of the conformation of the active site (the way in which it is shaped), only a certain substrate or combination of substrates can bind with it.

Because only one substrate (or substrate combination) can bind, there is only one possible reaction that can be catalysed. This is illustrated in figure 3.3.

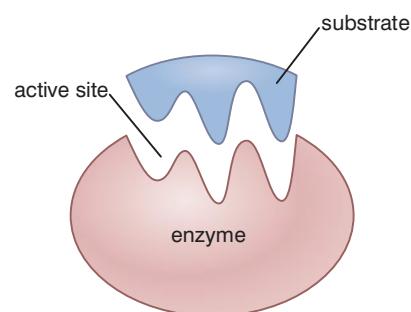
## How are enzymes affected by pH and by temperature?

Temperature affects enzyme action in two ways:

- a higher temperature gives the enzyme molecules (and their substrate molecules) more kinetic energy; they move around faster and form more enzyme–substrate complexes
- a higher temperature affects the chemical bonds holding the tertiary structure of the enzyme in place (particularly those in the active site); as more and more of these bonds break, the shape of the active site changes and it can no longer bind with its substrate

### DID YOU KNOW?

Not all biological catalysts are proteins. Recently, it has been shown that some RNA molecules can catalyse some biological reactions.



**Figure 3.3** An enzyme can only bind with one substrate because of the shape of its active site.

**Activity 3.1**

Make a poster which lists the four criteria for naming enzymes. Include a chart or key showing how you would classify the main types of enzymes (you will need to look at p83 before you can complete this activity).

pH affects the enzyme molecule in a similar way to high temperatures. A pH that is too low (too acid) or too high (too alkaline) will cause charges on the active site to alter and cause the active site to lose its conformation. The substrate cannot bind and so the reaction is no longer catalysed.

**How do we name and classify enzymes?****Common or working enzyme nomenclature (naming of enzymes)**

Table 3.1 gives some examples of enzymes and the reactions they catalyse.

**Table 3.1 Examples of enzymes and the reactions they catalyse**

Name of enzyme	Reaction catalysed
Lipase	Hydrolysis of lipids
ATPase	Hydrolysis of ATP
Succinate dehydrogenase	Removal of hydrogen ions from succinate (during respiration)
DNA polymerase	Joining of nucleotides to form DNA
Pepsin	Digestion of proteins in the stomachs of mammals

Different enzymes are named in different ways.

- Most commonly enzymes are named by adding 'ase' to part of the name of the substrate. For example, *lipase* (lipid hydrolysing enzyme), *sucrase* (sucrose hydrolysing enzyme).
- Sometimes the enzymes are named on the basis of the reaction that they catalyse. For example, *polymerase* (aids in polymerisation – joining similar units together), *dehydrogenase* (removal of hydrogen atoms or ions).
- Some enzymes have been named based on the source from which they were first identified. For example, *papain* from papaya. Others are named according to where they act. For example, *intestinal* protease acts on proteins in the intestine.
- The names of some enzymes end with 'in', indicating that they are basically proteins. For example, *pepsin*, *trypsin*, etc. These enzymes usually have alternative names that tell you rather more about them. For example, the alternative name for pepsin is *gastric protease*. This tells you that it acts on proteins and it does so in the stomach.

Because of the varied ways in which enzymes had been named, biologists at the **Enzyme Commission** decided to produce a systematic way of naming enzymes, based on the ways in which the enzymes act. To appreciate this, we must first look at how enzymes are classified.

**KEY WORD**

**Enzyme Commission body**  
set up to produce a systematic way of naming enzymes

## Enzyme classification and the systematic nomenclature of enzymes

Enzymes are generally classified on the basis of the type of reactions that they catalyse. Six groups of enzymes can be recognised on this basis. Table 3.2 lists these groups along with examples.

**Table 3.2 Classification of enzymes**

Class	Reaction catalysed	Examples
1. Oxidoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another	Dehydrogenases Oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl, etc.) from one substrate to another	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate	Esterases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate	Phosphohexoisomerase Fumerase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds	Citric acid synthetase

Each class of enzymes contains several different, but related, subclasses. Each subclass is further divided into sub-subclasses. Within the sub-subclasses, each enzyme has a number.

So, in the systematic naming of enzymes, an enzyme will have a 'name' such as EC 3.4.11.1. Each part of the description tells us something about the enzyme:

- EC stands for Enzyme Commission
- the first number shows to which of the six main classes the enzyme belongs
- the second figure indicates a subclass
- the third figure gives a sub-subclass
- the fourth figure is the serial number of the enzyme in its sub-subclass.

Enzyme EC 3.4.11.1 is:

- a hydrolase – all the enzymes in class 3 hydrolyse some kind of bond
- a peptidase – all the enzymes in subclass 4 of class 3 are peptidases and hydrolyse peptide bonds
- an amino-peptidase – all the enzymes in sub-subclass 11 of subclass 4 are amino-peptidases; they hydrolyse peptide bonds at the amino end of a polypeptide chain
- leucyl-amino-peptidase – this particular amino-peptidase is number 1 of this sub-subclass

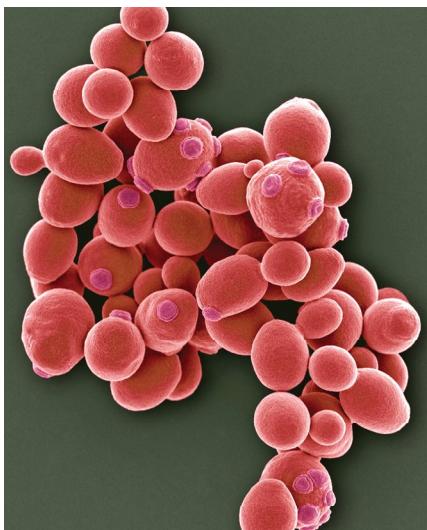


Figure 3.4 Yeast cells

### Activity 3.2: Library search

If you have access to the internet, you could visit site: [www.chem.qmul.ac.uk/iubmb/enzyme/](http://www.chem.qmul.ac.uk/iubmb/enzyme/) and find out more about the naming of enzymes. What is enzyme 1.1.1.1?

### What do enzymes do for you?

We have been using enzymes for thousands of years – although the people who used them then didn't know quite what they were using! Unknowingly, they used enzymes (in yeast) to make bread and beer. These are almost certainly the first uses of 'enzyme technology'.

Yeast is a unicellular fungus that ferments carbohydrates to produce carbon dioxide and alcohol. The enzymes in yeast control the reactions of fermentation. Figure 3.4 shows some yeast cells.

### DID YOU KNOW?

#### How long people have been brewing beer?

The oldest proven records of brewing are about 6000 years old in the ancient country of Sumeria, in the Middle East. A document 4000 years old is a Sumerian 'Hymn to Ninkasi', who was the goddess of brewing! The 'hymn' is also a recipe for making beer.

Of course, these people did not know they were using enzymes. They did not know at first that they were using yeast! But as time progressed people found that it was the yeast that fermented carbohydrates into alcohol. Now we know that several enzymes are involved in the brewing process and can control it much more efficiently.

Figure 3.5 An ancient Egyptian tomb model showing a woman brewing beer

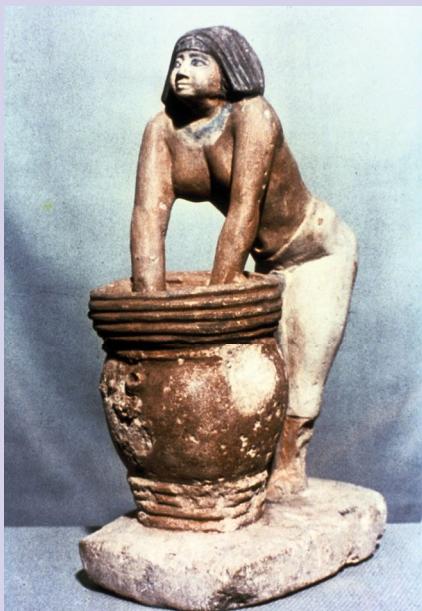


Figure 3.6 Injera

We still use yeast to brew alcoholic drinks, such as tella, and to bake breads, such as injera. Both these Ethiopian products are often made at home, as well as professionally.

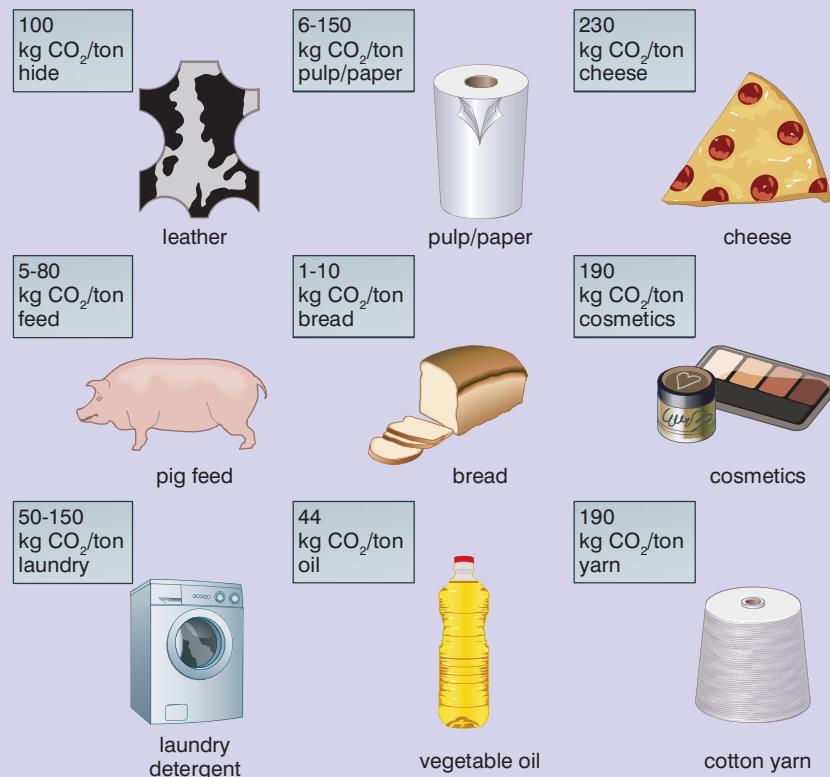
When dough is baked to produce the bread, the tiny amount of alcohol formed is lost and the carbon dioxide expands to make the dough 'rise' to form a loaf of bread. When beer is brewed, it is the carbon dioxide that is lost and the alcohol remains!

However, enzyme technology is now very big business. Enzymes are used in many industries. They are used to produce washing powders – the enzymes in the washing powders digest the stains in the clothes. 'Stone-washed' denim jeans are now given their stone-washed look by the action of enzymes. Table 3.3 shows just a few of the areas where enzyme technology is used.

**Table 3.3 Some industrial uses of enzyme technology**

Sector	Application area	Benefits
Dairy	<ul style="list-style-type: none"> <li>• Biochymosin to produce cheese</li> <li>• Lactase to produce lactose-free milk</li> </ul>	<ul style="list-style-type: none"> <li>• Supplies of natural rennet from calves livers are limited</li> <li>• Lactose-intolerant people suffer fewer cramps</li> </ul>
Detergents	<ul style="list-style-type: none"> <li>• Use of proteases, lipases and amylases in biological washing powders</li> <li>• Use of proteases and amylases in dishwasher detergents</li> </ul>	<ul style="list-style-type: none"> <li>• Many biological stains are removed efficiently at low temperatures (saving energy)</li> <li>• Remove food particles at lower temperatures and require fewer bleaching products to be added</li> </ul>
Textiles	<ul style="list-style-type: none"> <li>• Proteases to remove hair and lipases to degrease animal hides</li> <li>• Use of cellulase to 'bio-polish' cotton fabrics</li> <li>• Use of cellulase to 'bio-stone' denim</li> </ul>	<ul style="list-style-type: none"> <li>• Process is carried out much quicker than by traditional methods</li> <li>• Produces a smoother and glossier finish</li> <li>• The enzyme gives the 'stone-washed' effect much more easily</li> </ul>
Food processing	<ul style="list-style-type: none"> <li>• Pectinase to process fruit juice</li> <li>• Invertase to produce liquid-centre chocolates</li> </ul>	<ul style="list-style-type: none"> <li>• Clarifies fruit juice</li> <li>• Sucrose paste in the chocolate is made liquid by injection of the enzyme</li> </ul>
Pulp and paper	<ul style="list-style-type: none"> <li>• Amylases used in starch conversion</li> <li>• Use of xylanase enzymes in pre-bleaching the pulp</li> <li>• Use of esterases in control of 'stickies' (glues introduced during paper recycling)</li> </ul>	<ul style="list-style-type: none"> <li>• Reduces the quantity of starch in the paper and improves quality</li> <li>• Produces a whiter paper</li> <li>• Stickies would otherwise clog the machinery and reduce the quality of the paper</li> </ul>
Medicine	<ul style="list-style-type: none"> <li>• Glucose oxidase in clinstix strips, tests for glucose</li> <li>• Liver enzymes</li> <li>• Pulmozyme to treat cystic fibrosis</li> </ul>	<ul style="list-style-type: none"> <li>• Allows easy diagnosis of diabetes by testing urine</li> <li>• Testing for high levels of these in the blood confirms liver damage</li> <li>• Reduces viscosity (stickiness) of mucus</li> </ul>
Pharmaceutical	<ul style="list-style-type: none"> <li>• Streptokinase to dissolve clots of heart-attack patients</li> <li>• Production of abacavir sulphate is controlled by enzymes</li> </ul>	<ul style="list-style-type: none"> <li>• Restores blood supply to area of heart muscle</li> <li>• Abacavir sulphate is an important anti-AIDS drug</li> </ul>

One of the appeals of using enzymes in industry is that they allow the reactions involved in the processes to be carried out at much lower temperatures. This means less energy (and therefore less money) is spent on heating the reactants. Because less heating is required, less carbon dioxide is produced and this can benefit the environment as carbon dioxide is a greenhouse gas and its accumulation in the atmosphere can lead to global warming.



## DID YOU KNOW?

Using enzymes in industry can help the environment. Because enzymes allow some manufacturing processes to be carried out at lower temperatures, less carbon dioxide is produced in raising the temperature of the reactants. Figure 3.7 shows the mass of carbon dioxide emissions saved in some processes that now use isolated enzymes rather than the traditional method.

**Figure 3.7** Carbon dioxide emission reductions in some industrial processes that now use enzymes

### Activity 3.3: Discussion

#### The importance of enzymes in local manufacturing

As you have seen from the material presented in this book, some enzymes have been used for many hundreds of years in manufacturing processes and the number being used is increasing all the time. In this activity, you will discuss the importance of enzymes in these local processes. You might bear in mind:

- the importance of enzymes as catalysts in the processes
- whether or not there are other options to using enzymes in the processes that might be more cost-effective

The activity will follow the following procedure:

Your teacher will describe some of the uses of enzymes in the manufacture of products in your locality.

Your teacher will then ask you for your opinions as to how crucial you think the role of enzymes is in these processes. You may then make your point of view but, during this stage, it is important that:

- you do not interrupt anyone else; they also have the right to put their point of view
- you only put your point of view when your teacher allows you to – the discussion cannot degenerate into a row!

At the end of the discussion, your teacher will summarise the views of the class.

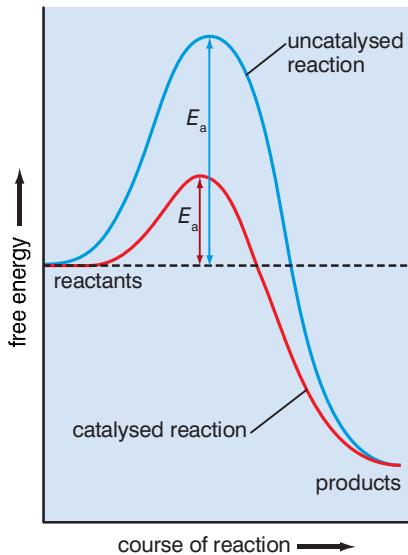
You will write a summary of the main views held by different people in the group.

## Review questions

Choose the correct answer from A to D.

- All enzymes are:
  - globular proteins that digest large molecules
  - globular proteins that catalyse reactions
  - fibrous proteins that catalyse reactions
  - fibrous proteins that digest large molecules
- Enzymes are specific because:
  - they are globular proteins
  - they are affected by temperature and pH
  - their tertiary structure gives them a uniquely shaped active site
  - they may have cofactors
- The shape of an enzyme's active site and its substrate are:
  - complementary
  - the same
  - similar
  - related
- Catalysts:
  - speed up a reaction and are used up in the process
  - slow down a reaction and are used up in the process
  - slow down a reaction and are not used up in the process
  - speed up a reaction and are not used up in the process
- The name of an enzyme often ends in:
  - ase
  - ese
  - ise
  - ose
- Some enzymes' names are derived from their substrate. An example of this is:
  - pepsin
  - papain
  - nuclease
  - amylase
- How many classes of enzymes are there in the Enzyme Commission classification?
  - 3
  - 4
  - 5
  - 6
- Which of the following is NOT a reason why enzymes are often used in industrial processes?
  - they allow reactions to be carried out at lower temperatures
  - they reduce heating costs
  - more energy is used and so more carbon dioxide is produced during the process
  - less energy is used and so less carbon dioxide is produced during the processes
- An environmental benefit of using enzymes in industrial processes is that it can:
  - reduce use of paper in packaging the product
  - reduce carbon dioxide emissions
  - increase purity of the product
  - reduce the costs involved
- One advantage of the Enzyme Commission systematic naming of enzymes is:
  - all enzymes are 'named' in the same way
  - biologists from all countries can understand the 'name' equally
  - no local knowledge is necessary to understand the name
  - all of the above

## 3.2 Functions of enzymes



**Figure 3.8** Activation energy for an uncatalysed reaction and the same reaction with a catalyst

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

- Explain how enzymes lower activation energy.
- Explain the mechanism of enzyme action.
- Discuss the action of apo- and coenzymes.
- Give examples of vitamins and minerals in food that act as cofactors.

### How do enzymes act as catalysts?

Catalysts speed up chemical reactions. In order for molecules to react, they must have sufficient energy. This energy to start off the reaction is called **activation energy** (or  $E_a$ ). Imagine a reaction in which substance A reacts with substance B to form substance AB. We can write an equation for this as:  $A + B \rightarrow AB$

However, this does not tell the whole story. The equation gives only the reactants (starting materials) and the products. It does not show how the energy changes as the reaction takes place.

The reactant must 'climb an activation energy hill' before anything happens. Under normal conditions, very few molecules of A and B have sufficient kinetic energy to 'climb the activation energy hill', so the reaction proceeds slowly. A catalyst lowers the activation energy required for the reaction. More reactant molecules can meet this lower energy requirement and so the reaction proceeds more quickly. Because the enzyme molecule is unaltered by the reaction, it can be used over and over, and so a small amount of enzyme can affect a large amount of substrate. This is shown in figure 3.8.

### KEY WORDS

**activation energy** the energy required to start off a chemical reaction

**lock-and-key model** proposes that the shapes of the substrate molecules are complementary to that of the active site

**induced-fit model** the active site and substrate do not complement each other but the binding of substrate molecules produces a change in shape in the active site, allowing the substrate to fit the active site

### How do enzymes lower activation energy?

There are two models of enzyme action; the **lock-and-key model**, first proposed in 1894 by a German biochemist named Fischer and the **induced-fit model**, proposed in 1958 by Koshland. Both of these models suggest that the enzyme catalyses the reaction by lowering the activation energy. However, they differ in the way that they explain how this happens. In particular, they differ in explaining how the substrate binds to the active site of the enzyme.

#### The lock-and-key model

This model proposes that the shapes of the substrate molecules are *complementary* to that of the active site, rather like the shape of a key is complementary to that of the lock it fits. A useful way of thinking of complementary shapes is to think of an egg sitting in an egg cup. The egg can sit inside the egg cup because the shapes are complementary. One egg cannot sit inside another egg because the shapes are the same.

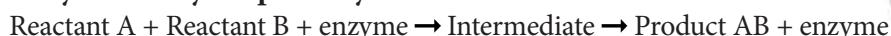
The complementary substrate molecule binds with the active site of the enzyme to form the enzyme–substrate complex. The complex causes the reactants to enter a transition state in which the activation energy of the reaction is lowered. The reaction takes place and the products formed are released. The lock-and-key model of enzyme action suggests that the enzyme lowers the activation energy by providing an alternative pathway for the reaction.

For example:

**Non-catalysed pathway:**



**Enzyme-catalysed pathway:**



This model sees the enzyme–substrate complex as the intermediate, which is part of a pathway that requires less energy than the normal pathway. However, a weakness of this model is that it does not explain how the intermediate reduces activation energy.

**The induced-fit model**

This model suggests that the active site and the substrate aren't naturally complementary in shape, but the binding of substrate molecules produces a conformational change (change in shape) in the active site. This allows the substrate and active site to bind fully. The conformational change also puts the substrate molecules under tension, so they enter a 'transition state' and are able to react because of the lowered activation energy. In the transition state, bonds in the reactants are put under strain and break more easily and rejoin with other bonds to form the products. The products formed leave the active site. This is shown in figure 3.11.

Most biologists now prefer the induced-fit model over the lock-and-key model as it explains other properties of enzymes, such as enzyme inhibition, in a more complete manner than the lock-and-key model.

The rate of a chemical reaction is the rate at which reactants are converted into products. In the case of an enzyme-controlled reaction, this is determined by how many molecules of substrate bind with enzyme molecules to form enzyme–substrate complexes. The number of molecules of reactants that form enzyme–substrate complexes with each molecule of an enzyme, per second, is the **turnover rate** of the enzyme.

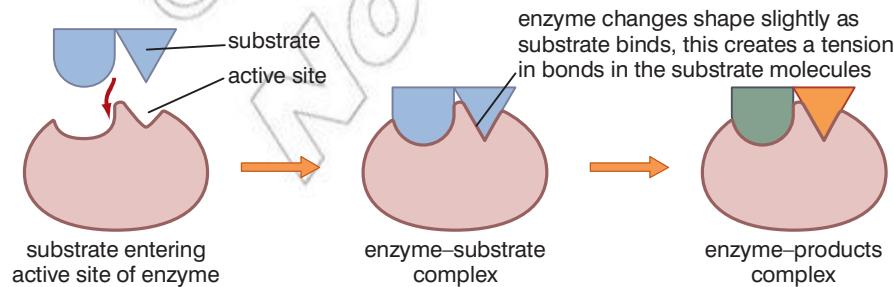


Figure 3.11 The induced-fit model of enzyme action



Figure 3.9 Complementary shapes

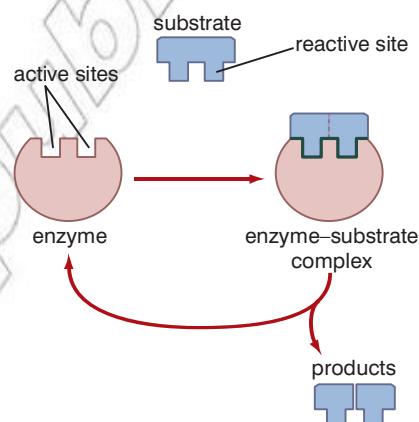
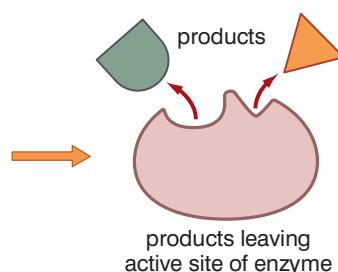


Figure 3.10 The lock-and-key model of enzyme action

**KEY WORD**

**turnover rate** the number of molecules of reactants that form enzyme–substrate complexes with each molecule of an enzyme, per second



**DID YOU KNOW?****Just how much faster enzyme-catalysed reactions proceed**

Table 3.4 shows how much faster reactions proceed with the enzymes than without the enzymes.

**Table 3.4 The rate enhancement of some enzymes**

Enzyme	Rate enhancement
OMP decarboxylase	$1.4 \times 10^{17}$
Staphylococcal nuclease	$5.6 \times 10^{14}$
Adenosine deaminase	$2.1 \times 10^{12}$
AMP nucleosidase	$6.0 \times 10^{12}$
Cytidine deaminase	$1.2 \times 10^{12}$
Phosphotriesterase	$2.8 \times 10^{11}$
Carboxypeptidase A	$1.9 \times 10^{17}$
Ketosteroid isomerase	$3.9 \times 10^{17}$
Triosephosphate isomerase	$1.0 \times 10^9$
Chorismate mutase	$1.9 \times 10^6$
Carbonic anhydrase	$7.7 \times 10^6$
Cyclophilin, human	$4.6 \times 10^5$

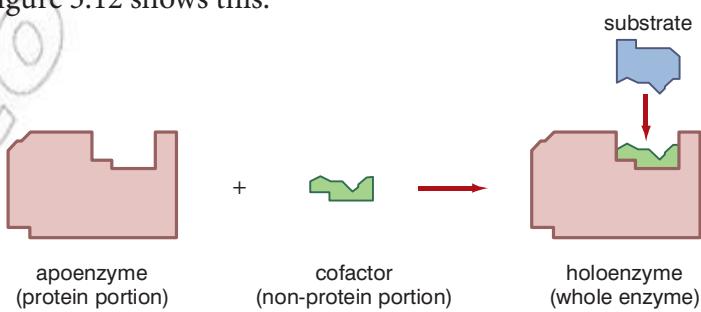
**Why do some enzymes need cofactors?**

Sometimes an active enzyme isn't just a single molecule, but is made from two molecules, neither of which has enzymic activity without the other. The two parts are the apoenzyme and the cofactor. We can define these in the following way:

**Apoenzyme** – a protein that combines with a cofactor, to form an active enzyme. The protein is inactive on its own.

**Cofactor** – a small non-protein particle essential for the activity of some enzymes. The cofactor combines with the apoenzyme to produce an active enzyme.

Where an active enzyme molecule comprises an apoenzyme and a cofactor, the whole is sometimes referred to as the **holoenzyme**. Figure 3.12 shows this.



**Figure 3.12** The apoenzyme and the cofactor make the holoenzyme.

Cofactors include:

- coenzymes
- mineral ions

**Coenzymes** are organic molecules and many are derived from vitamins. They bind with the enzyme to give catalytic activity.

Table 3.5 shows some common co enzymes, the vitamins they are derived from, the enzyme with which they bind, and their functions.

**Table 3.5 Common coenzymes and their functions**

Coenzyme	Vitamin	Enzyme	Function
Nicotinamide adenine dinucleotide (NAD)	Niacin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration
Flavin adenine dinucleotide (FAD)	Riboflavin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration

Some enzymes can only function in the presence of certain mineral ions. These bind loosely with the enzyme to give it its catalytic activity. Table 3.6 shows some examples of enzymes that require mineral ions as cofactors.

**Table 3.6 Enzymes that require mineral ions as cofactors**

Enzyme	Mineral ion	Function
Carbonic anhydrase	Zinc ions ( $Zn^{++}$ )	Causes $CO_2$ to react with water to form hydrogen carbonate
Alcohol dehydrogenase	Zinc ions ( $Zn^{++}$ )	Oxidises alcohol
Cytochrome oxidase	Copper ions ( $Cu^{++}$ or $Cu^+$ )	Transfers electrons to oxygen during respiration

### Activity 3.4: Field visit

**Field visit to study the use of enzymes by local manufacturers**

You may be able to visit a nearby manufacturing plant that uses enzymes in some of its processes. If this is possible you should:

- make careful notes when you are there about:
- the processes themselves
- the role of enzymes in these processes
- write a report on your return that describes how important the use of enzymes is in this particular manufacturing plant

### Review questions

Choose the correct answer from A to D.

1. Enzymes speed up biological reactions by:
  - reducing the kinetic energy of the reacting molecules
  - reducing the activation energy of the reaction
  - increasing activation energy of the reaction
  - increasing the kinetic energy of the reacting molecules
2. Which of the following statements about a lock and key model of enzyme action are not true?
  - the substrate and the active site bind because they have shapes that fit together like a key fits in a lock
  - the substrate and the active site have complementary 3-D shapes
  - nothing can interfere with the way the substrate and the active site bind together
  - high temperatures stop enzymes working as they denature the protein and change the shape of the active site

**Activity 3.5**

Design a 3-dimensional model to show how an enzyme works. You can plan to use a variety of resources from a carved fruit to modelling clay, from papier mache to paper and card. You may have the opportunity to actually make your model and display it to the rest of the class.

3. The induced-fit model of enzyme action suggests that, when enzyme and substrate bind, there is a conformational change in:
  - A the substrate
  - B the active site
  - C both substrate and active site
  - D neither substrate nor active site
4. An apoenzyme is:
  - A a protein with enzymic activity
  - B a non-protein with enzymic activity
  - C a non-protein with no enzymic activity
  - D a protein with no enzymic activity
5. Which of the following does not act as a cofactor to an enzyme?
  - A niacin
  - B copper ions
  - C pepsin
  - D riboflavin
6. A coenzyme is:
  - A an organic molecule that binds tightly with the apoenzyme
  - B an organic molecule that binds loosely with the apoenzyme
  - C an inorganic molecule that binds loosely with the apoenzyme
  - D an inorganic molecule that binds tightly with the apoenzyme
7. Mineral ions needed for enzyme activity:
  - A bind tightly with the apoenzyme
  - B bind loosely with the apoenzyme
  - C bind loosely with the coenzyme
  - D bind tightly with the coenzyme
8. Many coenzymes are derived from:
  - A vitamins
  - B hormones
  - C lipids
  - D proteins
9. The turnover rate of an enzyme is:
  - A the number of enzyme molecules used per second
  - B the number of product molecules formed per second
  - C the number of reactant molecules used per second
  - D all of the above
10. According to the induced-fit model of enzyme action, reacting molecules enter a transition state in which:
  - A reacting molecules assume a complementary shape
  - B apoenzyme and cofactor assume a complementary shape
  - C bonds in reacting molecules are put under tension
  - D bonds in the apoenzyme and coenzyme are put under tension

### 3.3 Factors affecting the functions of enzymes

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

- Explain factors that affect enzyme activity.
- Investigate the destruction of an enzyme by heat.
- Show how temperature, pH, substrate concentration and enzyme concentration affect enzyme activity.
- Explain allosteric regulation and the feedback control mechanism of enzyme activity.
- Appreciate the role of enzymes in controlling our metabolic activities.

The turnover rate and, therefore, the activity of the enzyme are influenced by a number of external factors, including:

- temperature
- pH
- substrate concentration
- the presence of inhibitors

#### How hot must it be?

When the temperature is raised, particles are given more kinetic energy. This has two main effects:

- ‘Free’ particles move around more quickly. This increases the probability that a substrate particle will collide with an enzyme molecule.
- Particles within a molecule vibrate more energetically. This puts strain on the bonds that hold the atoms in place. Bonds begin to break and, in the case of an enzyme, the shape of the molecule, and the active site in particular, begin to change. The enzyme begins to lose its tertiary structure (remember it is a protein) and **denature**. Figure 3.13 shows this.

#### KEY WORD

**denature** *the alteration of the tertiary structure of a protein; in living cells this is reversible*

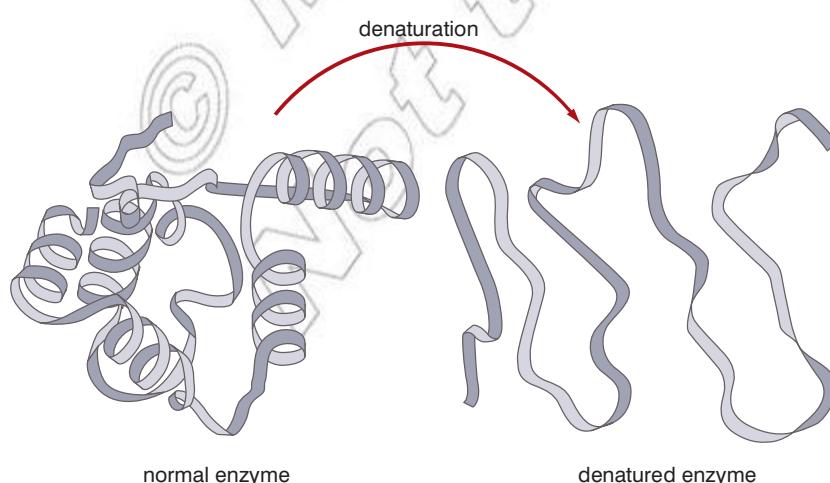
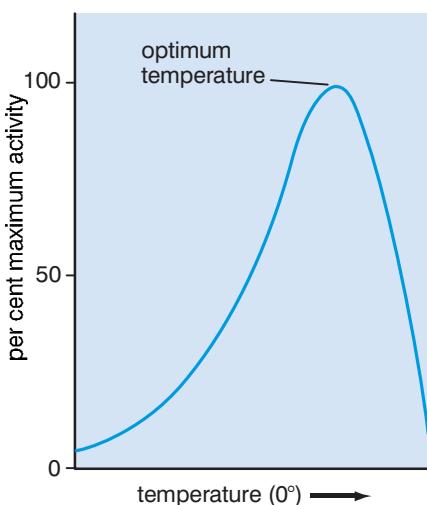


Figure 3.13 How an enzyme denatures



**Figure 3.14** The effect of temperature on enzyme activity

### DID YOU KNOW?

#### Optimum temperatures

Enzymes do not all have the same optimum temperature; they are adapted to work most efficiently within the organism in which they are found. For example, the optimum temperature for enzymes:

- in human beings is around 37 °C (normal body temperature)
- in plants growing in the Arctic may be less than 5 °C
- in bacteria that live in hot springs (thermophilic bacteria) may be over 90 °C.

The activity of an enzyme at a given temperature is a balance between these two effects. If the raised temperature results in little denaturation but a greatly increased number of collisions, the activity of the enzyme will increase. If the higher temperature causes significant denaturation then, despite the extra collisions, the activity of the enzyme will probably decrease. The temperature at which the two effects just balance each other is the **optimum temperature** for that enzyme. Any further increase in temperature will cause increased denaturation that will outweigh the effects of extra collisions. A decrease in temperature means that fewer collisions will occur. Figure 3.14 shows this.

Note that the graph is not symmetrical. Above the optimum temperature, the enzyme denatures very quickly to the point at which the shape of the active site has changed so much that an enzyme–substrate complex cannot form. At this point the reaction rate is zero.

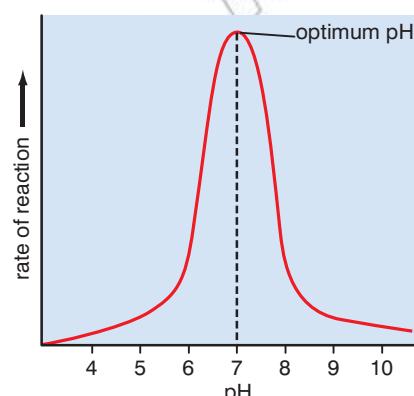
### How acidic must it be?

The **pH scale** is a measure of the hydrogen ion concentration of a solution or other liquid system. The pH scale ranges from 0 to 14. Solutions with a pH of less than 7 are acidic, those with a pH of more than 7 are alkaline and a solution with a pH of exactly 7 is neutral.

The majority of enzymes in most mammals function most efficiently within the pH range 6.0–8.0, although the optimum pH of pepsin (an enzyme found in the stomach) is between pH 1.0 and pH 3.0. Significant changes in pH can affect an enzyme molecule by:

- breaking ionic bonds that hold the tertiary structure in place; this leads to denaturation of the enzyme molecule
- altering the charge on some of the amino acids that form the active site; this makes it more difficult for substrate molecules to bind

These effects occur if the pH becomes either more acidic or more alkaline. Figure 3.15 shows this effect.



**Figure 3.15** The effect of pH on enzyme activity

### DID YOU KNOW?

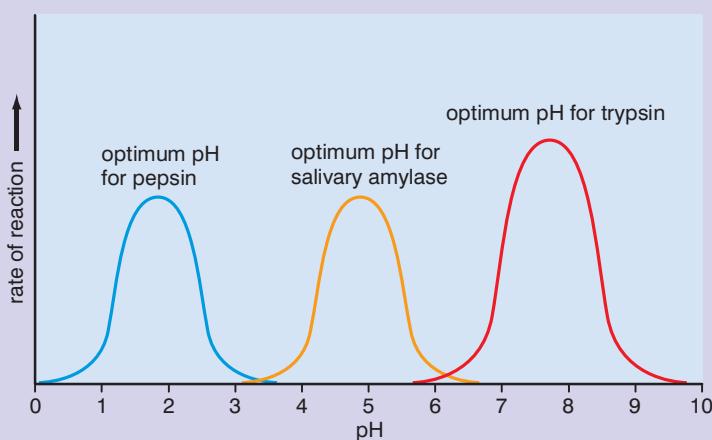
#### About pH

The pH scale of acidity/alkalinity is an inverse logarithmic scale! Each pH unit represents a tenfold change in hydrogen ion ( $H^+$ ) concentration. pH 0 represents the highest  $H^+$  concentration and is the most acid. A pH 1.0 solution has one-tenth (0.1) of this  $H^+$  concentration; a pH 4 solution has one ten-thousandth (0.0001). pH 14 represents the lowest  $H^+$  concentration and is the most alkaline. pH 7 is neutral.

## DID YOU KNOW?

### About the pH in your gut

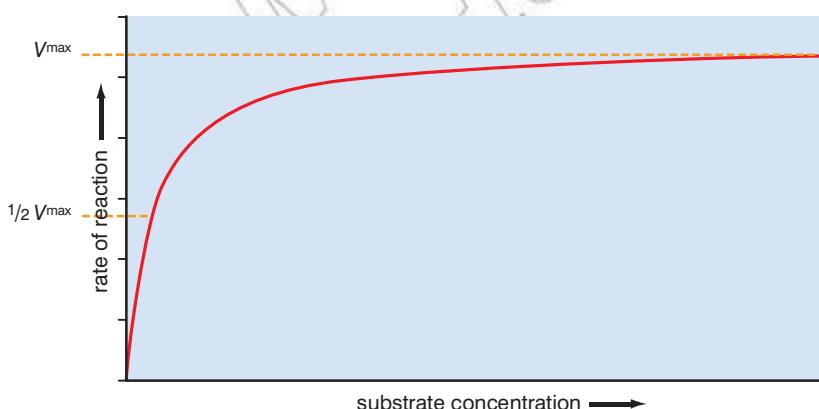
The pH of the intestinal tract of humans changes from one region to the next. The pH in the mouth varies from being slightly alkaline (pH 7.5) to quite acidic (pH 5.0) depending on whether or not we have eaten and also what we have eaten. The pH in the stomach can be as low as pH 1.5, whereas the pH of the small intestine is slightly alkaline at pH 7.5. Digestive enzymes from the different regions have optimum pHs that reflect the region in which they are secreted. Figure 3.16 shows the optimum pHs of salivary amylase (mouth), pepsin (stomach) and trypsin (small intestine).



**Figure 3.16** The optimum pHs of some human digestive enzymes

## Does the concentration of the substrate matter?

The activity of an enzyme depends on the number of substrate molecules per second that bind to form enzyme–substrate complexes. So the number of substrate molecules present must have an effect. A small number of substrate molecules means few collisions and so only a few enzyme–substrate complexes form. Increasing the concentration of the substrate means more collisions and more enzyme–substrate complexes. So, the overall rate of reaction is increased. Eventually, because of the high substrate concentration, each enzyme molecule could be working at maximum turnover – that is, each active site is binding with substrate molecules all the time and there is no ‘spare capacity’ in the system. Increasing the substrate concentration beyond this point will have no effect on the activity of the enzyme because all the active sites are occupied all the time. Figure 3.17 shows this effect.



**Figure 3.17** The effect of substrate concentration on enzyme activity.  $V_{\max}$  is the maximum rate of enzyme action.

## KEY WORDS

### optimum temperature

temperature at which an enzyme works most efficiently

### pH scale

measure of the hydrogen ion concentration of a solution

## KEY IDEA

Think about what will happen to the concentration of substrate molecules as an enzyme-controlled reaction takes place. As the reaction proceeds, more and more of the substrate molecules react, so there will be fewer remaining. The concentration of the substrate will decrease. With fewer substrate molecules left, the number of collisions per second between enzyme and substrate will also decrease, and the rate of reaction will slow down. This is because the turnover rate of each enzyme molecule decreases with time.

## How much enzyme should there be?

Assuming a constant large supply of substrate molecules, each enzyme molecule will work at maximum turnover. Therefore, the reaction rate will be directly proportional to the number of enzyme molecules – the concentration of the enzyme. Increasing the concentration will increase the reaction rate.

However, increasing the concentration of the enzyme will not increase the activity of the enzyme. Each enzyme molecule will be working at maximum turnover, so the activity of the enzyme is likely to remain constant.

### Activity 3.6: How can we measure the rate of an enzyme-controlled reaction?

We can do this in one of two ways. We can:

- measure the rate at which the substrate is used up, or
- measure the rate at which the product is formed.

Usually, it is more convenient to do the latter – measure the rate at which product is formed.

The enzyme catalase is commonly used in these sorts of investigations. This is because it is found in almost all cells and there are many readily available sources that contain significant amounts of catalase. These include:

- yeast
- liver
- potato

Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen. The equation for the reaction is:



Because oxygen is a gas, the volume of oxygen collected in a certain time is a measure of how fast the reaction is proceeding. There are several ways of carrying out the investigation. One of these is shown in figure 3.18. This investigation uses potato, but it could just as easily be carried out with yeast or pieces of liver.

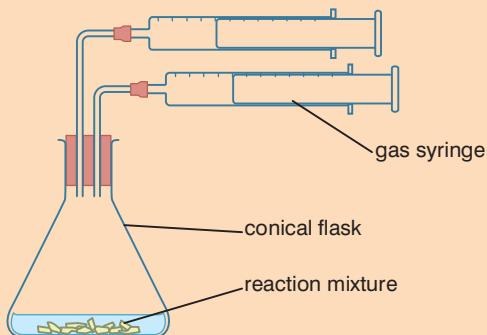


Figure 3.18 Apparatus set-up

#### You will also need:

- a potato
- access to a balance
- 10 volume hydrogen peroxide solution (safety note: this is an oxidising agent, take care)
- a scalpel and a tile on which to cut the potato
- a stopwatch

#### The experiment is carried out as follows:

1. Peel a potato and chop into small pieces (less than 1 cm square).
2. Weigh out 10 g of the potato and place it in the conical flask.
3. Attach the gas syringe (you will need to support it with a clamp and stand).
4. Make sure that the gas syringe:
  - is horizontal
  - reads zero

- Draw 20 cm<sup>3</sup> hydrogen peroxide into the second syringe and attach it to the conical flask.
- Check again that all seals are tight, the gas syringe reads zero and is horizontal.
- Add all the hydrogen peroxide solution to the potato quickly and start the stopwatch.
- Record the volume of gas collected in the gas syringe every minute for ten minutes.

Now that you have a set of results, you can plot them as a graph. You may well end up with a graph that looks like the one below:

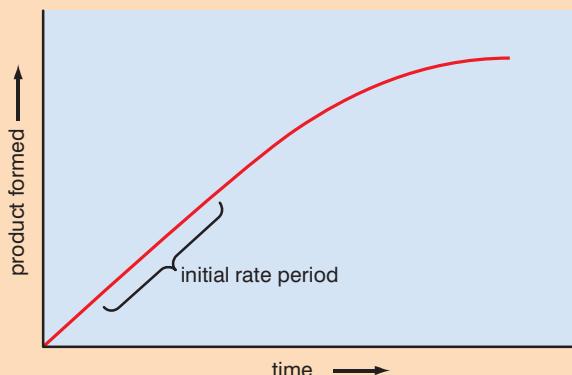


Figure 3.19 A graph of the results.

Notice that the line is starting to level off (yours may have completely levelled off). This is because as the reaction proceeds, substrate is used up, fewer enzyme substrate complexes form and the reaction rate slows down. Not as much oxygen is formed per minute as a result.

This procedure is a very basic one and there are a number of reasons why the results obtained might not be reliable. These include:

- We did not control the temperature; it might have increased or decreased during the investigation, speeding up the reaction or slowing it down.
- We did not control the pH of the reaction mixture; it too might have increased or decreased during the investigation, speeding up the reaction or slowing it down.
- We only carried out the investigation once; we may have obtained an anomalous (freak) result.

However, we did control:

- The concentration of the substrate (we used 10 volume hydrogen peroxide).
- The concentration of the enzyme (we used a specific mass of potato).

We can improve our investigation fairly easily, as shown in table 3.7.

Table 3.7 Improving the investigation

Factor controlled	How controlled	Note
Temperature	Use of water bath at the required temperature	Stand the potato pieces in the conical flask and hydrogen peroxide in the water bath separately for 10 minutes. This is called equilibration.
pH	Use of buffer solutions at required pH	Buffer solutions resist changes in pH and maintain a more or less constant pH. Add the buffer solution to the potato pieces at the start.
Repeats	Carry out the experiment three or five times	Carrying out the experiment more than once allows us to spot anomalous results and eliminate them. This is easier if you have an odd number of results.

We can use this basic experiment, with the improvements, to investigate how the different factors affect the rate of enzyme action. Before we do, however, we must be quite clear about what we are trying to find out.

**Reminder from Unit 1**

- The factor that you change is the independent variable (IV).
- The factor that you record as the results is the dependent variable (DV).

**KEY IDEA**

*The 'rate of enzyme action', like any rate, means 'how much per unit of time'. We cannot just say 12 cm<sup>3</sup> oxygen. We must convert this to volume per minute, or volume per second. Then we have a rate.*

*It is also best if we can compare the rates of enzyme action when they are working to maximum or near maximum capacity for the conditions. So, before we proceed to the main investigations, we should:*

- carry out our improved basic experiment three times
- plot the graphs of our results
- determine the point on each where the graph starts to level
- take an average of these times

*This is the time we will use for our main investigations.*

*You are now in a position to use this procedure to design your own investigations into:*

- the effect of temperature on enzyme activity
- the effect of pH on enzyme activity
- the effect of substrate concentration on enzyme activity

*When you are investigating one factor, then all the others need to be controlled – kept constant – so that they cannot influence the results. If you were investigating the effect of temperature then pH, substrate concentration and enzyme concentration would need to be controlled, as would the duration of the experiment.*

For each of your investigations, you should think about each of the following:

- How will I change the independent variable?
- How will I measure the dependent variable?
- What other factors need to be controlled?
- How will I control them?
- How many different values of the IV shall I use? Usually five is the minimum requirement.
- What values shall I use? These need to be reasonably spaced, for example, temperatures of 20 °C, 30 °C, 40 °C, 50 °C, 60 °C are better than temperatures of 20 °C, 22 °C, 30 °C, 52 °C, 60 °C. Can you see why?
- How many times shall I repeat each condition? Usually three times is the minimum requirement.
- How will I record my results? You should have a table prepared before you commence the investigation.

If, for some reason, you were unable to carry out the investigation, here are some results you could analyse.

*Substrate concentration*

Concentration of hydrogen peroxide/volume	Reaction rate/cm <sup>3</sup> s <sup>-1</sup>			
	Trial 1	Trial 2	Trial 3	Mean
0	0	0	0	
5	0.7	0.7	0.4	
10	1.6	1.9	1.6	
15	2.5	3.1	2.8	
20	2.9	3.0	3.7	

You can copy the table, calculate the mean and plot a graph of the mean reaction rate against the concentration of hydrogen peroxide.

*Temperature*

These results come from a class who varied the procedure slightly. They timed how long it took to produce 30 cm<sup>3</sup> oxygen at different temperatures. So before you can plot your graph of reaction rate, you must first:

- calculate the mean result for each temperature, and
- convert this to a volume per second (or per minute).

Temperature/°C	Time taken to collect 30 cm <sup>3</sup> oxygen in seconds			
	Trial 1	Trial 2	Trial 3	Mean
10	54	47	43	
20	12	14	16	
30	5	5	5	
35	9	5	4	
40	9	6	9	
45	14	11	11	
50	73	71	57	
55	119	109	132	

Once you have calculated the mean rates for each temperature, plot a graph of reaction rate against temperature.

**How do other substances affect enzyme activity?**

**Inhibitors** are substances that bind to enzymes and prevent them from forming enzyme–substrate complexes and, as a result, stop, or slow down, the reaction. There are two main types of inhibitors:

- irreversible inhibitors, and
- reversible inhibitors.

Irreversible inhibitors bind strongly to enzymes, usually by a covalent bond, permanently altering the structure of the enzyme

**Activity 3.8**

Plan an investigation into the effect of pH on the rate of an enzyme controlled reaction such as the breakdown of starch by the enzyme amylase in saliva or the breakdown of hydrogen peroxide by the enzyme catalase in potato or liver. Explain how you would vary the pH of the reacting mixture and suggest what results you would expect to see.

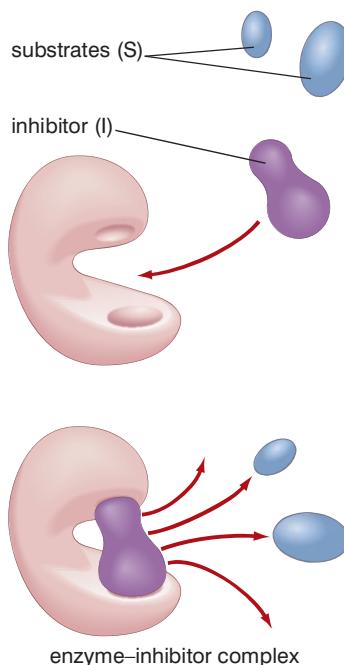


Figure 3.20 Competitive inhibition

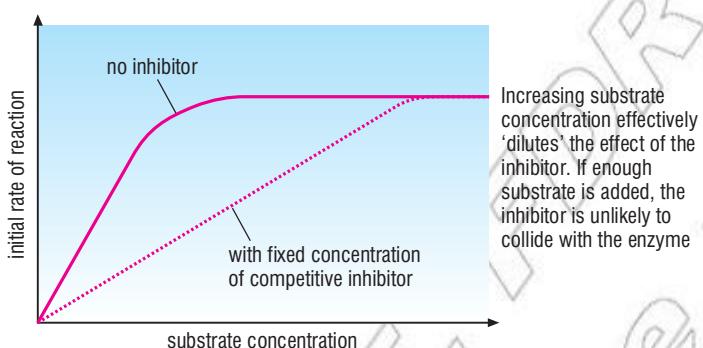


Figure 3.21 Effect of substrate concentration on inhibition by a competitive inhibitor

molecule and inactivating it. The painkiller aspirin is an example of an irreversible inhibitor. It binds with the enzyme cyclo-oxidase-2, which is an important enzyme in producing prostaglandins which give the sensation of pain.

Reversible inhibitors bind to enzymes only weakly and the bond that holds them breaks easily releasing the inhibitor. This allows the enzyme to become active again. There are two main kinds of reversible inhibitors:

- **competitive inhibitors**, and
- **non-competitive inhibitors**.

### Competitive inhibitors

Competitive inhibitors have molecules with shapes that are complementary to all, or part, of the active site of an enzyme. They are often similar in shape to the substrate molecules. They can bind with the active site and prevent substrate molecules from binding. The binding is only temporary and the competitive inhibitor is quickly released. A competitive inhibitor blocks the active site so substrate molecules cannot bind.

The overall effect on the rate of reaction depends on the relative concentrations of substrate and inhibitor molecules. Each molecule of competitive inhibitor can inhibit (temporarily) one enzyme molecule – but only if it can collide with the enzyme molecule and bind with the active site. To do this, it must compete with the substrate molecules for the active site – hence the name, competitive inhibitor. If there were 99 substrate molecules for every inhibitor molecule, then 99% of the collisions would be between enzyme and substrate and the reaction would proceed at 99% of the maximum rate. If the ratio were 90 substrate molecules to ten inhibitor molecules, there would be 10% inhibition and the reaction rate would fall to 90% of maximum.

The painkiller ibuprofen acts as a competitive inhibitor of the enzyme cyclo-oxidase-2, competing with the precursors of prostaglandins, which are the substrate of cyclo-oxidase-2. The metabolic poison cyanide acts as a competitive inhibitor of the enzyme cytochrome oxidase, an important enzyme in the release of energy in respiration.

### Non-competitive inhibitors

Non-competitive inhibitors do not compete for the active site. Instead, they bind to another part of the enzyme called the allosteric site. This produces a conformational change in the part of the enzyme molecule that includes the active site. Because of this, the active site is a different shape and can no longer bind with the substrate to catalyse the reaction.

### KEY WORDS

**competitive inhibitor** a molecule that inhibits enzyme activity by competing with the substrate for the active site

**non-competitive inhibitor** a molecule that alters the conformation of the active site by binding with the allosteric site of the enzyme; it prevents the substrate from binding and inhibits enzyme activity

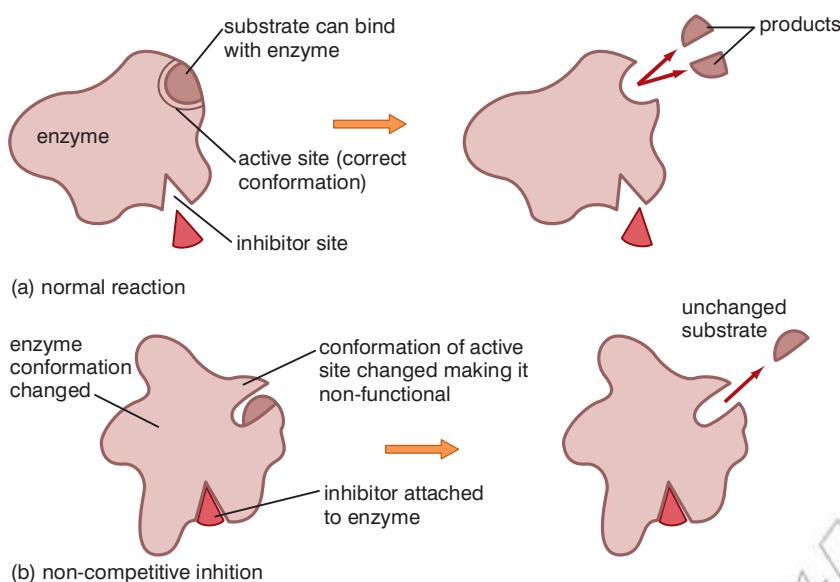


Figure 3.22 Non-competitive inhibition

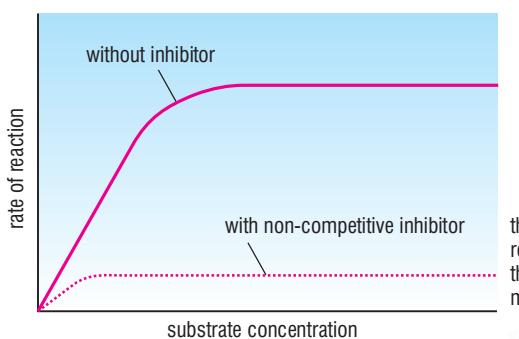


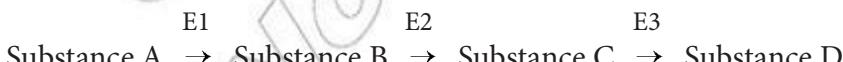
Figure 3.23 The effect of substrate concentration on a non-competitive inhibitor

The effectiveness of a non-competitive inhibitor is in no way affected by the concentration of the substrate. Suppose there are enough inhibitor molecules to bind with the allosteric sites of 80% of the enzyme molecules. 80% of the enzyme molecules will be inhibited irrespective of the number of substrate molecules (as the two are not competing for the same site) and the reaction rate will drop to 20% of maximum.

Non-competitive inhibitors are particularly important in regulating metabolic pathways in cells.

## How do inhibitors control enzyme activity in living cells?

Many substances are produced in cells as a result of a metabolic pathway (a series of reactions), which can be represented as:



E1, E2 and E3 are enzymes catalysing the reactions.

All the reactions in this sequence are enzyme controlled. Therefore, inhibition of any of these enzymes will interrupt the process. However, the main function of this pathway is to produce substance D for use by the cell. If the requirement for substance D in the cell decreases, then the concentration of D will increase. This is at

### KEY WORDS

**end-product inhibition** when an end product inhibits the enzyme controlling the first stage of a reaction sequence  
**activator** a substance that removes an inhibitor

least inefficient (producing something that is not being used) and may be potentially harmful because high concentrations could be toxic. Such reaction sequences are often controlled by **end-product inhibition**. The end product (D) inhibits the enzyme controlling the first stage of the reaction sequence, as shown in the diagram.

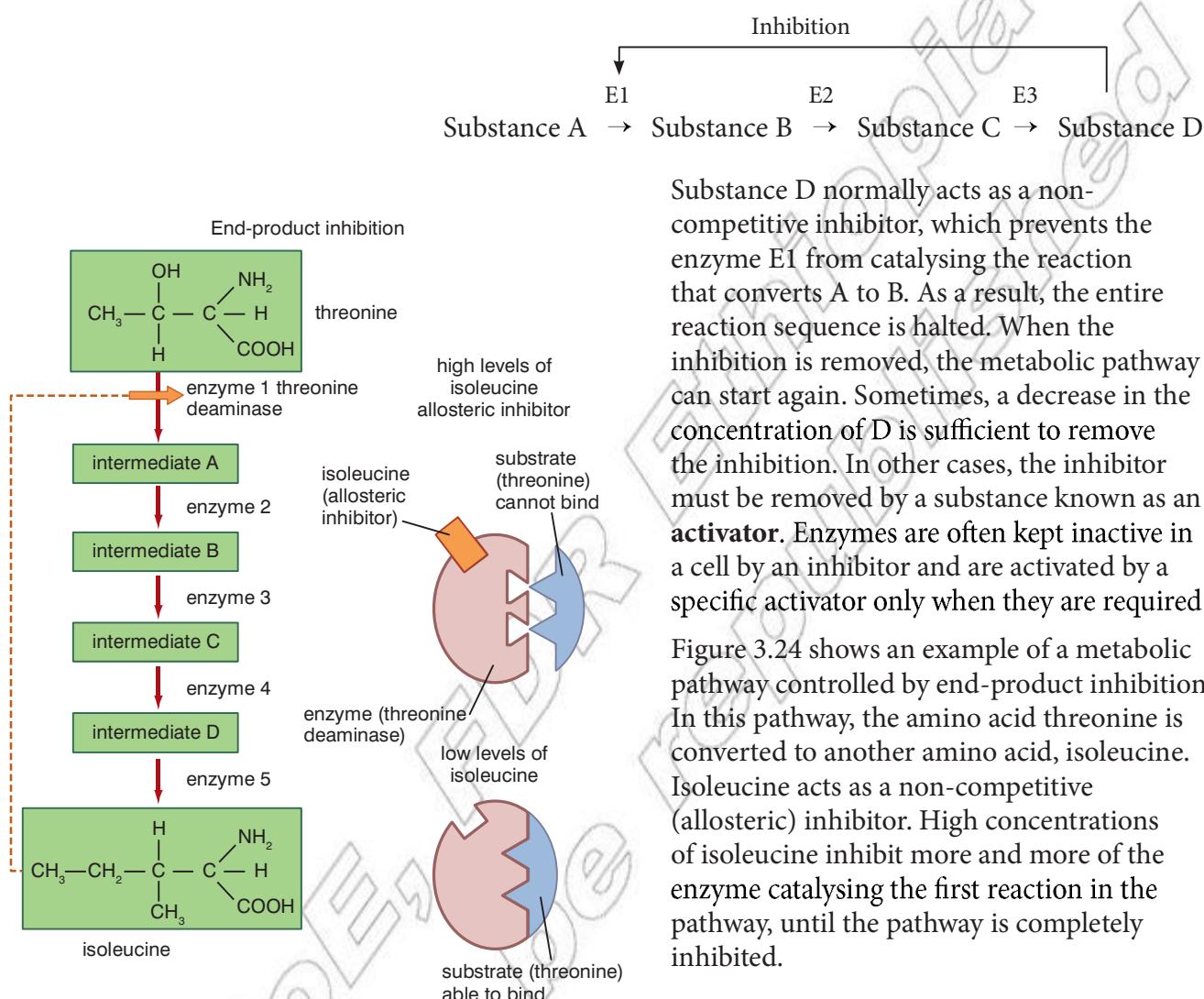


Figure 3.24 A metabolic pathway controlled by end-product inhibition

Substance D normally acts as a non-competitive inhibitor, which prevents the enzyme E1 from catalysing the reaction that converts A to B. As a result, the entire reaction sequence is halted. When the inhibition is removed, the metabolic pathway can start again. Sometimes, a decrease in the concentration of D is sufficient to remove the inhibition. In other cases, the inhibitor must be removed by a substance known as an **activator**. Enzymes are often kept inactive in a cell by an inhibitor and are activated by a specific activator only when they are required.

Figure 3.24 shows an example of a metabolic pathway controlled by end-product inhibition. In this pathway, the amino acid threonine is converted to another amino acid, isoleucine. Isoleucine acts as a non-competitive (allosteric) inhibitor. High concentrations of isoleucine inhibit more and more of the enzyme catalysing the first reaction in the pathway, until the pathway is completely inhibited.

### Review questions

Choose the correct answer from A to D.

- When an enzyme is subjected to excess heat:
  - bonds in the active site are strained
  - some of the bonds in the active site break
  - the active site undergoes a conformational change
  - all of the above

2. Extreme pHs can inactivate enzymes because they:
  - A alter the charge on the amino acids in the allosteric site
  - B alter the charge on the amino acids in the active site
  - C alter the charge on amino acids away from the active site and allosteric site
  - D all of the above
3. The optimum temperature of an enzyme is the temperature at which:
  - A there is no denaturation
  - B the maximum number of enzyme–substrate complexes are formed
  - C there is the maximum number of collisions between enzyme and substrate
  - D the particles have the most kinetic energy
4. A non-competitive enzyme inhibitor...
  - A does not compete for the active site
  - B binds with the allosteric site
  - C binds with the active site of the enzyme
  - D is not affected by the substrate concentration
5. If the ratio of non-competitive inhibitor molecules to substrate molecules is 3:7, the enzyme controlling the reaction will be:
  - A 70% inhibited
  - B 30% activated
  - C 30% inhibited
  - D three-sevenths inhibited
6. When investigating the effect of temperature on enzyme activity, we should control:
  - A pH
  - B substrate concentration
  - C enzyme concentration
  - D all of these
7. End-product inhibition of a metabolic pathway occurs when:
  - A the last product of the pathway inhibits the enzyme controlling the first reaction
  - B the last product of the pathway inhibits the enzyme controlling the last reaction
  - C the first product of the pathway inhibits the enzyme controlling the last reaction
  - D the last product of the pathway inhibits the enzyme controlling the first reaction

### Activity 3.9

Plan an investigation into the effect of changing the substrate concentration on the rate of an enzyme controlled reaction such as the breakdown of starch by the enzyme amylase in saliva or the breakdown of hydrogen peroxide by the enzyme catalase in potato or liver. Explain how you would vary the concentration of the substrate and suggest what results you would expect to see.

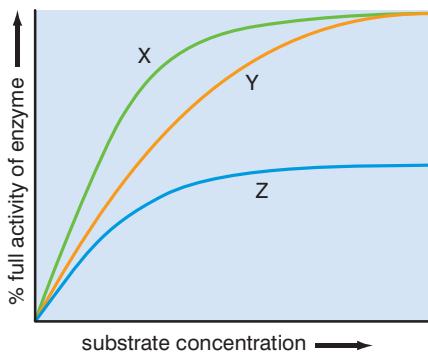


Figure 3.25

8. Figure 3.25 shows the activity of an enzyme at different substrate concentrations under three conditions:
- no inhibitor present
  - a competitive inhibitor present
  - a non-competitive inhibitor present
- Which of the following represents the correct interpretation of the graph:
- A X is with the competitive inhibitor, Y with the non-competitive inhibitor and Z with no inhibitor
  - B X is with the non-competitive inhibitor, Y with the competitive inhibitor and Z with no inhibitor
  - C X is with no inhibitor, Y with the non-competitive inhibitor and Z with the competitive inhibitor
  - D X is with no inhibitor, Y with the competitive inhibitor and Z with the non-competitive inhibitor
9. As temperature increases up to the optimum, the rate of an enzyme-controlled reaction increases because:
- A the particles have more kinetic energy
  - B there are more collisions between enzyme and substrate
  - C there are more enzyme–substrate complexes formed
  - D all of these
10. If substrate concentration is kept permanently high and the enzyme concentration is gradually increased, the rate of activity of the enzyme will:
- A increase
  - B increase and then decrease
  - C increase and then level off
  - D stay the same

## Summary

In this unit you have learnt that:

### Catalysts

- A catalyst speeds up a chemical reaction with no effect on:
  - the products formed
  - the energy change
  - the nature of the catalyst itself
- A catalyst speeds up a reaction by lowering the activation energy required for reactants to enter the transition state.
- Nearly all biological catalysts are enzymes. They are globular proteins with a specific tertiary shape, part of which forms an active site.

- A substrate molecule binds with the active site to form an enzyme–substrate complex. This then forms the products. The products are released from the enzyme molecule, which is unaltered.

### Models of enzyme action

- The lock-and-key model of enzyme action suggests a rigid structure for the enzyme molecule, with the shape of the substrate and active site being complementary to each other. This model explains enzyme specificity but not how the transition state is achieved.
- The induced-fit model of enzyme action suggests that binding of the substrate induces a conformational change in enzyme structure, which puts the substrate molecule under tension, causing it to enter the transition state.
- The number of substrate molecules that bind to the active site of an enzyme molecule per second is the turnover rate.

### Factors affecting enzyme activity

- Temperature – below the optimum temperature, the low level of kinetic energy limits the number of enzyme–substrate complexes formed; above the optimum temperature, denaturation of the enzyme prevents binding of the substrate.
- pH – above and below the optimum pH, changes occur in the tertiary structure of the enzyme molecule and in the charges on the amino acids making up the active site; both prevent binding of the substrate.
- Substrate concentration – if the concentration of enzyme remains constant, increasing the substrate concentration increases the number of enzyme–substrate complexes formed until, at any one time, all the active sites are occupied; the rate of reaction increases to its maximum.
- Enzyme concentration – if the substrate concentration is high and constant, increasing the enzyme concentration increases the rate of reaction.
- Inhibitors:
  - competitive inhibitors have molecules that are often similar in shape to the substrate molecules and that compete for the active site; the extent of the inhibition depends on the ratio of substrate molecules to inhibitor molecules
  - non-competitive (allosteric) inhibitors bind to a region away from the active site, producing a conformational change in the enzyme that prevents the substrate from binding; the extent of the inhibition is independent of the substrate concentration
  - allosteric inhibition can control metabolic pathways; the final product of a series of reactions inhibits the enzyme controlling the first reaction in the series; this is also known as end-product inhibition.

### Activity 3.10

You know that a high temperature can denature an enzyme but it can be difficult to imagine how this happens. However you can demonstrate the effect very easily. Take an egg and separate the yolk from the white. Then divide the white between two test tubes. Egg white is pure protein and the coiled protein molecules are similar to the protein molecules which form enzymes.

Keep one tube at room temperature. Place the other in a beaker of boiling water and leave it for several minutes. Observe what happens and explain what you see in terms of changes to the coiled protein molecules in the raw egg white.

## End of unit questions

1. a) What is a cofactor?  
 b) The table shows the various groups that can combine to form a holoenzyme. Copy and complete the table by placing a tick (✓) or a cross (✗) in each box.

Type of group	Organic	Protein	Binds tightly
Apoenzyme			
Coenzyme			
Ion			

2. When conducting investigations into the activity of enzymes, a number of factors need to be controlled. Copy and complete the table to describe the reasons for controlling these factors.

Factor controlled	How controlled	Reason for controlling factor
Temperature		
pH		Changes in pH can alter charge on amino acids in the active site.
Substrate concentration	Equal strength solutions	

3. Figure 3.26 shows the activity of two enzymes at different temperatures.

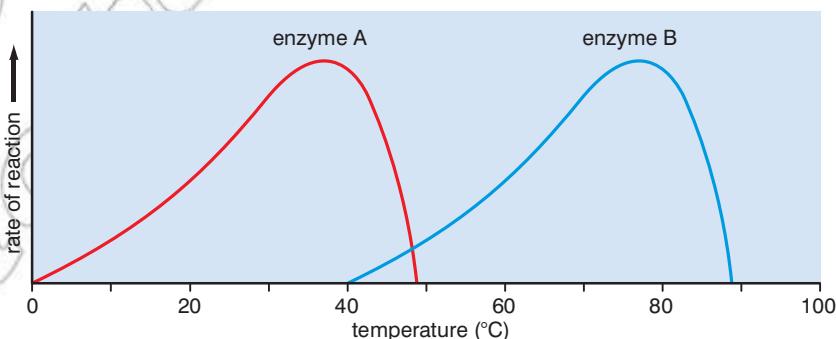


Figure 3.26

- a) What is the optimum temperature of each enzyme? Give reasons for your answers.  
 b) Which enzyme may have come from a thermophilic bacterium? Give the reasons for your answer.  
 c) Describe and explain the shape of the curve from 20 °C to 35 °C for enzyme A. Explain your answer.

4. Figure 3.27 shows the rate of reaction of an enzyme at 25 °C at different substrate concentrations.

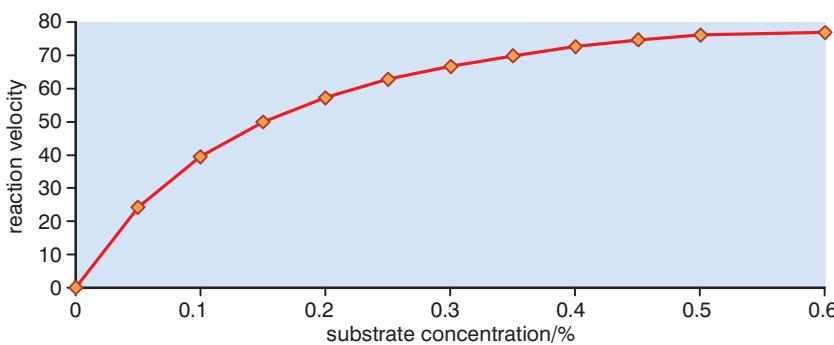


Figure 3.27

- a) Describe and explain the shape of the graph in terms of kinetic theory and enzyme–substrate complex formation:
- from substrate concentration 0.05% to 0.4%
  - from substrate concentration 0.4% to 0.6%
- b) Copy the graph and sketch, on your copy, the curve you would expect if the experiment had been carried out at 35 °C rather than 25 °C.
5. Figure 3.28 shows an energy level diagram of a reaction proceeding without an enzyme and the same reaction with an enzyme.
- Describe *two* ways in which the energetics of the reactions are similar.
  - Describe and explain the differences between the regions marked X and Y on the diagram.
  - Explain why enzymes speed up biological reactions.
6. Enzymes are increasingly being used in industrial processes.
- Give three examples of industrial processes that use enzymes.
  - Give two reasons why enzymes are being increasingly used in industrial processes.
  - Explain one way in which the increased use of enzymes may benefit the environment.
7. Students investigated the effect of temperature on the rate of activity of the enzyme catalase. They timed how long it took for potato tissue to produce 50 cm<sup>3</sup> oxygen at different temperatures. Figure 3.29 shows the graph that one student drew after averaging all the students' results.
- (i) According to this graph, what is the optimum temperature of catalase?
  - (ii) Explain why this might not be an accurate estimate of the optimum temperature.

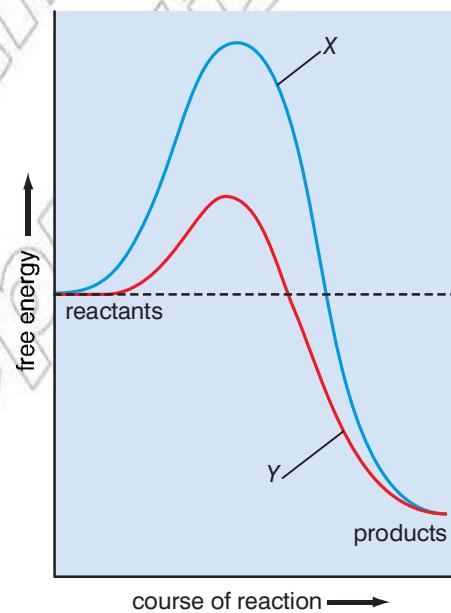


Figure 3.28

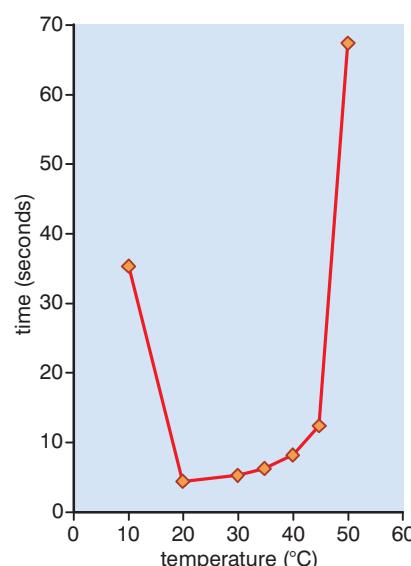


Figure 3.29

- b) In a control experiment (no enzyme present but all other factors the same as in the other experiments) carried out at 20 °C, 0.5 cm<sup>3</sup> of oxygen was collected. Assuming no experimental error, explain why this small amount of oxygen was produced.

c) (i) Explain the difference in the volumes of oxygen collected at 10 °C and at 20 °C.

(ii) Explain the difference in the volumes of oxygen collected at 35 °C and at 50 °C.

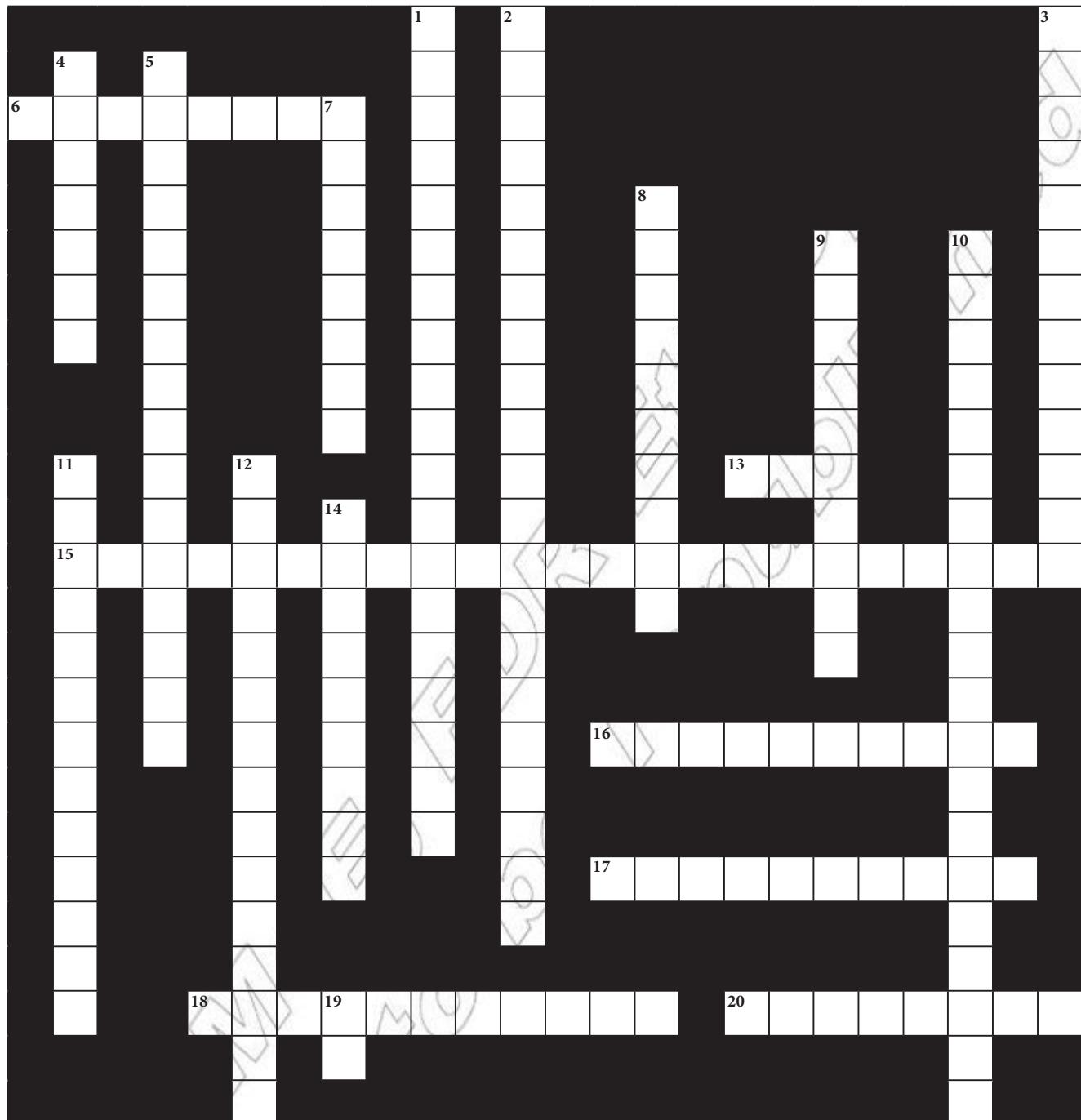
8. a) Explain what is meant by non-competitive (allosteric) inhibition.

b) A metabolic pathway consists of a series of reactions controlled by enzymes, as shown below.

E1      E2      E3  
Substance A → Substance B → Substance C → Substance D

- (i) Use this example to explain what is meant by end-product inhibition.
  - (ii) Explain how end-product inhibition can control enzyme activity in living cells.

Copy the crossword puzzle below into your exercise book (or your teacher may give you a photocopy) and solve the numbered clues to complete it.



**Across**

6. Means that enzymes only catalyse one reaction (8)
13. The names of most enzymes ends in these three letters (3)
15. This slows a reaction by binding to the allosteric site of an enzyme (3-11, 9)
16. A model of enzyme action in which the active site changes shape as the substrate binds (7, 3)
17. Literally means 'water-splitting' (10)

18. If this is too high, enzymes are denatured (11)
20. A substance (sometimes a vitamin) necessary for the functioning of an enzyme (8)

**Down**

1. Enzymes are sometimes described as these (10, 9)
2. This is formed when an enzyme and its substrate bind (6-8, 7)
3. This cleaning substance for clothes often contains enzymes (7, 6)
4. The temperature at which an enzyme is most active is called this (7)
5. The energy needed before a reaction will proceed (10, 6)
7. A substance that speeds up a chemical reaction, but remains unchanged itself (8)
8. A model of enzyme action in which enzyme and substrate fit together like an egg and egg cup (4, 3, 3)
9. The part of an enzyme that binds with its substrate (6, 4)
10. This slows a reaction by competing with the substrate for the active site of an enzyme (11, 9)
11. The amount of substrate per 100 cm<sup>3</sup> is its ... (13)
12. All enzymes are this type of molecule (8, 7)
14. The main part of an enzyme that consists of two molecules (9)
19. This can influence how active enzymes are (2)

### Contents

Section	Learning competencies
4.1 Cell theory (page 112)	<ul style="list-style-type: none"> <li>• Tell the history of cell biology.</li> <li>• Describe cell theory and investigate the size, structure and shape of cells.</li> <li>• State the basic functions of cells.</li> <li>• Appreciate that all life on Earth originates from cells.</li> </ul>
4.2 Types of cells (page 121)	<ul style="list-style-type: none"> <li>• Appreciate that there are just two basic types of cells: prokaryotic and eukaryotic cells.</li> <li>• Give examples and describe the basic structure of each type.</li> <li>• Explain the difference between prokaryotic and eukaryotic cells.</li> </ul>
4.3 Parts of the cell and their functions (page 125)	<ul style="list-style-type: none"> <li>• Discuss the importance of a cell membrane.</li> <li>• Describe the composition and arrangement of lipids and proteins in the membrane.</li> <li>• Compare the Davson–Danielli and fluid mosaic models.</li> <li>• Construct and show the arrangement of the phospholipids and proteins in the fluid mosaic model.</li> <li>• Explain the role of glycoprotein and other components in the cell membrane.</li> <li>• Name the different parts of the cell and explain their functions.</li> <li>• State and explain the mechanisms of substance transport across a cell membrane.</li> <li>• Conduct an experiment to show movement of solvent through a semi-permeable membrane.</li> <li>• Demonstrate osmosis at a semi-permeable membrane.</li> <li>• Explain that the size of a cell changes by osmosis because of the inflow and outflow of water.</li> <li>• Appreciate that osmosis is responsible for everyday life phenomena.</li> </ul>

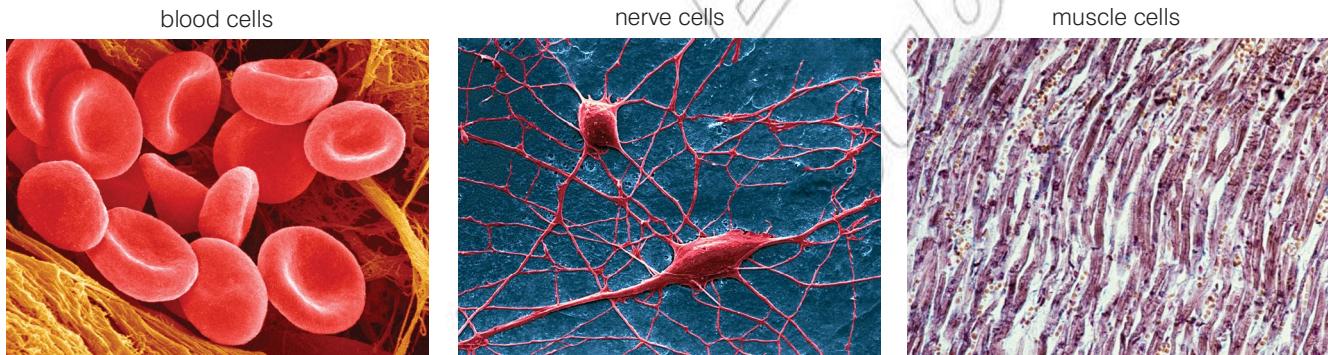
## 4.1 Cell theory

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

- Tell the history of cell biology.
- Describe cell theory and investigate the size, structure and shape of cells.
- State the basic functions of cells.
- Appreciate that all life on Earth originates from cells.

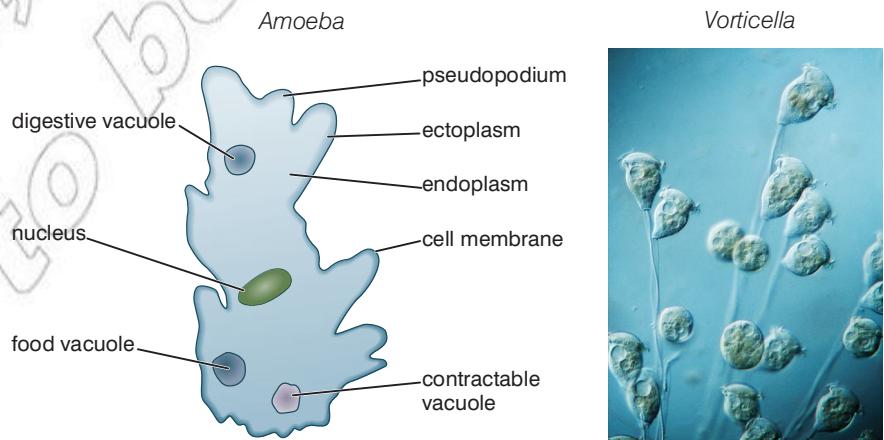
### How did the modern cell theory develop?

Today we take it for granted that living things are made of cells. Billions of cells in the case of human beings, just one cell in the case of organisms like amoeba. Figure 4.1 shows some of the different types of cells that make up our bodies.



**Figure 4.1** Some of the different cells in our bodies

There is only one cell in *Amoeba* and in *Vorticella*.

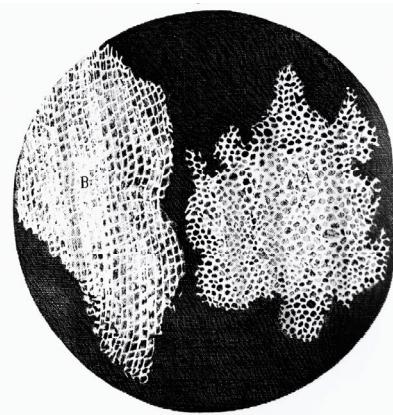


**Figure 4.2** The unicellular organisms Amoeba and Vorticella

It seems strange that the idea of organisms being made from cells is a relatively recent idea. Only a few hundred years ago, cells had not been discovered. Their discovery had to wait for the development of reliable microscopes that could magnify sufficiently to show the cellular structure of living organisms. Many biologists and other scientists contributed to the discovery of cells and the statement of the very first cell theory. The timeline below shows some of the major contributors.

## A timeline for the development of the cell theory

**1665** Robert Hooke, with one of the earliest compound microscopes, makes drawings of cork and sees tiny structures that he calls 'cells'. However, although his microscope is a compound microscope, the lenses are not very good and magnifications of more than 30 $\times$  are very blurred and do not show much detail. Also, Hooke saw only dead cells.



**Figure 4.3** Robert Hooke's drawing of cells in cork

**Figure 4.4** Robert Hooke's microscope

**1674** Anton van Leeuwenhoek sees living, moving unicellular organisms (protoctistans) in a drop of water. He is using a simple microscope with only one lens. It is really little more than a magnifying glass with a mount for the specimens.

However, van Leeuwenhoek is very skilled at grinding lenses and so his microscope can achieve magnifications of 300 $\times$ . He calls the moving organisms 'animalcules'. He also sees bacteria (from his teeth), which he also calls 'tiny animalcules'.



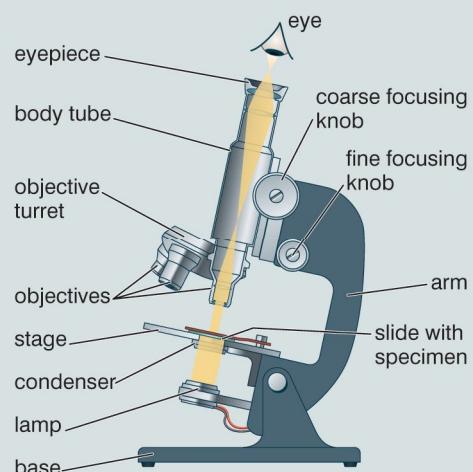
**Figure 4.5** Anton van Leeuwenhoek's microscope



**Figure 4.6** Anton van Leeuwenhoek

### About compound microscopes

A compound microscope is the sort of microscope we use in biology today. It has two lenses – the eyepiece and the objective lens (check back to unit 1 for more details) – that combine to produce the final image. Because two lenses are used, compound microscopes are capable of higher magnifications than simple microscopes, which use only one lens. The second lens (the eyepiece) magnifies the already magnified image produced by the objective lens. However, it also magnifies any 'aberrations' or faults in the image. So if the lenses are not well made, the final image, at high magnifications, will be blurred. The first compound microscope was made in 1595 by the Dutch scientist, Zaccharias Jansen.



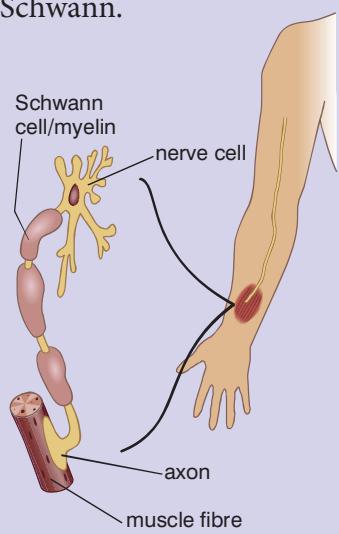
**Figure 4.7** A modern compound microscope

Check back to unit 1 to look at the work of Francesco Redi and Louis Pasteur in disproving spontaneous generation.

### DID YOU KNOW?

#### About Schwann cells

Schwann cells are special cells that contain a lot of a fatty substance called myelin. They wrap themselves around the axons of nerve cells as the nerve cells are growing and insulate the axons. They are named in honour of Theodor Schwann.



**Figure 4.8** Schwann cells around the axon of a nerve cell

**1824** The French biologist Rene Dutrochet concludes that all organisms are composed of cells. This follows many years work in which he also discovers:

- the stomata in the epidermis of leaves
- the process of osmosis
- chlorophyll is needed for photosynthesis to occur
- respiration occurs in both animals and plants

In many ways, Dutrochet is the man who first states the cell theory by recognising that all organisms are made of cells and that 'all growth occurs because of the increase in volume of cells or by the addition of more little cells'.

**1839** Matthias Schleiden and Theodor Schwann put forward the first clearly stated cell theory. It states that:

- the cell is the unit of structure, physiology and organisation in living things
- the cell retains a dual existence as:
  - a distinct entity, and
  - a 'building block' in the formation of organisms
- cells form by free-cell formation (spontaneous generation)

Although we still accept the first two ideas, the final idea of spontaneous generation has now been proved false.

**1858** Rudolf Virchow, a German doctor who develops many surgical techniques and promotes several fields of modern medicine, declares that: '*Omnis cellula e cellula*', which means that a cell can only arise from another cell like it. With this he completes the first accepted version of the cell theory:

- all organisms are made up of one or more cells
- all cells come from pre-existing cells
- the cell is the unit of structure, physiology and organisation in living things
- the cell retains a dual existence as a distinct entity and a building block in the construction of organisms

Today, this has been modified and extended in the light of our increased knowledge of genetics and cell biology and now reads:

- all known living things are made up of cells
- the cell is a structural and functional unit of all living things

- all cells come from pre-existing cells by division (there is no spontaneous generation of cells)
- cells contain hereditary information which is passed from cell to cell during cell division
- all cells have basically the same chemical composition
- all energy flow (the metabolism and biochemistry of life) occurs within cells

Besides these major steps in the development of a cell theory, there have been other developments in the study of cell biology. Some of these are listed below.

### Key events in the study of cell biology

**Table 4.1** Key events in cell biology

	Event
1595	Jansen builds the first compound microscope.
1626	Redi postulates that living things do not arise from spontaneous generation.
1665	Hooke describes 'cells' in cork.
1674	Leeuwenhoek discovers protozoa.
1833	Brown describes the cell nucleus in cells of an orchid.
1839	Schleiden and Schwann propose a cell theory.
1857	Kolliker describes mitochondria.
1858	Virchow states <i>omnis cellula e cellula</i> .
1869	Miescher isolates DNA.
1879	Fleming describes chromosome behaviour during mitosis.
1898	Golgi describes the Golgi apparatus in cells.
1939	The first transmission electron microscope.
1953	Watson and Crick propose the double-helix structure of DNA.
1965	The first scanning electron microscope.
2000	Human genome DNA sequence draft.

### Activity 4.1

Work in groups for this activity. Each group is going to make a presentation to the class about one of the following scientists who all made a major contribution to our modern understanding of cells: Robert Hooke, Anton van Leeuwenhoek, Rene Dutrochet, Matthias Schleiden, Theodor Schwann and Rudolf Virchow. Use this textbook as a resource and use other books and the internet for your research if it is available. Make your presentations as interesting and lively as possible.

### How big are cells?

It all depends on what kind of cell you are talking about. The contents of a chicken's egg are just one huge cell packed with food – it's a pretty big cell, up to 5 cm (0.05 m) in length. On the other hand, the smallest bacterial cells are only just over 100 nm in length. This is approximately one hundred-thousandth of the size of the chicken's egg. That's quite a range of sizes!

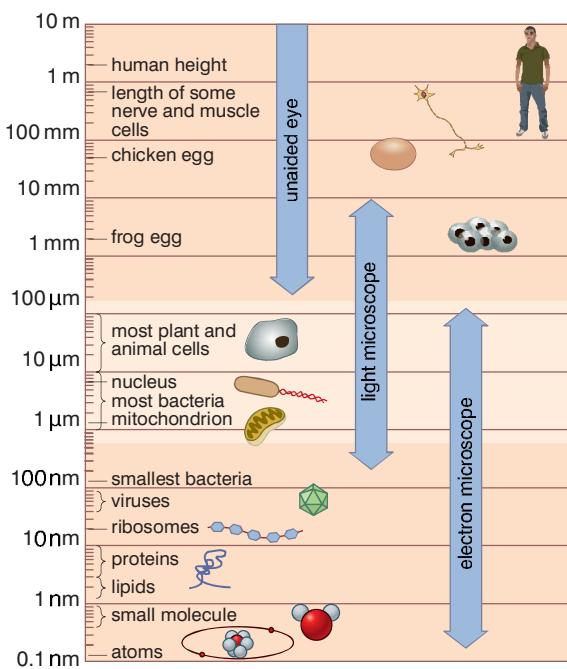


Figure 4.9 Size and scale in living things

## What units shall we measure cells in?

Again, it all depends on which cells, but first we should understand which units are available and which ones would be convenient to use. We could measure cells in metres, but the size of a red blood cell in metres would be approximately 0.000007 m.

All those 0's are very confusing and we don't easily work with such small numbers. So we use other, smaller units to measure the size of cells and molecules. These smaller units give us numbers that are more convenient to work with. There are three smaller units commonly used:

- millimetres (mm) – 1/1000 of a metre
- micrometres ( $\mu\text{m}$ ) – 1/1000 of a millimetre, and 1/1 000 000 of a metre
- nanometres (nm) – 1/1000 of a micrometre, 1/1 000 000 of a millimetre, and 1/1000 000 000 of a metre

We can convert the units from one to another as shown below:

$$\begin{array}{ccccccc}
 & \times 1000 & & \times 1000 & & \times 1000 & \\
 \text{m} & \xrightleftharpoons[\div 1000]{\quad} & \text{mm} & \xrightleftharpoons[\div 1000]{\quad} & \mu\text{m} & \xrightleftharpoons[\div 1000]{\quad} & \text{nm} \\
 & \div 1000 & & \div 1000 & & \div 1000 & \\
 \end{array}$$

To convert a larger unit to the next smaller unit, multiply by 1000:

For example, convert 3.5 mm to  $\mu\text{m}$ .

$$3.5 \text{ mm} = 3.5 \times 1000 = 3500 \mu\text{m}$$

To convert a smaller unit to the next larger unit, divide by 1000:

For example, convert 87 nm to  $\mu\text{m}$ .

$$87 \div 1000 = 0.087 \mu\text{m}$$

So, our red blood cell that was 0.000007 m in diameter is 0.007 mm or 7  $\mu\text{m}$  in diameter. This is a much more comprehensible number.

Most cells fall within a much narrower range of sizes than the chicken's egg and the smallest bacterium. As you can see from figure 4.9, the length of most animal and plant cells fall within a range of 10  $\mu\text{m}$  to 100  $\mu\text{m}$ . Most bacteria are about one-tenth of this length.

Figure 4.10 shows the relative sizes of an animal cell, a bacterium and a virus in a slightly different way. This diagram makes it clear just how much bigger an animal cell is than a bacterium.

The animal cell may be just ten times as long – but it is also ten times as wide and ten times as deep. This makes it 1000 times bigger than the bacterium!

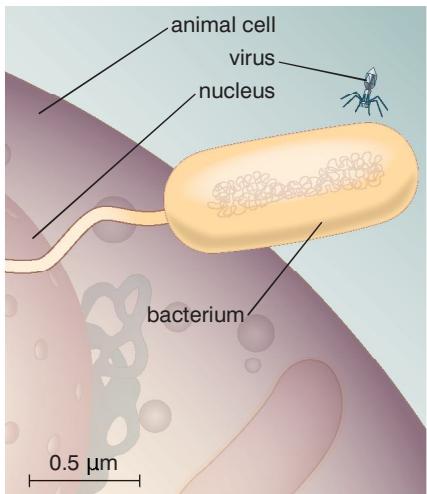


Figure 4.10 The relative size of an animal cell, a bacterial cell and a virus

### KEY WORDS

**calibrating** correlate the readings of an instrument with a standard

### Activity 4.2: How can we find out how big cells are?

To do this properly, you need to use two measuring devices with your microscope:

- a stage micrometer – this is really a microscope slide with a very precise scale etched onto it, and
- an eyepiece graticule – this is a piece of plastic with a less accurate scale than the graticule that fits inside the eyepiece of the microscope.

When you put the micrometer onto the slide and look at it through the eyepiece containing the graticule, you see something like figure 4.11.

The smallest divisions on the stage micrometer slide are 100  $\mu\text{m}$ . So each large division on the stage micrometer is 10 times that – 1 mm.

If we look at the two scales, we can see that the range 50–60 on the stage micrometer corresponds with the range 35–72 on the graticule. Ten divisions on the micrometer scale correspond to 37 divisions on the graticule scale and 1 micrometer division therefore corresponds to 3.7 graticule divisions. But we know that 1 micrometer division = 100  $\mu\text{m}$  (or 0.1 mm).

So 1 division of the eyepiece graticule =  $100 \mu\text{m} \div 3.7 = 27 \mu\text{m}$  (or 0.027 mm).

This is called **calibrating** the eyepiece graticule. If we now put an object on an ordinary slide (having removed the stage micrometer) and view it at the same magnification, we can calculate its size. If it takes up 26 graticule divisions, then this length is:  $26 \times 27 = 702 \mu\text{m}$  (or 0.702 mm).

However, the graticule will need recalibrating if it is to be used at a different magnification.

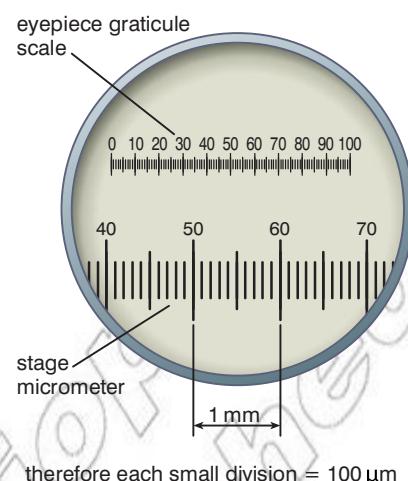


Figure 4.11 An eyepiece graticule and stage micrometer

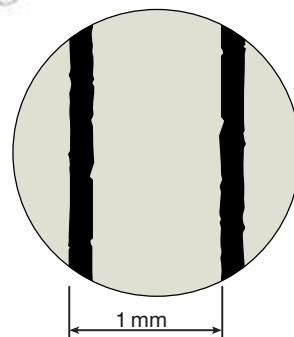


Figure 4.12 Estimating the field of view

### How you can make a rough estimate of cell size

It's a way of using the same principle as the micrometer. It isn't as accurate, but it's a lot cheaper and easier.

- Place your slide of cells (for example, onion epidermis cells) under the microscope and get the cells in focus at about magnification  $\times 100$ .
- Now take the slide away and replace it with a transparent plastic ruler. (Keep the magnification the same.)
- Focus on the millimetre scale on the ruler. You will see something like figure 4.12.

- Use this to estimate the width of 'field of view'. You would probably estimate the field of view as shown in figure 4.12 at about 2 mm.
- Now replace your slide of the onion cells and refocus.
- Count how many cells fit lengthways and widthways into the field of view.
- If 8 cells fit across the field of view the width of each cell is  $2 \text{ mm} \div 8 = 0.25 \text{ mm}$  (or 250  $\mu\text{m}$ ). However, this is only one estimate and you should repeat the procedure in several areas of the slide and find the average.

**KEY WORD**

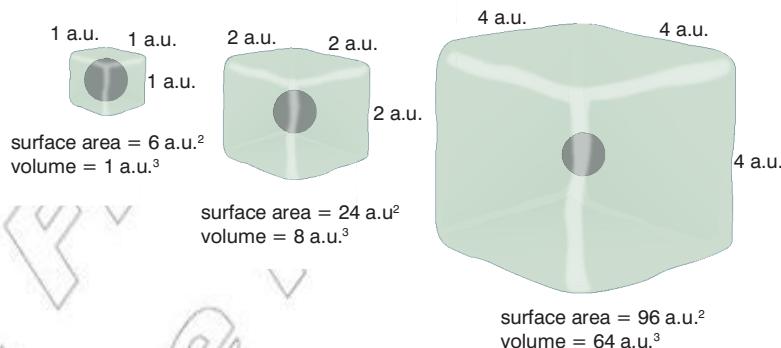
**arbitrary units** are units we use when we don't know actual dimensions but we know the mathematical relationship between different conditions

## What are the consequences of the different sizes of cells?

When a cell gets bigger, all its dimensions change. It is easy to be tricked into thinking that when a cell doubles all its dimensions it is twice as big. But, in fact, there is now eight times more cell as a result! This is most easily explained if we pretend that our cell is a cube, but the same principles hold true for other shapes also.

Look at figure 4.13. It shows three cubic 'cells' of different sizes. The linear dimensions double from the first cell to the second and double again from the second cell to the third. But the surface area and volume of the cell doesn't double.

There are six sides to a cube. We calculate the area of each side by multiplying length by breadth. In the case of the first cell, this is 1 arbitrary unit (a.u.)  $\times$  1 a.u., so the area of one side is 1 a.u.<sup>2</sup>. The volume of a cube is length  $\times$  breadth  $\times$  height. In this case 1 a.u.  $\times$  1 a.u.  $\times$  1 a.u. So the volume of the first cell is 1 a.u.<sup>3</sup>. The ratio of the surface area to the volume is 6:1.



**Figure 4.13** How increasing size affects surface area and volume. The measurements are in arbitrary units (a.u.).

So what about the second cell? The linear dimensions have doubled, but the surface area is 24 a.u.<sup>2</sup> and the volume is 8 a.u.<sup>3</sup>. The ratio of the surface area to volume is now  $24 \div 8 = 3:1$ . It is half that of the smaller 'cell'.

The ratio of the surface area to volume of the third cell is in fact 1.5:1. Smaller again and half the value of the ratio of the cell with linear dimensions that are half of this one.

### Activity 4.3: Can you spot a trend?

Calculate the surface area, volume and surface-area-to-volume ratio for cubes with linear dimensions ranging from 1 to 10. You could use a table like the one below.

Now plot a graph of your results. Plot linear dimensions on the x (horizontal) axis and surface-area-to-volume ratio on the y (vertical) axis. Describe the trend carefully.

Linear dimensions	Area of one side ( $l \times b$ )	Total surface area ( $6 \times l \times b$ )	Volume ( $l \times b \times h$ )	Surface-area-to-volume ratio
1				6
2				3
3				
etc.				
10				

### So, does it matter if the surface-area-to-volume ratio changes?

To answer this question, we must think about the functions of the surface area of the cell and of the volume of the cell. It is best understood if we think of just one function – that of respiration. A cell respires to release energy to drive all the other cellular processes that take place. If it can't release enough energy, these other processes will slow down and the cell may die. In order to respire, the cell needs oxygen, which enters through the surface of the cell.

- The volume determines how much activity there is in a cell. A large cell will have more processes happening, or at least the same processes happening faster, than a smaller cell. The amount of energy that must be released in respiration is therefore decided largely by the volume.
- The amount of oxygen that can be delivered into the cell is decided largely by how much 'surface' there is, since it is through the surface of the cell that the oxygen enters.

A large surface-area-to-volume ratio means that it is likely that the surface will be able to supply the oxygen demands of the cell. But as cells increase in size, *the volume increases faster than the surface area* and the surface-area-to-volume ratio decreases. How will this affect the ability of the cell to release the energy it needs?

#### KEY IDEA

*Think of this surface-area-to-volume ratio in terms of 'supply' and 'demand'. The volume of the cell creates the 'demand' for oxygen, which is 'supplied' through the surface (area) of the cell.*

### Review questions

Choose the correct answer from A to D.

- A compound microscope differs from a simple microscope in that it always has:
  - more than one objective lens
  - more than one ocular lens
  - both ocular and objective lenses
  - a condenser
- The word 'cell' was first coined by Robert Hooke when he examined:
  - living cells in cork tissue
  - dead cells in cork tissue

### Activity 4.4: Debate

Most biologists believe that a high surface area to volume ratio is advantageous to a cell.

Your teacher will divide the class into three groups:

- Group 1 – this group will present arguments to support the idea that a high surface area to volume ratio is advantageous to a cell
- Group 2 – this group will present arguments to support the idea that a low surface area to volume ratio is advantageous to a cell
- Group 3 – this group will form the ‘audience’ who will:
  - question the members of each of the other groups after their presentation
  - vote to decide the outcome of the debate

The debate will follow the following procedure:

- Group 1 will present their case (2 minutes)
- Group 2 will present their case (2 minutes)
- Groups 1 and 2 can question the other group and try to disprove their ideas (2 minutes)
- Group 3 (the audience) can question any members of any group (4 minutes)
- Group 3 votes on the issue

C dead cells in skin

D living cells in skin

3. Anton van Leeuwenhoek saw what he called ‘animalcules’ and ‘tiny animalcules’. These were, respectively:
  - A bacteria and viruses
  - B bacteria and protoctistans
  - C protoctistans and viruses
  - D protoctistans and bacteria
4. In 1824, Rene Dutrochet stated that:
  - A all living cells come from other cells
  - B all living things are made of cells
  - C cells can be spontaneously generated
  - D cells cannot be spontaneously generated
5. The first cell theory stated by Schleiden and Schwann was not completely accurate because it held that:
  - A all living things are made of cells
  - B the cell is the basic unit of living things
  - C the cell retains a dual existence
  - D cells form by cell-free formation
6. The first correct cell theory was proposed by:
  - A Koliker
  - B Virchow
  - C Fleming
  - D Golgi
7. To convert mm to  $\mu\text{m}$  we:
  - A multiply by 100
  - B divide by 100
  - C divide by 1000
  - D multiply by 1000
8. 40 divisions on the scale of an eyepiece graticule correspond to 16 small divisions on the stage micrometer. Each small division on the stage micrometer = 10  $\mu\text{m}$ . 4 cells fit across 40 divisions of the eyepiece graticule. The length of each cell is:
  - A 10  $\mu\text{m}$
  - B 40  $\mu\text{m}$
  - C 40 mm
  - D 10 mm

9. Cube A has a side measuring 3 mm. Cube B has a side measuring 12 mm. The surface-area-to-volume ratio of cube A when compared to cube B is:
- two times bigger
  - two times smaller
  - four times smaller
  - four times bigger
10. The surface-area-to-volume ratio of a cell is important because it is a measure of:
- how efficiently the cell releases energy in respiration
  - how efficient the cell is in conserving energy
  - how efficient the cell is in obtaining the oxygen it needs for respiration
  - how efficiently the cell uses the energy it releases in respiration

#### KEY WORD

**division of labour** *the specialisation of different parts to carry out certain functions*

## 4.2 Types of cells

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

- Appreciate that there are just two basic types of cells: prokaryotic and eukaryotic cells.
- Give examples and describe the basic structure of each type.
- Explain the difference between prokaryotic and eukaryotic cells.

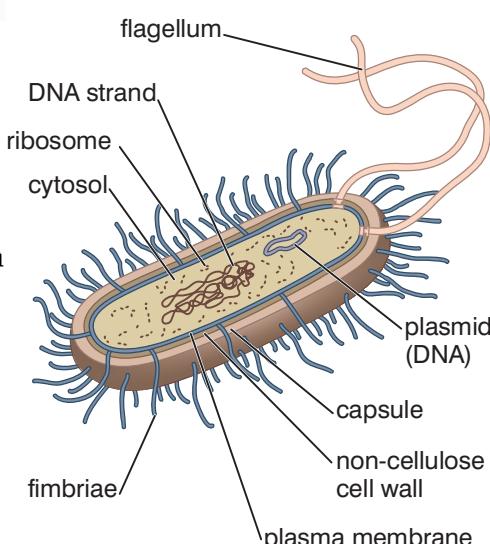
#### DID YOU KNOW?

The structure of the cell wall and capsule vary between different bacteria. You will learn more about this in grade 12.

## What are prokaryotic and eukaryotic cells?

Many biologists believe that **prokaryotic cells** were the first type of cells to be formed when life first evolved. Prokaryotic cells are much smaller and simpler than **eukaryotic cells**; even so these cells must carry out all the same functions that a eukaryotic cell carries out in order to survive. There is therefore some **division of labour** within the cell. There are specialised regions for certain functions. You can see this in figure 4.14.

However, there is much more division of labour in a eukaryotic cell. There are different types of eukaryotic cell, but all of them have a number of features in common. Figure 4.15 shows a generalised animal cell. Most of the structures shown are found in all eukaryotic cells. Plant cells have a number of other structures in addition to these, as figure 4.16 shows.



**Figure 4.14** A generalised prokaryotic cell

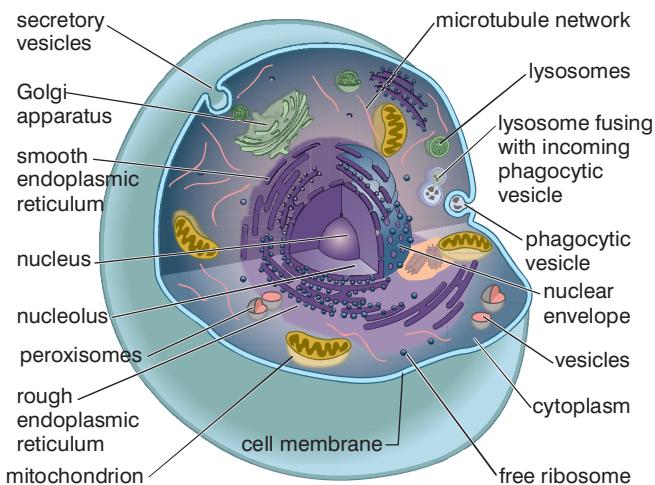


Figure 4.15 A generalised animal cell

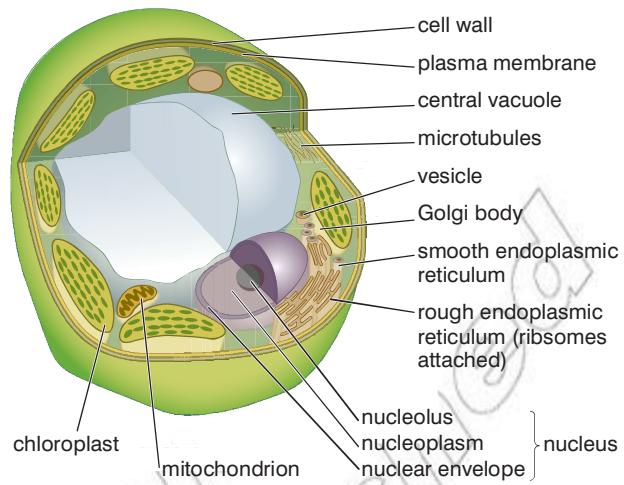


Figure 4.16 A generalised plant cell

### KEY WORDS

**prokaryotic cell** a type of cell that does not have a nucleus. The word *prokaryotic* is derived from Greek *pro* (before) and *karyos* (nuclear)

**eukaryotic cell** a type of cell that has a nucleus. The word *eukaryotic* is derived from Greek *eu* (true) and *karyos* (nuclear)

**organelles** individual structures in a cell with a specific function

**endoplasmic reticulum** the network of membranes in a cell

**membrane-bound organelles** organelles surrounded by membranes

These are clearly much more complex cells than the prokaryotic cell. What is really different about them is that there are many more different individual structures, called **organelles**, in the cell. Also, there are many more membranes in the cell. Some of these form the complex membrane system that is found throughout the cell – the **endoplasmic reticulum**. In addition to these, several of the organelles are surrounded by membranes. These are the:

- nucleus
- mitochondria
- chloroplasts (if present)
- lysosomes
- Golgi apparatus

These are called **membrane-bound organelles**.

These membranes make the cell able to function more efficiently. Because each mitochondrion is enclosed by a membrane (actually by two membranes!), the reactions that take place here are not affected by other cellular reactions. The same applies to the other membrane-bound organelles. Also, membranes of the endoplasmic reticulum separate areas of the cytoplasm and allow them to function independently.

### How did eukaryotic cells originate?

One theory is that the ‘modern’ eukaryotic cell was formed when several of the more primitive prokaryotic cells ‘got together’. Over millions of years ancestral prokaryotic cells became more membranous. The plasma membrane around the cell became more and more ‘infolded’ until there was an extensive membrane system in the cell. This would eventually evolve into the endoplasmic reticulum (EPR) of eukaryotic cells. The next stage in the theory suggests that this membranous cell engulfed other smaller cells that were better at respiration to release energy.

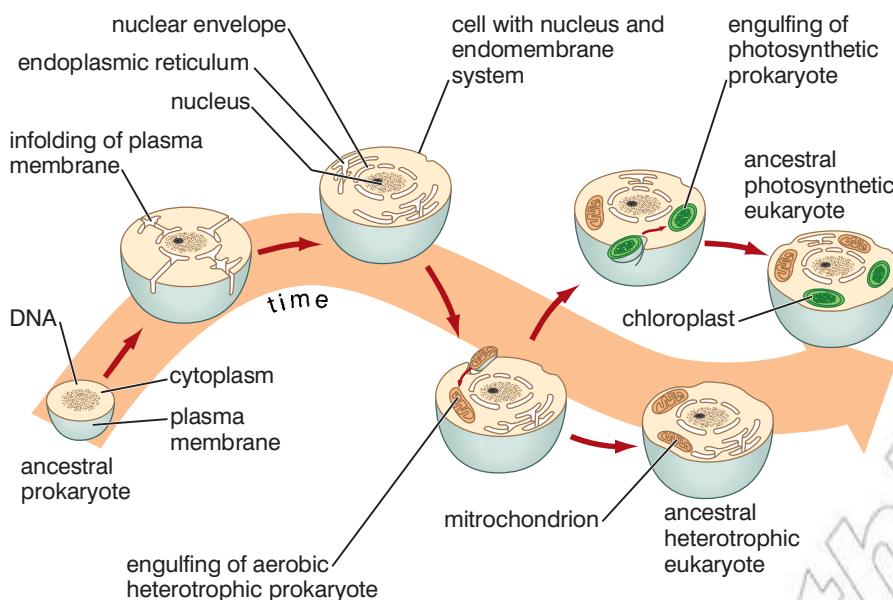


Figure 4.17 The origin of eukaryotic cells

These 'engulfed' prokaryotes would evolve into the mitochondria of eukaryotic cells. The cells that contained them were **heterotrophic** and the forerunners of animal, fungal and prototistian cells. One further stage suggests that some of these cells with their 'primitive mitochondria' also engulfed other, smaller, prokaryotic cells. These were prokaryotic cells that could photosynthesise and would, in time, evolve into chloroplasts. The cells that contained these would be **autotrophic** and the forerunners of plant cells. This theory of the origin of eukaryotic cells is called the **endosymbiont theory** and was first proposed by the biologist Lynn Margulis.

### KEY WORDS

**heterotrophic** these cells must absorb organic molecules 'ready-made'

**autotrophic** autotrophic cells are capable of making their own organic molecules from inorganic ones, usually by photosynthesis

**endosymbiont theory** theory of the origin of eukaryotic cells

### DID YOU KNOW?

#### Which organisms have eukaryotic cells and which have prokaryotic cells?

Figure 4.18 shows the main groups of living things and which of these have prokaryotic and which have eukaryotic cells. The **archaeabacteria** are thought to be the oldest organisms on Earth. They evolved when conditions on Earth were very harsh and are still only found where it is very hot, or where there are large concentrations of gases like methane or sulphur dioxide. The **eubacteria** are what you and I really mean when we talk about bacteria. These are the bacteria that inhabit our intestines, decay organisms, convert milk to yoghurt and so on.

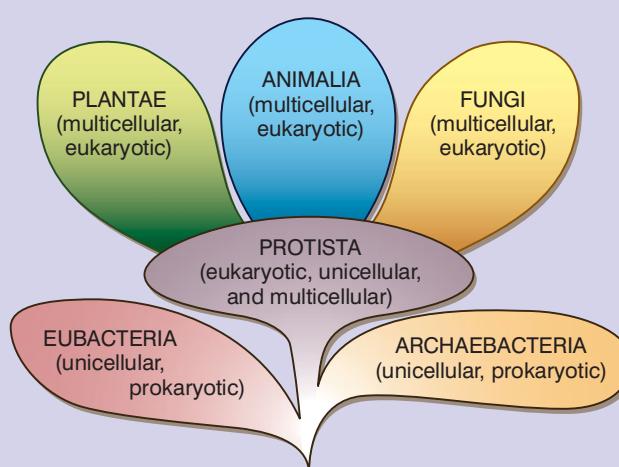


Figure 4.18 The main groups of living things

**Activity 4.5**

Make a table to compare and contrast the main features of eukaryotic and prokaryotic cells.

What are the differences between prokaryotic and eukaryotic cells?

**Table 4.2** A summary of the main differences between prokaryotic and eukaryotic cells

Feature	Prokaryotic cells	Eukaryotic cells
Size	1–10 $\mu\text{m}$	10–100 $\mu\text{m}$
Nucleus	No membrane-bound nucleus	Nucleus surrounded by nuclear envelope
DNA	<ul style="list-style-type: none"> <li>• In a continuous loop</li> <li>• Not associated with protein to form chromosomes</li> </ul>	<ul style="list-style-type: none"> <li>• Linear DNA</li> <li>• Associated with histone proteins in chromosomes</li> </ul>
Mitochondria	Absent	Present
Chloroplasts	Absent (but some prokaryotic cells contain a kind of chlorophyll and can photosynthesise)	Present in some cells (some plant cells and some algal cells)
Ribosomes	Present, but smaller than in eukaryotic cells (70S)	Present, but larger than in prokaryotic cells (80S)
Cell wall	<ul style="list-style-type: none"> <li>• Always present</li> <li>• Not made from cellulose (often made from peptidoglycan)</li> </ul>	<ul style="list-style-type: none"> <li>• Present in plant cells, algal cells and fungal cells</li> <li>• Cellulose in plant cells, various materials in other cells</li> </ul>

**KEY WORDS**

**70S and 80S ribosomes** the 'S' stands for 'sedimentation coefficient' and is a measure of their mass

**Review questions**

Choose the correct answer from A to D.

1. Differences between prokaryotic and eukaryotic cells are:
  - prokaryotic cells are smaller but contain more organelles
  - prokaryotic cells are larger and contain more organelles
  - prokaryotic cells are larger and contain fewer organelles
  - prokaryotic cells are smaller and contain fewer organelles
2. The DNA in prokaryotic cells is:
  - linear and bound with proteins
  - linear and not bound with proteins
  - circular and not bound with proteins
  - circular and bound with proteins

3. Membrane-bound organelles include:
  - A ribosomes and mitochondria
  - B mitochondria and chloroplasts
  - C chloroplasts and peroxisomes
  - D peroxisomes and nuclei
4. The cell wall of prokaryotic cells is made from:
  - A protein
  - B cellulose
  - C peptidoglycan
  - D another substance
5. It is thought that eukaryotic cells originated by several prokaryotic cells becoming associated. This theory is the:
  - A endosymbiont theory
  - B membrane association theory
  - C both of the above
  - D neither of the above

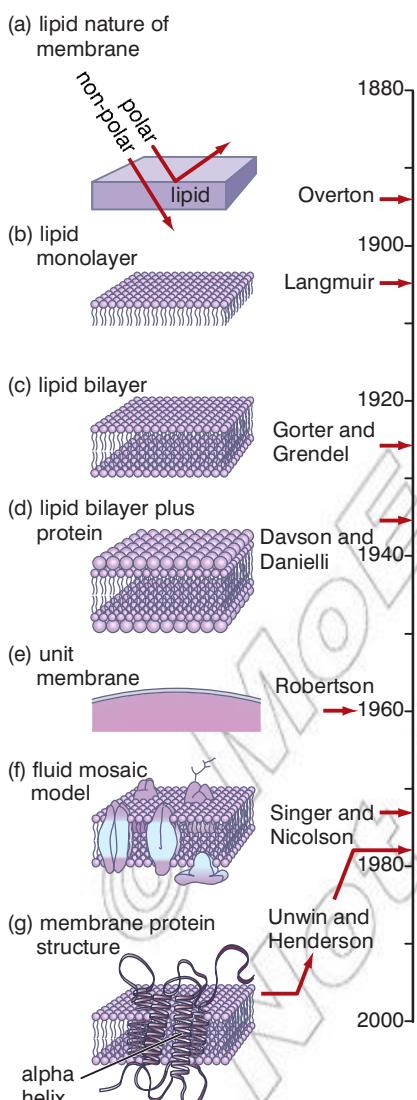
### 4.3 Parts of the cell and their functions

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

- Discuss the importance of a cell membrane.
- Describe the composition and arrangement of lipids and proteins in the membrane.
- Compare the Davson–Danielli and fluid mosaic models.
- Construct and show the arrangement of the phospholipids and proteins in the fluid mosaic model.
- Explain the role of glycoprotein and other components in the cell membrane.
- Name the different parts of the cell and explain their functions.
- State and explain the mechanisms of substance transport across a cell membrane.
- Conduct an experiment to show movement of solvent through a semi-permeable membrane.
- Demonstrate osmosis at a semi-permeable membrane.
- Explain that the size of a cell changes by osmosis because of the inflow and outflow of water.
- Appreciate that osmosis is responsible for everyday life phenomena.

## KEY IDEA

*The cell's environment could be fluids inside the body of an animal, plant, fungus or alga, or it could be the ocean, a river, a pond, soil or just about anything you care to think of! The plasma membrane of a cell must isolate the cell from that environment, but, at the same time, allow exchange with the environment.*



**Figure 4.19** A timeline of the development of our understanding of the structure of the plasma membrane

## What is the importance of the cell membrane?

The membrane that surrounds and encloses a cell is sometimes called the **cell surface membrane**, but most biologists now refer to it as the **plasma membrane**. Although this membrane has little mechanical strength to support the cell, it plays a crucial role in:

- controlling what enters and leaves the cell; the plasma membrane moves substances in and out of the cell by:
  - simple diffusion
  - facilitated diffusion
  - osmosis
  - active transport
  - endocytosis
  - exocytosis
- cell signalling; various molecules in the membrane allow the cell to be recognised by hormones and the immune system (in animals) and (in plants) growth regulator substances, such as auxins.

The plasma membrane clearly has a vital role in isolating the cell from its environment, whilst allowing necessary exchanges with that environment.

## What is the plasma membrane like?

We already found out in unit 2 that the basis of plasma membranes is a phospholipid bilayer. But a plasma membrane is much more complex than a simple bilayer. There have been several models of the structure of the plasma membrane. Table 4.3 shows some key events in developing the current model of membrane structure. Figure 4.19 illustrates this history.

**Table 4.3** Key events

Year	Event
1665	Robert Hooke discovers cells, but only sees dead cells and has no idea of a cell membrane.
1895	Charles Overton shows that the cell membrane is composed of some kind of lipids.
1905	Langmuir proposes a lipid monolayer as the basic membrane structure.
1910–1920	Evidence accumulates to show that the lipid in the membrane must be a phospholipid.
1925	E Gorter and G Grendel suggest that the plasma membrane is a phospholipid bilayer.
1935	Davson and Danielli know that proteins are also found in plasma membranes and suggest a 'sandwich' model.
1959	Based on electron microscope evidence that appears to support the Davson–Danielli model, J D Robertson proposes the unit membrane model – he suggests that all membranes are essentially the same.

Year	Event
1972	S J Singer and G L Nicholson propose the fluid mosaic model of membrane structure. As more and more supporting evidence accumulates, this is essentially the model we accept today.

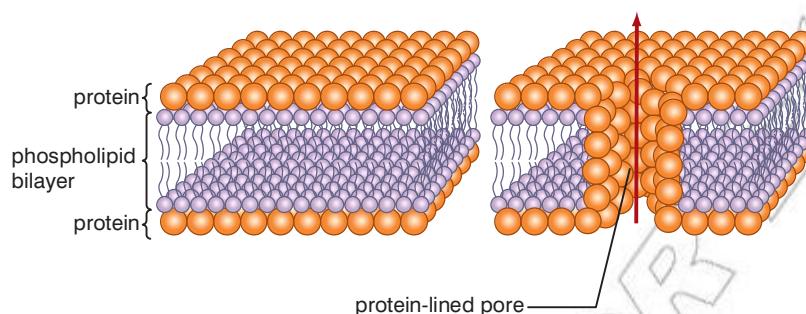
**KEY IDEA****Scientific models**

Models such as the Davson–Danielli membrane model and the fluid mosaic model are very important in science. But why do we call it a model, and what are scientific models?

- Models are conceptual plans of some system that try to explain experimental observations and relate the various observations to each other.
- A good model incorporates what is known about some concept and adds in the best guesses about missing parts.
- A good model will allow you to make predictions about some aspect of the way in which a system works that can be tested experimentally.
- Models may, therefore, turn out to be wrong, if the evidence from experiments does not match the predictions. Even so, the model will have served a valuable purpose in eliminating one possible idea and, perhaps, hinting at how to change the model to explain the system we are interested in.
- As more evidence accumulates to support a model, it becomes accepted by scientists as the best explanation for the system they are investigating.

**The Davson–Danielli model**

In 1935, Davson and Danielli knew that both proteins and phospholipids were involved in the structure of plasma membranes. Without any direct observational evidence to assist them (the very first electron microscopes were only just being built and they could not reveal membrane structure) Davson and Danielli suggested a kind of ‘sandwich’ of protein and phospholipid.



**Figure 4.20** The Davson–Danielli models of 1935 and 1954

This was based on what they knew of the proportions of the two substances in the membrane. The protein was to form the ‘bread’ of the sandwich with the phospholipid forming the ‘filling’. In 1954 they proposed a revised model in which they included protein-lined pores. Figure 4.20 shows the Davson–Danielli models of 1935 and 1954.

As more and more evidence accumulated about how molecules moved across membranes, it became clear that the Davson–Danielli model could not adequately explain all the new evidence. The model therefore had to be rejected.

In 1972, Singer and Nicholson proposed a totally different arrangement of the phospholipids and proteins in the plasma membrane. They retained the idea of a phospholipid bilayer, but rejected the sandwich arrangement. Instead, they suggested that proteins were ‘studded’ into the bilayer at different points. They also suggested that the arrangement was not static, but was fluid and constantly changing. Figure 4.21 shows the difference between the Davson–Danielli model and the original fluid mosaic model.

**KEY WORDS**

**plasma membrane/cell surface membrane** the membrane at the surface of all cells that isolates the cell from the environment and controls the exchange of substances between cell and environment

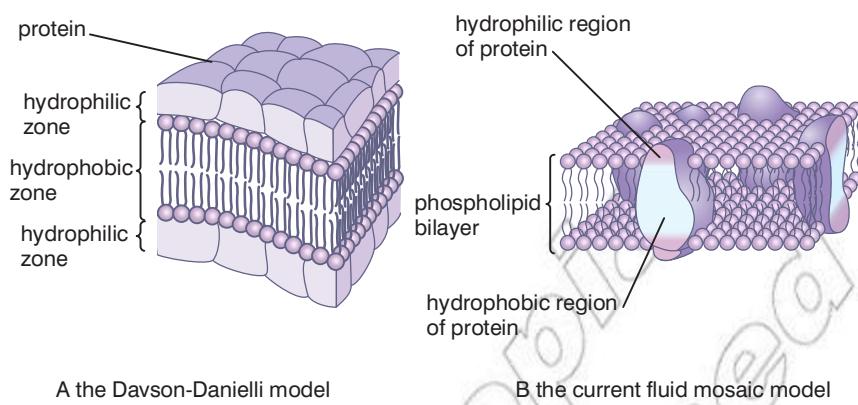


Figure 4.21 The Davson-Danielli model and the fluid mosaic model

As our understanding of processes that occur at the plasma membrane has increased and we have learned more of cell structure and function, the fluid mosaic model has become more sophisticated. Our current idea of membrane structure still assumes this fluid-mosaic nature, but there is now much more detail to the model, as figure 4.22 shows.

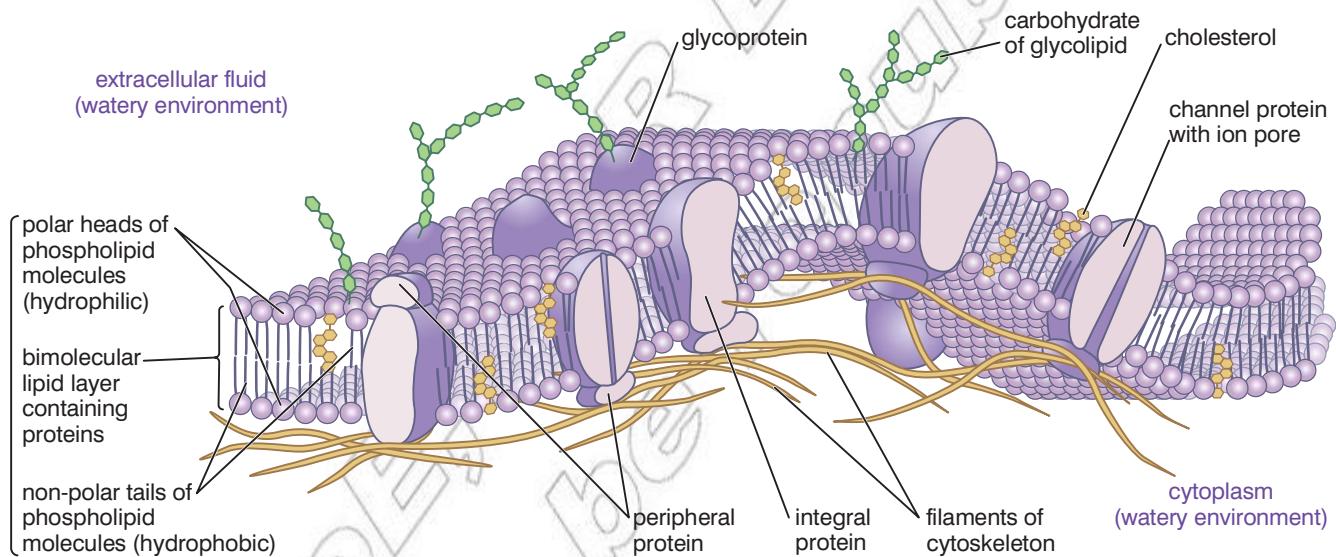


Figure 4.22 The current fluid mosaic model of membrane structure

### KEY WORDS

**integral proteins** proteins that span both phospholipid layers in a plasma membrane

**channel proteins** integral proteins with pores that allow ions to pass through the membrane

**carrier proteins** integral proteins that move medium-sized particles across the membrane

The key features of the model as we currently understand it are:

- The phospholipid bilayer as the basis for the membrane
- **Integral proteins** (also known as intrinsic proteins and transmembrane proteins) that span the membrane. Some of these proteins play an important role in moving substances across the membrane. There are three main types of these transport proteins:
  - **channel proteins** – these proteins have a channel through them along which a specific ion can pass; there are different channel proteins for different ions
  - **carrier proteins** – these proteins act in a more sophisticated way to move larger molecules through the membrane by facilitated diffusion or active transport; the ones involved in active transport are often referred to as pumps

- **peripheral proteins** (also known as extrinsic proteins) that span only one layer (or sometimes less) of the membrane. They have a range of functions; some are enzymes, others anchor integral proteins to the cytoskeleton
- **Glycoproteins** and **glycolipids** – protein and lipid molecules that have carbohydrate chains attached to them and often serve as signals to other cells. They also act as receptor sites for hormones and drugs. The carbohydrate component of each can be cell-specific and so allow identification of the cell by the immune system.
- **Cholesterol** – reduces the fluidity of the membrane.

### DID YOU KNOW?

**Receptors are the 'way in' for unwanted substances**

Some viruses are able to bind with some of the receptors on the plasma membrane and gain entry to the cell as a consequence. Some bacterial toxins enter in the same way.

### DID YOU KNOW?

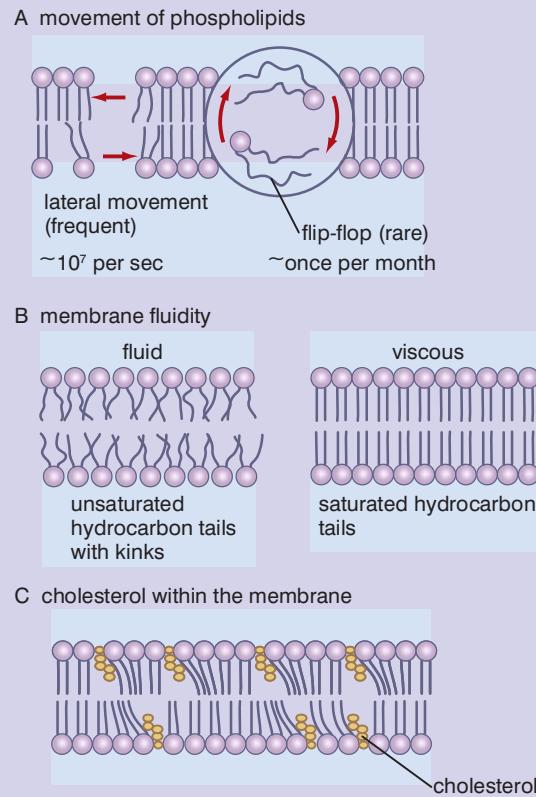
#### Why is the fluid mosaic model called the fluid mosaic model?

It is fluid mainly because the phospholipids in the membrane can move and change position. Figure 4.23 shows some ways in which they do this.

The nature of the fatty acids (saturated or unsaturated) and the amount of cholesterol in the membrane both influence the fluidity of the membrane.

The 'mosaic' part of the name comes from the way the proteins in the membrane give a patchwork appearance when viewed from the inside or outside. This is rather like a mosaic.

**Figure 4.23 Fluidity of the plasma membrane**



### How do substances cross the plasma membrane?

Not all particles can actually pass through a plasma membrane unaided. This is because of the largely lipid nature of the membrane. To pass through the plasma membrane by simple diffusion particles must be:

- small
- lipid soluble
- non-charged

This excludes particles such as ions (they are charged), sugars and amino acids (they are not lipid soluble and are not small particles) and any of the really large particles, such as proteins.

### KEY WORDS

**peripheral proteins** proteins that span only one of the two phospholipid layers

**glycoproteins** proteins with chains of sugars attached

**cholesterol** a sterol lipid

We can group the processes by which substances cross plasma membranes into two main types:

- **passive processes** – these processes rely only on the kinetic energy of the particles of the substances and on concentration gradients; they need no extra energy from the cell's metabolism
- **active processes** – these require energy from the cell's metabolism in the form of ATP to drive the transport.

Table 4.4 summarises the transport processes.

**Table 4.4 The transport process**

Passive processes		Active processes	
Process	Brief description	Process	Brief description
Simple diffusion	Particles move from a high concentration to a low concentration.	Active transport	Particles move from a low concentration to a higher concentration using a carrier protein (pump).
Facilitated diffusion	Particles move from a high concentration to a low concentration through an ion pore or carrier protein.	Endocytosis	Large particles are engulfed by the plasma membrane invaginating and forming a vesicle.
Osmosis	Water molecules move from a high water potential to a lower one.	Exocytosis	Large particles are secreted by a vesicle in the cell merging with the plasma membrane to release the substance.

### Activity 4.6

Work in groups. EITHER make a big poster to show the fluid mosaic model of the structure of the membrane of a eukaryotic cell OR make a 3-D model of the fluid mosaic model of the structure of the membrane of a eukaryotic cell.

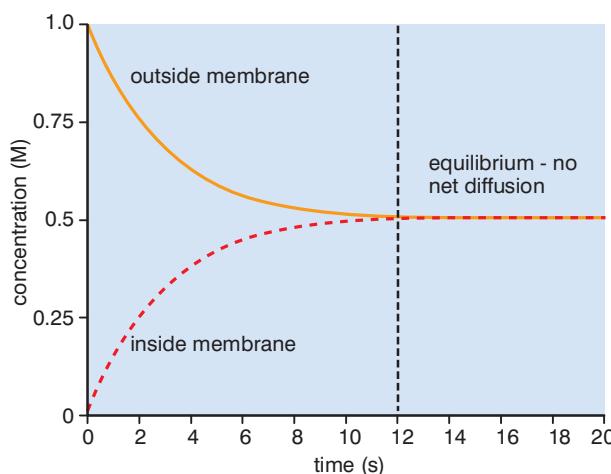
### Passive processes

#### Simple diffusion

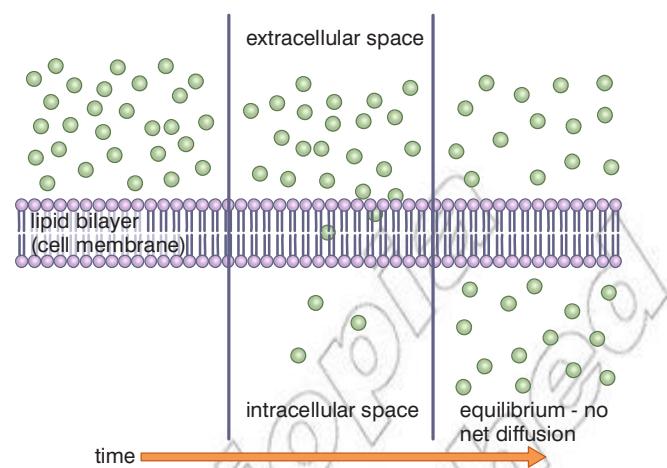
In fluids – liquids and gases – the particles that make up the fluid are free to move around. This kinetic energy is what drives diffusion. If particles are, for some reason, concentrated in a small area, they will move in such a way that the particles 'spread out' and occupy all the space that is available to them. This is a result of random particular motion. Diffusion need not involve a membrane.

When particles diffuse across a plasma membrane, there must be a concentration difference between the two sides of the membrane (a concentration gradient) to drive the process. As diffusion proceeds, the high concentration will decrease and the low concentration will increase until the two concentrations are the same. At this point there will be no further net diffusion.

This means that although particles will still move across the membrane, they will move equally in both directions, so there will be no overall effect. We say that the concentrations are in equilibrium. Figures 4.24 and 4.25 show this.



**Figure 4.24** The change in concentrations as diffusion proceeds



**Figure 4.25** The change in concentrations across a membrane as diffusion proceeds

The rate at which diffusion across a membrane takes place is influenced by:

- the concentration gradient – a bigger difference in concentration results in faster diffusion than a smaller gradient
- the thickness of the membrane – as all plasma membranes are the same thickness, this is not really an issue when considering diffusion into and out of cells, but for other situations where particles must cross some kind of barrier, a shorter distance results in faster diffusion
- the surface area of the membrane – clearly if there is more membrane where diffusion can take place, diffusion will happen faster

These features are all related in an equation called Fick's law of diffusion:

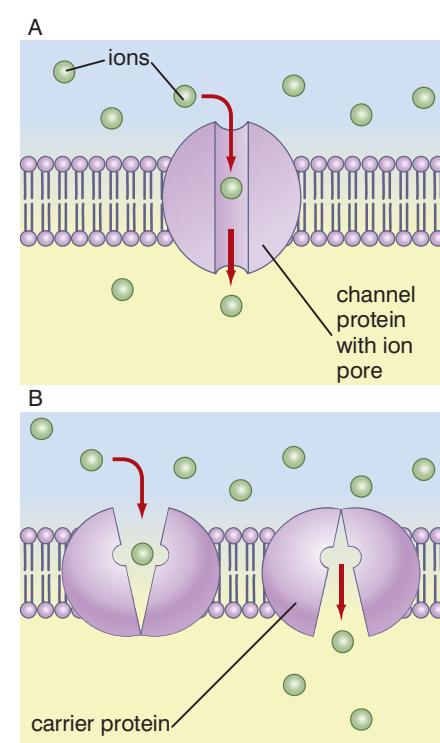
$$\text{Rate of diffusion} \propto \frac{\text{Surface area of membrane} \times \text{Concentration difference}}{\text{Diffusion distance}}$$

The rate of diffusion is also influenced by temperature. Diffusion occurs faster at higher temperatures because the particles have more kinetic energy and so move faster.

### Facilitated diffusion

Facilitated diffusion is essentially the same process as diffusion, in that it depends on a concentration gradient to allow particles to cross the membrane. However, it differs in that the particles must be helped to diffuse across the membrane (their diffusion must be 'facilitated') by a carrier protein or a channel protein with an ion pore. Figure 4.26A shows facilitated diffusion of an ion through an ion pore. Figure 4.26B shows a carrier protein moving particles across a membrane.

Note in both cases that the particles are moving from a high concentration to a low concentration (as with simple diffusion).



**Figure 4.26** Facilitated diffusion through A an ion pore and B a carrier protein.

**DID YOU KNOW?****Saturation of carrier proteins**

Although increasing the concentration gradient will increase the rate of both simple diffusion and facilitated diffusion, a point will come with facilitated diffusion when all the carrier proteins are transferring particles as fast as they can. At this point we say that the carrier proteins are saturated and facilitated diffusion can go no faster.

**KEY WORD**

**water potential** *the concentration of water molecules*

However, also note that whilst the ions can simply move straight through the ion pore of a channel protein, the carrier protein must undergo a conformational change (change in shape) to move particles through the membrane.

The rate of facilitated diffusion is affected by the same factors that affect simple diffusion with the exception that it is not the actual surface area of the membrane that determines the rate, but the number of carrier proteins (or channel proteins) present.

**Osmosis**

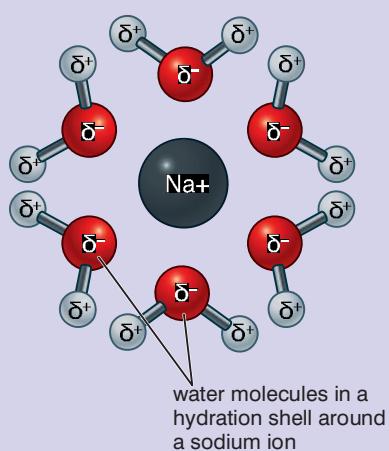
Osmosis is the process by which water moves across a partially permeable membrane. It is, effectively, the diffusion of water. However, we do not refer to the concentration of water molecules, but to **water potential**. We can say that osmosis is the movement of water from a system with a high water potential to a system with a low water potential across a partially permeable membrane.

The symbol for water potential is the Greek letter  $\Psi$  (psi). Water potential is measured in units of pressure – pascals (Pa), kilopascals (kPa) or megapascals (MPa). Pure, liquid water has a higher water potential than any other system. It is defined as zero:

$$\Psi(\text{pure water}) = 0 \text{ Pa}$$

All other systems (cells, solutions and suspensions) have a water potential that is lower than that of water. Therefore, their water potential values must be negative. So we can define osmosis more accurately as follows:

*Osmosis is the movement of water from a system with a high (less negative) water potential to one with a lower (more negative) water potential, across a partially permeable membrane.*



**Figure 4.27** Adding a solute reduces the water potential of a system

**DID YOU KNOW?****Why water potential values are negative**

The water potential of a system is due to the concentration of free water molecules in that system. In pure water, there are only water molecules. When a solute is added, some of the water molecules form 'hydration shells' around the solute molecules. This reduces the number of (free) water molecules in the system and so the water potential is reduced. Since pure water is assigned a water potential of zero, the solution must have a negative water potential. A more concentrated solution will take more free water molecules out of the system and lower the water potential still further, making it more negative.

The rate at which osmosis proceeds is influenced by the same factors as simple diffusion:

- surface area of the membrane
- difference in water potential
- distance the molecules must travel

*What happens to cells placed in solutions of different concentrations?*

This depends on what type of cell. Animal cells have no cell wall, whereas plant cells do and this has a significant influence on the outcome. The difference in water potential between cell and solution will determine whether water enters or leaves by osmosis.

When comparing the water potential of a solution to that of a cell, we could describe it as:

**isotonic** – having the same water potential as the cell

**hypertonic** – having a lower (more negative) water potential than the cell

**hypotonic** – having a higher (less negative) water potential than the cell

## KEY WORDS

**isotonic** solution having the same water potential as the cell

**hypertonic** solution having lower water potential than the cell

**hypotonic** solution having a higher water potential than the cell

### Activity 4.7: Osmosis in an Egg

In this investigation, you will use a hen's egg which has had the shell removed by soaking in vinegar (the acid in the vinegar dissolves the calcium carbonate that makes up the shell)

#### You will need:

- 1-2 fresh hen eggs (shells removed)
- masking tape & marker
- distilled water,
- concentrated salt (sodium chloride) solution
- clear jar with lid,
- tongs,
- electronic balance,

#### Procedure:

- use tongs to *carefully* remove an egg to a paper towel & pat it dry.
- record the size & appearance of your egg in a table
- place the egg on an electronic balance & record
- *carefully* place the egg into the jar and cover the egg with distilled water
- loosely re-cap the jar & allow it to sit for 24 hours
- repeat the procedure with another egg, but cover this egg in concentrated sodium chloride solution (rather than distilled water)
- open the jars & discard the distilled water/sodium chloride solution
- use tongs to carefully remove the eggs to a paper towel and pat them dry
- record the size & appearance of the eggs in your data table.
- weigh the egg on an electronic balance & record the mass in your table

#### What happened to:

- the size of the egg
  - the mass of the egg
- after soaking in:
- distilled water?
  - concentrated sodium chloride solution?

Explain these changes using your knowledge of osmosis.

#### Results table

Solution used	Original mass / g	Final mass / g	Size and appearance of egg
Distilled water			
Concentrated salt			

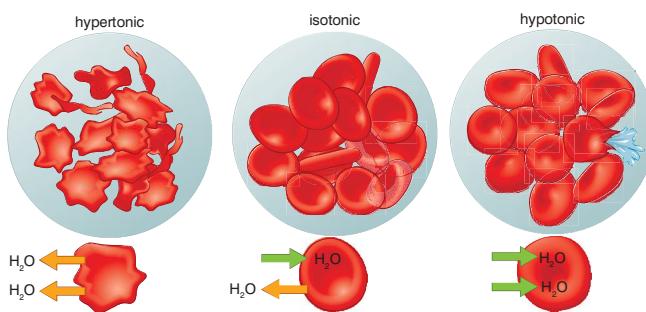


Figure 4.28 The effect of different solutions on red blood cells

### Animal cells

Figure 4.28 shows what happens when red blood cells are placed in different solutions.

In the hypertonic solution, the cells lose water by osmosis and shrink.

In the hypotonic solution, the cells gain water by osmosis and swell. The pressure will eventually burst the weak plasma membrane: this is called haemolysis.

There is no change in the isotonic solution.

### Plant cells

Figure 4.29 shows what happens when plant cells are placed in different solutions.

In the hypertonic solution, the cytoplasm of the cells loses water by osmosis and shrinks. Because of this, there is no pressure from the cytoplasm on the cell wall. The cell is said to be flaccid. If the cytoplasm shrinks too much, it loses contact with the cell wall and we say the cell has been plasmolysed.

In the hypotonic solution, the cells gain water by osmosis and swell. However, because of the cell wall, the cell cannot become much larger. Plant cells in this condition are turgid.

There is no change in the isotonic solution.

Turgidity is important in supporting young, non-woody plant stems. If the plant is kept well watered, the cells will remain turgid. The turgid cells will press against each other and this pressure will keep the plant upright. If the plant is not watered, the cells will be plasmolysed and become flaccid. They will no longer press against each other and the support will be lost. The plant will wilt.

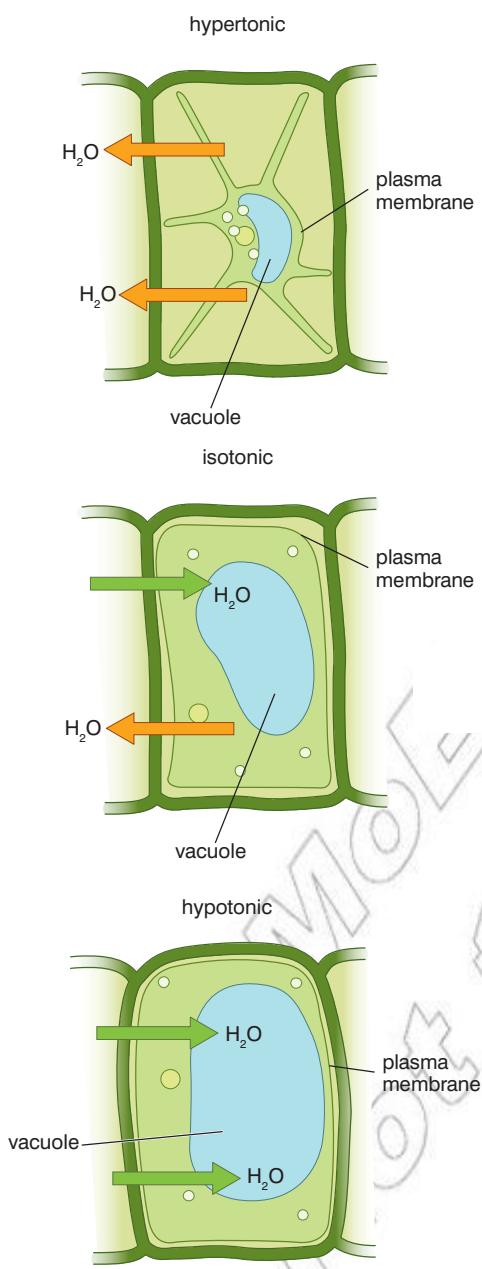


Figure 4.29 The effect of different solutions on plant cells

### Activity 4.8: Investigation showing the pressure generated by different solutions

#### You will need:

- a thistle funnel
- clamp and stand
- cellophane to act as the membrane
- distilled water
- 0.2M, 0.4M, 0.6M, 0.8M and 1.0M solutions of sucrose
- a ruler

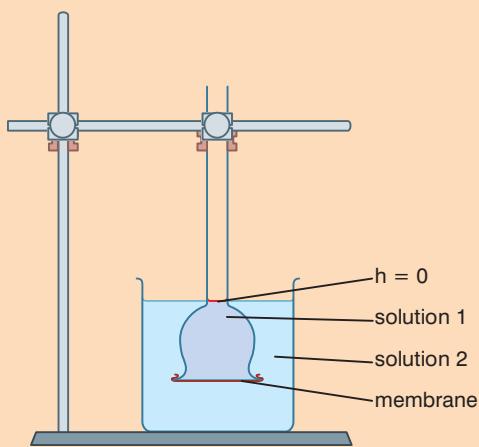


Figure 4.30 Investigating the osmotic pressure generated by different solutions

**Method**

1. Set up the apparatus as shown with distilled water on both sides of the membrane.
2. Leave for 30 minutes.
3. Measure the increase in height of the water inside the thistle funnel.
4. Repeat with 0.2M, 0.4M, 0.6M, 0.8M and 1.0M solutions of sucrose inside the membrane (solution 1) and distilled water outside the membrane (solution 2).
5. Plot a graph of your results.
6. What conclusion can you draw?



**Figure 4.31** The effect of watering a plant

**Activity 4.9: Finding the water potential of potato cells****Method**

1. Collect about  $100\text{ cm}^3$  1M sucrose and  $100\text{ cm}^3$  water in separate beakers.
2. Label six boiling tubes A–F.
3. Make up dilutions of the 1M sucrose solution supplied as below. Use a separate  $10\text{ cm}^3$  syringe for the water and for the sucrose.

Tube	Amount of sucrose/ $\text{cm}^3$	Amount of water/ $\text{cm}^3$	Final concentration/M
A	20	0	1.0
B	16	4	0.8
C	12	8	0.6
D	8	12	0.4
E	4	16	0.2
F	0	20	0.0

4. Prepare a table for your results; you will need columns for initial mass, final mass, change in mass and % change in mass.

Solution	Mass at the start/g	Mass at the end /g	Change in mass /g	% Change in mass
Distilled water				
0.2M sucrose				
0.4M sucrose				
0.6M sucrose				
0.8M sucrose				
1.0M sucrose				

5. Using a cork borer obtain six cylinders of potato tissue.
6. Trim each to a length of 5 cm. Cut off any 'skin' from the ends.
7. Roll each on absorbent paper to remove any surface water.
8. Weigh each and note the mass.
9. Place each cylinder in one of the solutions and leave for 30 minutes.
10. Remove each from the solution; roll each on absorbent paper to remove any surface water and reweigh.
11. Calculate the % change in mass for each.
12. Plot a graph of % change in mass against molarity and use the graph to estimate the molarity of the solution that has the same water potential as the potato cells.

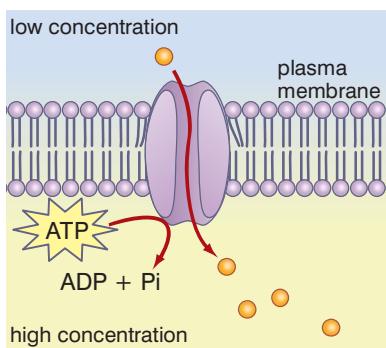


Figure 4.32 Active transport

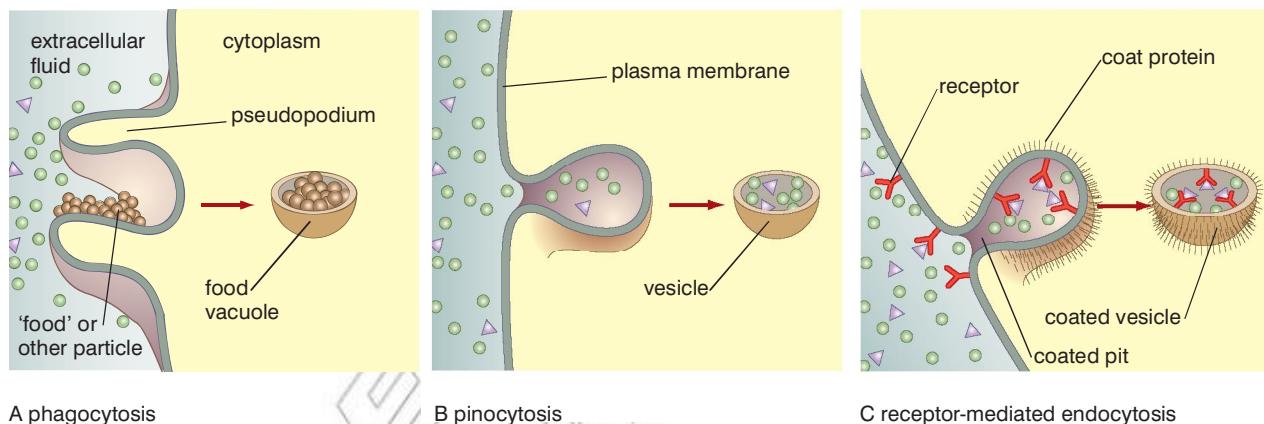
## Active processes

### Active transport

Sometimes, substances must be moved against a concentration gradient – from a low concentration to a higher one. This cannot happen by diffusion, since it would tend to concentrate particles rather than spread them out. It can only happen if metabolic energy is used to drive the process. In living organisms, this energy is released from the ATP produced in respiration. When the energy is released from ATP, it is broken down into ADP and  $P_i$  (inorganic phosphate). The proteins used to actively transport substances across plasma membranes are called pumps.

### Endocytosis

In this process, large particles are engulfed by a cell. There are several ways in which it can happen, but, essentially, part of the plasma membrane surrounds the particles to form a vesicle which is then processed by the cell. Figure 4.33 shows three different types of endocytosis. All of them require ATP to move the membrane around the particles to form the vesicle.



### Phagocytosis

This involves the creation of pseudopodia (extensions of the plasma membrane) to enclose large particles or even whole organisms outside the cell. Once enclosed by the pseudopodia, they form an internal vesicle which is then moved further inside the cell.

### Pinocytosis

This differs from phagocytosis only in scale. It involves the ingestion of smaller particles (but particles that are still too large to cross the membrane by other methods) and does not require the formation of large pseudopodia to engulf the particles.

### Receptor-mediated endocytosis

The membrane infolds to form vesicles only in regions where particles have bound to specific receptors. The binding stimulates the infolding.

## Exocytosis

In this process, substances are moved from the inside to the outside of the cell in what is, effectively, the reverse of endocytosis. It is the process by which enzymes and hormones are secreted. Again, ATP is used to alter the configuration of the membrane.

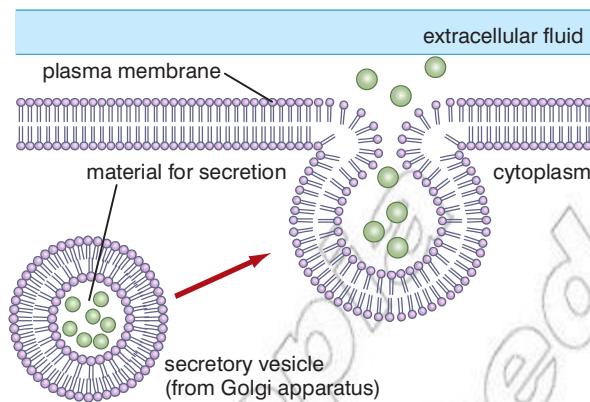


Table 4.5 The transport processes compared

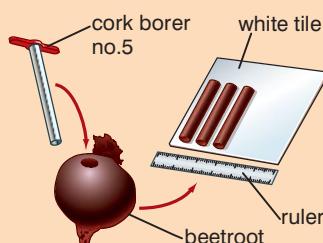
Process	Influence of concentration	Requirement for ATP?	Type of particles moved	Transport proteins needed?
Simple diffusion	Occurs from high to low	No	Lipid-soluble, small, non-polar	No
Facilitated diffusion	Occurs from high to low	No	Ions and medium-sized particles	No
Osmosis	Occurs from high to low (water potential)	No	Water molecules	No
Active transport	Occurs from low to high	Yes	Ions and medium-sized, non-lipid-soluble particles	Yes
Endocytosis	Can occur either way	Yes	Very large particles	Yes
Exocytosis	Can occur either way	Yes	Very large particles	Yes

Figure 4.34 Exocytosis

## Activity 4.10: How does temperature affect the permeability of a plasma membrane?

### Method

1. Set up water baths at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C and 70 °C.
  2. Using a cork borer, obtain 10 cylinders from beetroot (you may have to use more than one).
  3. Cut off the 'skin' at the ends.
  4. Cut each into 1 cm lengths – you will need 30.
  5. Place them in a beaker and rinse under running water until the water no longer shows any colouration.
- DO NOT PUT THE BEETROOT IN THE BOILING TUBES YET.



6. Place 10 cm<sup>3</sup> water into each of six boiling tubes; label them 20 °C, 30 °C, 40 °C, 50 °C, 60 °C and 70 °C.
7. Stand each in the appropriate water bath for five minutes to equilibrate to temperature.
8. Add five beetroot discs to each tube and leave for 15 minutes (still in the water bath). Start to clear away whilst you are waiting.
9. After 10 minutes, remove the tubes and shake them for 10 seconds to distribute any pigment.
10. Transfer a sample of the liquid to a cuvette.
11. Obtain a reading of absorbance for each sample. Remember to zero the colorimeter with a reference cuvette of distilled water each time.
12. Record your results in a table then use them to plot a line graph. Explain your results using your knowledge of membrane structure.

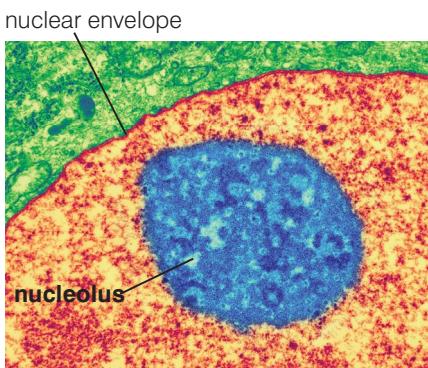


Figure 4.35 An electron-micrograph of the nucleus

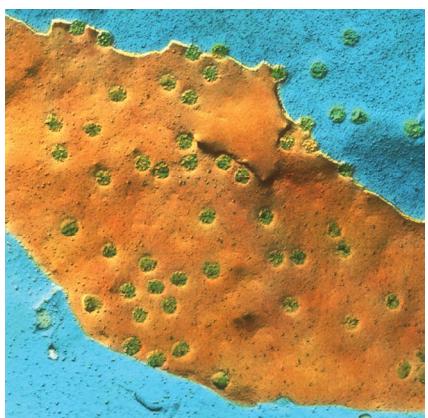


Figure 4.36 An electron-micrograph showing pores in the nuclear membrane

### KEY WORDS

**cristae** partial partitions in mitochondria

**fluid matrix** where some of the reactions of aerobic respiration take place in mitochondria

### DID YOU KNOW?

ATP is the 'energy storage molecule' of cells. Energy released in respiration is stored in ATP molecules, to be released and used when needed. Cells that are very active (such as muscle cells or epithelial cells that absorb molecules from the gut) use a great deal of ATP and, therefore, contain many mitochondria.

## The other cell organelles – what are they like and what do they do?

In this section, we will discuss the other organelles in outline only. We shall discover their functions in more detail as we discuss the metabolic processes they carry out.

### The nucleus

The nucleus typically occupies about 10% of the volume of a cell. It has several components:

- The nuclear envelope is a double membrane that surrounds the nucleus. There are many nuclear pores, which allow the passage of some molecules between the nucleus and the cytoplasm.
- The nucleolus is an organelle within the nucleus. It is not membrane-bound. Its function is to synthesise the components of ribosomes, which then pass through the nuclear pores into the cytoplasm.
- Chromatin consists of DNA molecules bound with proteins called histones. For most of the cell cycle, the chromatin fibres are loosely dispersed throughout the nucleus. Just before a cell is about to divide, the chromatin condenses into distinct, recognisable structures called chromosomes.

### Mitochondria

Mitochondria are the sites of most of the reactions of aerobic respiration. They are surrounded by two membranes. The inner membrane is folded into **cristae** to increase the available surface area. Some of the reactions of aerobic respiration take place in the **fluid matrix**. The folded inner membrane provides a large surface area for the electron-transport system, which produces most of the ATP.

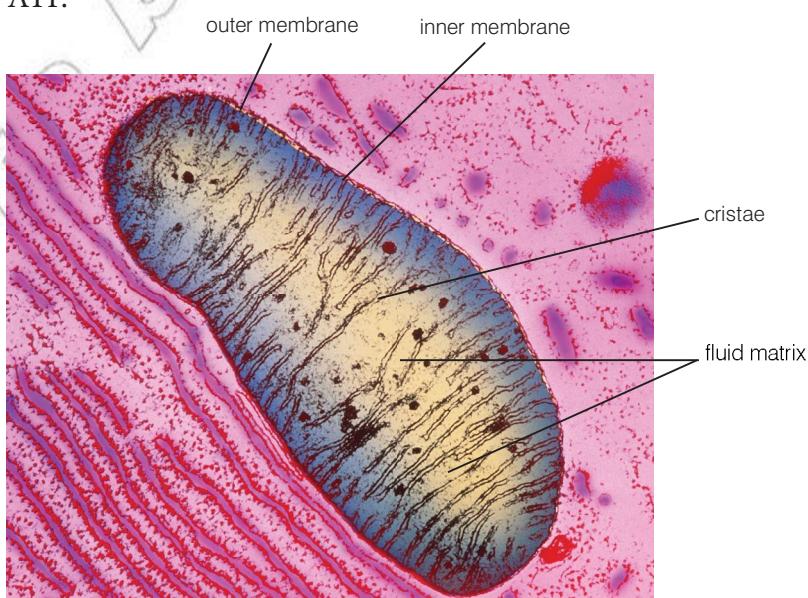
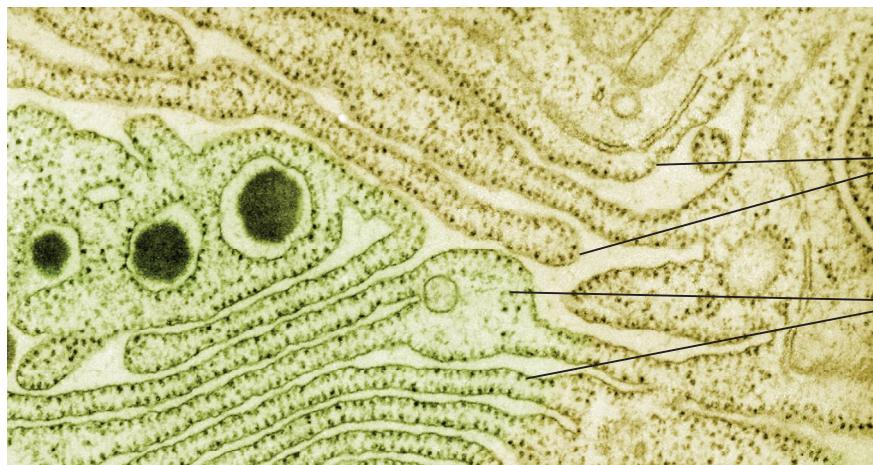


Figure 4.37 An electron-micrograph of a mitochondrion

## Ribosomes

Ribosomes are the sites of protein synthesis. They can be found free in the cytoplasm, but are also bound to the membrane system of the endoplasmic reticulum, forming rough endoplasmic reticulum. Each ribosome comprises two subunits that are made from RNA and protein. The subunits are manufactured in the nucleolus. They leave the nucleus through nuclear pores and combine in the cytoplasm.



**Figure 4.38** Rough endoplasmic reticulum with ribosomes attached

## Endoplasmic reticulum

Endoplasmic reticulum (ER) is a membrane system found throughout the cytoplasm of eukaryotic cells. There are two types of endoplasmic reticulum:

- **Rough ER** has ribosomes on its surface and is responsible for the manufacture and transport of proteins. Protein molecules manufactured by the ribosomes pass through small pores into the lumen (inner space) of the ER. They are then moved in a vesicle to the Golgi body. Rough ER is extensive in cells that manufacture a lot of protein, such as cells that manufacture enzymes to be secreted into the lumen of the intestine.
- **Smooth ER** has no ribosomes on its surface. It is concerned with the synthesis of lipids. It is also associated with carbohydrate metabolism and detoxification.



**Figure 4.39** An electron-micrograph of the Golgi apparatus

## Golgi apparatus (or Golgi body)

The Golgi apparatus consists of a number of flattened membrane-bound sacs in which proteins are modified. Proteins may be converted into glycoproteins, for example. Many of the modifications added in the Golgi apparatus act as a kind of 'tag', which determine the final destination of the molecule. Think of the Golgi apparatus as a cellular post office that labels and then distributes molecules!

Many of the modified molecules are released from the Golgi apparatus in vesicles to be carried to other parts of the cell or to the plasma membrane to pass out of the cell by exocytosis to be used elsewhere. Some vesicles form the lysosomes.



**Figure 4.40** An artist's impression of the Golgi apparatus

**KEY WORDS**

**grana** membranous regions in a chloroplast

**thylakoids** flattened sacs inside a chloroplast where photosynthesis takes place

**Lysosomes**

Lysosomes have no specialised internal structure and are surrounded by a single membrane. They are formed in the Golgi apparatus and contain digestive enzymes that break down cellular waste and debris. Lysosomes are particularly abundant in phagocytic white blood cells. Here, enzymes from the lysosomes digest foreign cells that have been engulfed.

The organelles we have described so far are found in all eukaryotic cells. However, not all eukaryotic cells are the same. In particular, there are important differences between plant and animal cells.

**Organelles found in plant cells****Cell wall**

We have studied the molecular structure of the cell wall in unit 2. The criss-cross arrangement of cellulose fibres in the cell wall gives it both strength and elasticity. Because there are large 'gaps' (on a molecular scale) between the fibres, the cell wall is freely permeable.

**Vacuole**

The vacuole in a plant cell is a fluid-filled sac that stores a range of solutes. It is also important in maintaining the turgidity, or turgor, of a cell. When the vacuole is full of liquid (mainly water), it exerts pressure on the cytoplasm and, in turn, on the cell wall. If the vacuole loses water by osmosis, the pressure reduces and turgor is lost. The cell becomes flaccid (see the section on osmosis).

**Chloroplast**

Figure 4.41 is an electron-micrograph showing the structure of a chloroplast. Chloroplasts are surrounded by two membranes, like mitochondria, but, unlike mitochondria, the inner membrane is not folded. There are two main regions in chloroplasts that are linked to the stages of photosynthesis:

- membranous regions called **grana** (each of which is a stack of **thylakoids**) where the light-dependent reactions occur, and
- a fluid stroma – where the light-independent reactions occur.

**How have biologists been able to study the different organelles?**

This has been possible because of a technique called cell fractionation. The technique is based on the fact that the masses of organelles vary and depend on their size. When a mixture of organelles is spun in a centrifuge, the various types settle out at different speeds of spinning. The large nucleus requires a relatively low centrifuge speed to make it settle out; the much smaller ribosomes require a much higher speed. The technique is carried out as follows:



**Figure 4.41** An electron-micrograph of a chloroplast

- The cell sample is stored in a suspension that is:
  - buffered – the neutral pH prevents damage to the structure of proteins, including enzymes
  - isotonic (of equal water potential) – this prevents osmotic water gain or loss by the organelles; gaining too much water could rupture the organelles
  - cool – this reduces the overall activity of enzymes released later in the procedure

- The cells are homogenised in a blender and filtered to remove debris.
- The homogenised sample is placed in an ultracentrifuge and spun at low speed. The nuclei settle out, forming a pellet.
- The supernatant (the suspension containing the remaining organelles) is spun at a higher speed – chloroplasts settle out (if plant tissue is used).
- The supernatant is spun at a higher speed still – mitochondria settle out.
- The process is repeated at ever higher speeds until all the organelles have been separated.
- The process is shown in figure 4.43.

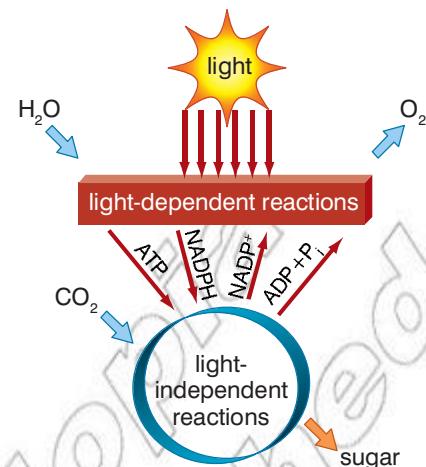


Figure 4.42 Light-dependent and light-independent reactions of photosynthesis

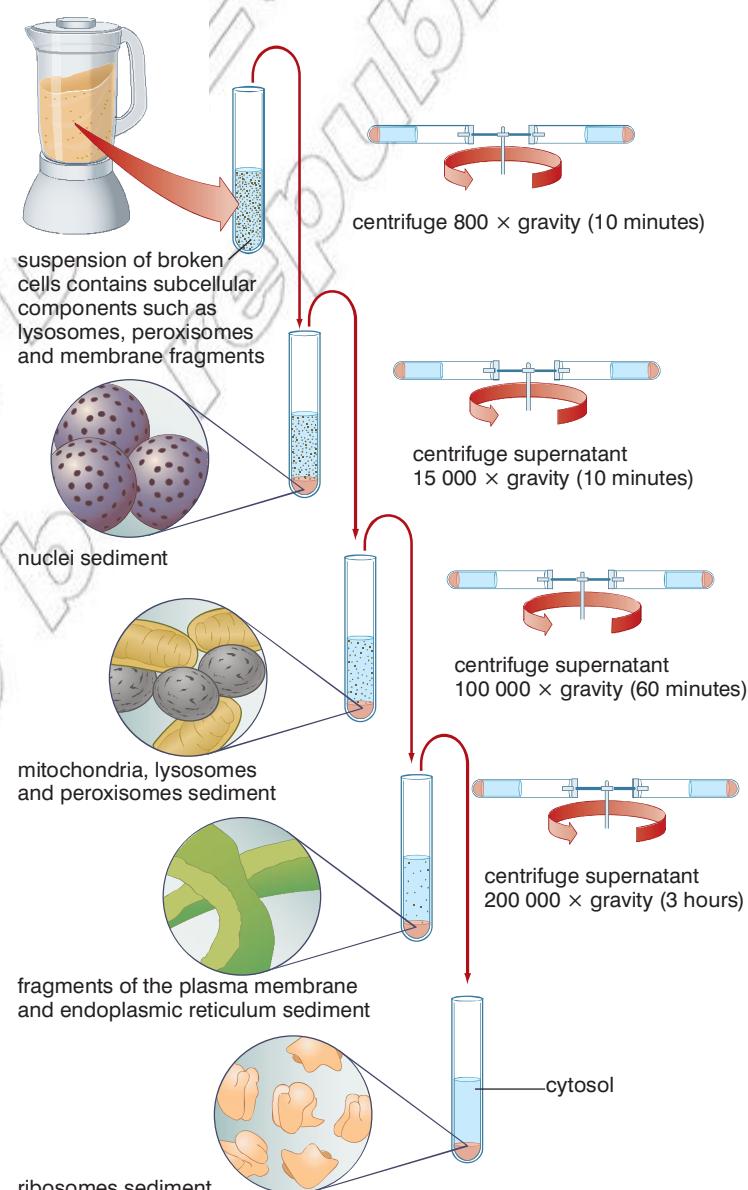


Figure 4.43 Cell fractionation

## Review questions

Choose the correct answer from A to D.

1. To convert millimetres to micrometres:
  - A multiply by 1000
  - B divide by 100
  - C divide by 1000
  - D multiply by 100
2. The functions of the rough endoplasmic reticulum and the Golgi body are related because:
  - A proteins synthesised by the rough endoplasmic reticulum are modified by the Golgi body
  - B proteins synthesised by the Golgi body are modified by the rough endoplasmic reticulum
  - C lipids synthesised by the Golgi body are modified by the rough endoplasmic reticulum
  - D lipids synthesised by the rough endoplasmic reticulum are modified by the Golgi body
3. In cell fractionation, the purpose of keeping the tissue sample in an isotonic solution in a refrigerator prior to homogenisation is:
  - A to prevent osmotic damage to the cells and to reduce the metabolic activity of the cells
  - B to prevent osmotic damage to the cells and to increase the metabolic activity of the cells
  - C to prevent osmotic damage to the organelles and to reduce the metabolic activity of the cells
  - D to prevent osmotic damage to the organelles and to increase the metabolic activity of the cells
4. Which of the following is not part of the structure of mitochondria?
  - A a double membrane surrounding the organelle
  - B a fluid matrix inside the organelle
  - C stacks of membranes known as thylakoids
  - D folds of membranes known as cristae
5. The plasma membrane provides:
  - A structural support for the cell and regulates which substances enter and leave
  - B structural support for the cell but does not regulate which substances enter and leave

- C no structural support for the cell and does not regulate which substances enter and leave
- D no structural support for the cell but regulates which substances enter and leave
6. The principle behind separating cell organelles by ultracentrifugation is that:
- A the various organelles have different masses
  - B the various organelles have different volumes
  - C the various organelles have different shapes
  - D the various organelles have different widths
7. Which of the following does not use energy in the form of ATP?
- A active transport
  - B endocytosis
  - C facilitated diffusion
  - D phagocytosis
8. Which of the following statements concerning mitochondria and chloroplasts is correct?
- A Only mitochondria are surrounded by two membranes.
  - B The inner membrane of chloroplasts is folded into cristae.
  - C Both organelles have a fluid interior.
  - D Mitochondria contain stacks of membranes called thylakoids.
9. If red blood cells are immersed in a hypotonic solution, they will:
- A take in water by osmosis, swell and become turgid
  - B lose water by osmosis and shrink
  - C lose water by osmosis and burst
  - D take in water by osmosis, swell and burst
10. In the fluid mosaic model of membrane structure, intrinsic proteins can be:
- A glycoproteins
  - B ion channel proteins
  - C carrier proteins
  - D all of the above

## Summary

In this unit you have learnt that:

- A cell is the smallest unit of life capable of independent existence.
- The cell theory proposed by Schleiden and Schwann stated:
  - the cell is the unit of structure, physiology and organisation in living things
  - the cell retains a dual existence as:
    - a distinct entity, and
    - a ‘building block’ in the formation of organisms.
- Virchow added another important idea which was that all cells come from pre-existing cells.
- Modern cell theory also includes the ideas that:
  - cells contain hereditary information which is passed from cell to cell during cell division
  - all cells have basically the same chemical composition
  - all energy flow (the metabolism and biochemistry of life) occurs within cells
- Dimensions of cells are measured in units derived from the metre, including:
  - millimetre, mm = 0.001 m
  - micrometre,  $\mu\text{m}$  = 0.000,001 m (0.001 mm)
  - nanometre, nm = 0.000,000,001 m (0.001  $\mu\text{m}$ )
- We can measure the size of cells using an eyepiece graticule calibrated by a stage micrometer.
- As cells increase in size, the surface-area-to-volume ratio decreases; this affects their ability to obtain the resources they need to carry out their metabolism.
- There are two main types of cells: prokaryotic cells and eukaryotic cells; the table shows the differences between them.

Feature	Prokaryotic cells	Eukaryotic cells
Size	1–10 $\mu\text{m}$	10–100 $\mu\text{m}$
Nucleus	No membrane-bound nucleus	Nucleus surrounded by nuclear envelope
DNA	<ul style="list-style-type: none"> <li>• In a continuous loop</li> <li>• Not associated with protein to form chromosomes</li> </ul>	<ul style="list-style-type: none"> <li>• Linear DNA</li> <li>• Associated with histone proteins in chromosomes</li> </ul>
Mitochondria	Absent	Present
Chloroplasts	Absent	Present
Ribosomes	Smaller than in eukaryotic cells (70S)	Larger than in prokaryotic cells (80S)
Cell wall	<ul style="list-style-type: none"> <li>• Always present</li> <li>• Not made from cellulose</li> </ul>	<ul style="list-style-type: none"> <li>• Present in some</li> <li>• Cellulose in plant cells</li> </ul>

- Animal cells contain a nucleus, mitochondria, lysosomes, ribosomes, ER (rough and smooth) as well as Golgi apparatus, all enclosed within a plasma membrane.
- Plant cells contain all the same organelles but also contain chloroplasts, a cellulose cell wall and a permanent vacuole.
- The organelles of cells have specific functions:
  - the nucleus contains DNA which controls the metabolism of the cell
  - mitochondria carry out aerobic respiration to release energy from organic molecules and store it in the ATP molecule
  - ribosomes synthesise proteins from amino acids
  - lysosomes contain hydrolytic enzymes that digest worn-out or damaged organelles as well as engulfed bacteria
  - the Golgi body modifies proteins and distributes them to the appropriate part of the cell
  - chloroplasts in plant cells carry out the reactions of photosynthesis
  - the plant cell wall supports and protects the contents of the cell; it is freely permeable to all molecules
  - the vacuole in plant cells contains a solution of mineral ions and sugars; it is important in maintaining the turgor of the cell
- The current model of the structure of the plasma membrane is called the fluid mosaic model; most biologists now prefer this model to Davson and Danielli's 'sandwich' model.
- The fluid mosaic model suggests that:
  - the plasma membrane is based on a phospholipid bilayer
  - cholesterol molecules in this bilayer reduce the fluidity of the membrane
  - the plasma membrane has protein molecules 'studded' in the bilayer
  - some proteins are intrinsic (trans-membrane), whilst others are extrinsic (only span part of the membrane)
  - proteins can be:
    - channel proteins with ion pores
    - carrier proteins for facilitated diffusion or for active transport
    - glycoproteins for cell signalling and cell recognition
- Molecules pass through the plasma membrane in several ways, summarised in the table overleaf.

### Activity 4.11

Work in groups. This is a revision exercise. Each group should draw or make a model of one cell organelle and research as much as possible about that organelle. Each group then presents the details of their organelle and its role in the cell to the rest of the class.

Process	Influence of concentration	Requirement for ATP?	Type of particles moved	Transport proteins needed?
Simple diffusion	Occurs from high to low	No	Lipid-soluble, small, non-polar	No
Facilitated diffusion	Occurs from high to low	No	Ions and medium-sized particles	No
Osmosis	Occurs from high to low (water potential)	No	Water molecules	No
Active transport	Occurs from low to high	Yes	Ions and medium-sized, non-lipid-soluble particles	Yes
Endocytosis	Can occur either way	Yes	Very large particles	Yes
Exocytosis	Can occur either way	Yes	Very large particles	Yes

- Cell fractionation separates the components of a cell by centrifugation, heavier organelles being isolated at lower centrifuge speeds.

### End of unit questions

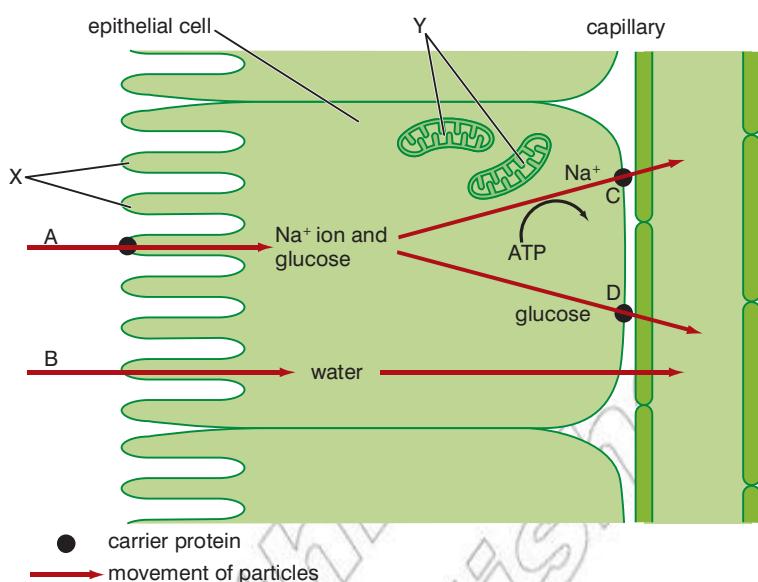
- a) Schleiden and Schwann were the first biologists to put forward a tenable cell theory.
  - State two ideas of this theory that we still accept today.
  - State one idea of this theory that we reject today.
  - Name the biologist who modified Schleiden and Schwann's theory to make it acceptable to us.
- b) State the cell theory as we understand it today.
- Describe the role of each of the following in the development of a cell theory:
  - Robert Hooke
  - Anton van Leeuwenhoek
  - Rene Dutrochet
- Copy and complete the table.

Feature	Prokaryotic cells	Eukaryotic cells
Size	1–10 $\mu\text{m}$	
Nucleus		
DNA	• in a continuous loop	• •
Mitochondria		
Ribosomes	70S ribosomes	

4. The diagram represents the uptake of glucose, sodium ions and water by an epithelial cell in a kidney tubule.

- a) Suggest how the structures labelled X help to maintain a high rate of absorption from the lumen of the kidney tubule.
- b) Explain how the presence of the organelles labelled Y is essential to the absorption of glucose.
- c) Name, with a reason, the transport process occurring at A, B, C and D.

5. The table below shows the percentage masses of protein, lipid and carbohydrate in four different plasma membranes.



Membrane	Percentage mass		
	Protein	Lipid	Carbohydrate
A	18	79	3
B	51	49	0
C	52	44	4
D	76	24	0

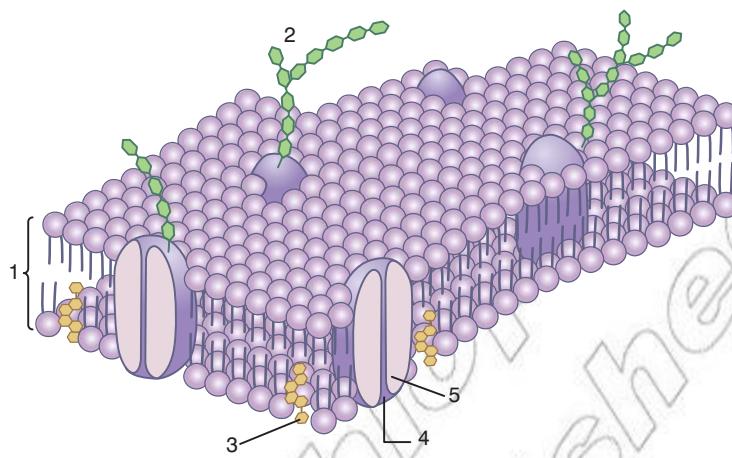
- (i) Calculate the mean ratio of protein to lipid for the four membranes.
- (ii) Describe two functions of proteins in plasma membranes.
- (iii) Describe one function of carbohydrates in plasma membranes.
- (iv) Suggest why plasma membrane D has a much higher protein content than plasma membrane A.

6. a) Copy and complete the table showing the functions of cell organelles.

Organelle	Function
	Contains DNA, regulates cell metabolism
Ribosome	
	Site of aerobic respiration, produces most of the ATP in a cell
	Modifies structure of protein molecules
Lysosome	
Chloroplast	
	Controls entry and exit of substances from cell
	Gives cell support and rigidity

- b) Describe the main stages in the process of cell fractionation. Explain why each stage is necessary.

7. The diagram shows the structure of a plasma membrane.



- a) Name the structures numbered 1, 2, 3 and 4.
- b) Describe the function of the structure labelled 5.
- c) This model of the structure of a membrane is called the fluid mosaic model. Explain why.
8. Write a short essay to describe the history of the development of modern cell theory. In your essay, try to explain why events happened as and when they did. You will be given credit for logical presentation and breadth of coverage as well as for scientific accuracy.

Copy the crossword puzzle below into your exercise book (or your teacher may give you a photocopy) and solve the numbered clues to complete it.



#### Across

1. The organelle in which proteins are modified (5, 4)
6. Process that moves particles across the plasma membrane against the concentration gradient (6, 9)
7. The process by which water moves across the plasma membrane (7)
10. The site of photosynthesis in a plant cell (11)
11. The organelle in which proteins are modified (5, 4)
12. Process that moves particles across the plasma membrane against the concentration gradient (6, 9)
13. The process by which water moves across the plasma membrane (7)
14. The organelle in which proteins are modified (5, 4)
15. The organelle in which proteins are modified (5, 4)
16. The organelle in which proteins are modified (5, 4)
17. The organelle in which proteins are modified (5, 4)
18. The organelle in which proteins are modified (5, 4)
19. The organelle in which proteins are modified (5, 4)
20. The organelle in which proteins are modified (5, 4)
21. The organelle in which proteins are modified (5, 4)
22. The organelle in which proteins are modified (5, 4)
23. The organelle in which proteins are modified (5, 4)

12. The model of plasma membrane structure proposed by Sanger and Nicholson in 1972 (5, 6)
15. The Germans who proposed the first cell theory (9, 3, 7)
18. Unit in which the size of cells and cell organelles is measured (10)
20. The membrane at the surface of the cell (6, 8)
21. The site of aerobic respiration in a cell (13)
22. This Englishman drew dead cork cells that he saw through his microscope (6, 5)
23. Cells with chromosomes and membrane-bound organelles (10)

**Down**

2. These biologists suggested that the plasma membrane was a 'sandwich' of protein and lipid (6, 3, 8)
3. The organelle that controls all the cell's activities (7)
4. Cells with no true nucleus (11)
5. Process by which small, lipid-soluble and non-polar particles cross the plasma membrane (6, 9)
7. The German who stated that 'a cell can only arise from another cell like it' (6, 7)
8. The site of protein synthesis in a cell (8)
10. A measure of the free energy of water molecules in a solution (5, 9)
11. The procedure that allows biologists to separate different cellular organelles (4, 13)
13. A protein in the plasma membrane that moves medium-sized particles across the membrane (7, 7)
14. Anton ... the Dutchman who saw 'animalcules' and bacteria through an early microscope (3, 11)
16. This famous organism has only one cell (6)
17. A protein in the plasma membrane that allows ions to cross (7, 7)
19. Organelles like the nucleus, mitochondrion and chloroplast are said to be ... bound (8)

### Contents

Section	Learning competencies
5.1 Respiration (page 152)	<ul style="list-style-type: none"><li>Describe the structure of ATP and its role in cellular metabolism.</li><li>Explain how ATP is adapted to its role as an energy transfer molecule within a cell.</li><li>Describe how ATP is produced in a cell.</li><li>Locate where the different processes of cellular respiration occur in the cell.</li><li>Explain the role of electron donors and acceptors.</li><li>Describe in detail each stage of aerobic respiration.</li><li>Draw and label the structure of a mitochondrion.</li><li>Explain the processes of alcoholic fermentation and lactate production.</li><li>Appreciate the importance of lactate production during running and other sports.</li><li>Summarise the metabolism of proteins, polysaccharides and lipids.</li></ul>
5.2 How do plants harness light energy in photosynthesis? (page 170)	<ul style="list-style-type: none"><li>Draw, label and describe a chloroplast.</li><li>Locate where light-dependent and -independent processes occur in the chloroplast.</li><li>Name the products of the light-dependent and -independent processes.</li><li>Explain how the structure of a photosystem is related to its function.</li><li>Explain what is meant by a photosynthetic unit.</li><li>Describe how glucose is synthesised in the light-independent reactions of photosynthesis.</li><li>Describe the factors that affect the rate of photosynthesis and explain why they affect the rate.</li><li>Separate photosynthetic pigments by paper chromatography.</li><li>Explain photorespiration and how it is related to higher temperatures.</li><li>Distinguish between C3 and C4 plants and give at least three examples of each.</li><li>Appreciate the importance of C4 plants in Ethiopia.</li><li>Describe the CAM photosynthetic pathway and explain why this brings added benefits to plants living in desert conditions.</li></ul>

## 5.1 Respiration

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

- Describe the structure of ATP and its role in cellular metabolism.
- Explain how ATP is adapted to its role as an energy transfer molecule within a cell.
- Describe how ATP is produced in a cell.
- Locate where the different processes of cellular respiration occur in the cell.
- Explain the role of electron donors and acceptors.
- Describe in detail each stage of aerobic respiration.
- Draw and label the structure of a mitochondrion.
- Explain the processes of alcoholic fermentation and lactate production.
- Appreciate the importance of lactate production during running and other sports.
- Summarise the metabolism of proteins, polysaccharides and lipids.

### What is the ATP molecule like?

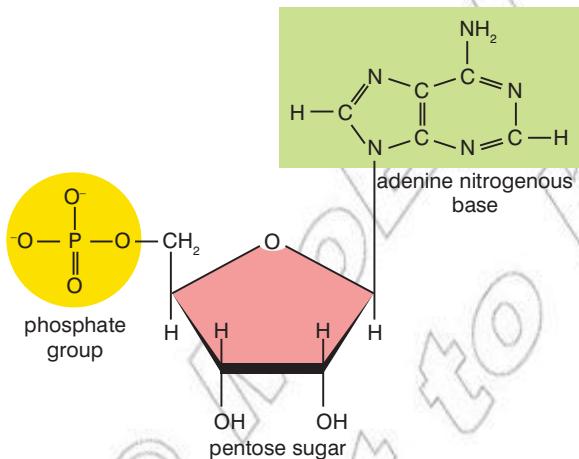


Figure 5.1 A nucleotide containing the nitrogenous base adenine

The full name for ATP is Adenosine Tri-Phosphate – but you will not need to use this name, all biologists always refer to it as ATP. So why bother telling you? Well, it helps to understand about the structure of the molecule.

In unit 2, we learned that nucleic acids are built from nucleotides, like the one shown in figure 5.1.

All nucleotides contain:

- a nitrogenous base (this one contains adenine)
- a pentose sugar
- a phosphate group

The ATP molecule is based on this nucleotide. ATP is sometimes described as a **phosphorylated nucleotide**. If you look at figure 5.2, you can perhaps work out why. When you ‘phosphorylate’ a molecule, you add one or more phosphate groups to it. ATP is essentially the adenine nucleotide with two extra phosphate groups added on – making three in all. Adding the extra phosphates requires energy, particularly when the third phosphate is added. As a result, energy is stored in the ATP molecule and when the bonds that hold this third phosphate in place are broken, the energy is released again. When the third phosphate is removed from ATP,

we are still left with a phosphorylated nucleotide, but this one only has two nucleotides. It is **Adenosine Di-Phosphate** – or **ADP**.

The phosphate group that is split off is usually written as  $P_i$  as a kind of shorthand to save writing out the full formula.

The inter-conversion of ADP and ATP is shown in figure 5.3. Notice that the diagram says that the energy to form ATP can come from 'sunlight or from food'. This is because ATP is formed in both photosynthesis and in cellular respiration.

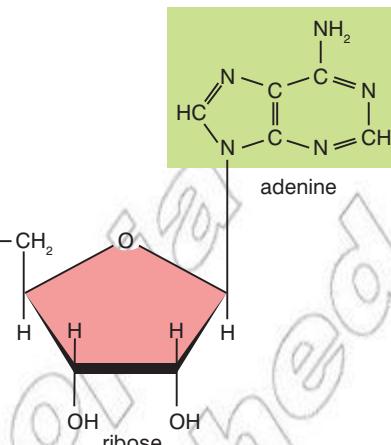
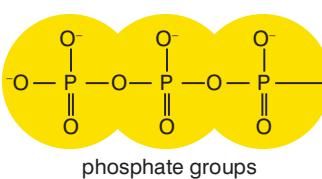


Figure 5.2 The structure of the ATP molecule

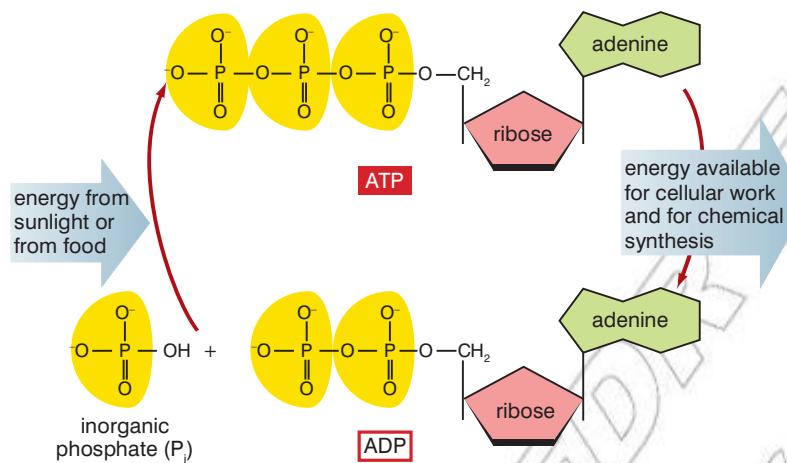


Figure 5.3 The inter-conversion of ADP and ATP

## How is ATP adapted to its role as an energy transfer molecule in cells?

First, we must explain what we mean by an energy transfer molecule. Sunlight energy cannot be used directly by plants (and certainly not by other organisms) to 'drive' the synthesis of proteins – or any other molecules. The same applies to the energy held in a glucose molecule. These two energy sources must be used to produce ATP, which is used to transfer the energy to the relevant cellular process. We say that it is coupled to these processes.

### KEY WORDS

**phosphorylated nucleotide**  
the adding of one or more phosphate groups to a molecule

**adenosine di-phosphate**  
removing the third phosphate from ATP leaves a phosphorylated nucleotide with two nucleotides

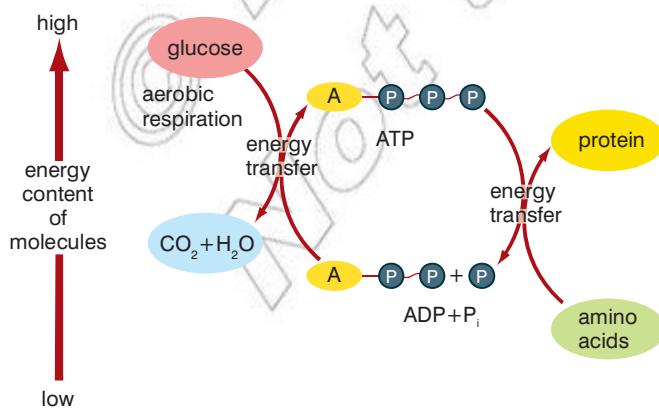


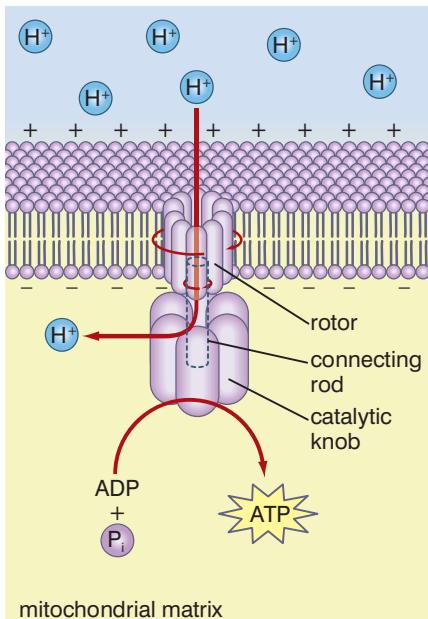
Figure 5.4 Coupled reactions transfer energy in cells

### DID YOU KNOW?

All living cells respire all the time to produce the ATP they need. There are no exceptions.

**KEY WORD**

**ATP synthase enzyme involved in the formation of ATP**



**Figure 5.5 The structure of ATP synthase**

**DID YOU KNOW?****About the ADP/ATP inter-conversion**

More than one eminent biologist has said 'the whole biological world turns on the coupling and uncoupling of the third phosphate of ATP'. This is because this process is virtually the only way in which energy can be harnessed and then released to drive metabolic processes in cells.

ATP is adapted to this role because it:

- releases energy in relatively small amounts that are closely matched to the amounts of energy required in many biological processes occurring inside cells
- releases energy in a single-step hydrolysis reaction, so the energy can be released quickly
- is able to move around the cell easily, but cannot escape from the cell

The following processes are examples of processes that require energy from ATP:

- the synthesis of macromolecules – such as proteins
- active transport across a plasma membrane (see unit 4 for details)
- muscle contraction
- conduction of nerve impulses
- the initial reactions of respiration (the later reactions release energy from glucose to form more ATP)

**How is ATP produced in a cell?**

Almost all the ATP produced in cells is formed in the same way. It obviously involves ADP and  $P_i$  joining to form ATP and this requires an input of energy. What we need to look at is just how it is made to happen.

The formation of ATP involves an enzyme called **ATP synthase**. Figure 5.5 shows the structure of the ATP synthase molecule. The ATP synthase in this diagram is in one of the membranes of a mitochondrion, but it could be in a membrane in a chloroplast.

To understand how it works, you should think of it as a kind of molecular 'water wheel'. When the rotor is made to spin by hydrogen ions passing through it, the energy of the spinning is used to activate sites in the catalytic knob that convert ADP and  $P_i$  to ATP.

In both photosynthesis and aerobic respiration, many of the reactions generate the hydrogen ions that will pass through the ATP synthase to produce ATP.

**How is ATP produced in respiration?**

There are two main pathways by which respiration can produce ATP:

- the aerobic pathway (aerobic respiration) – this requires the presence of oxygen, and
- the anaerobic pathway (anaerobic respiration and fermentation) – this can take place in the absence of oxygen.

## How is ATP produced in aerobic respiration?

A small amount of ATP is produced in a way that does not involve the ATP synthase molecule; this method is called **substrate level phosphorylation**. In this process, another molecule such as phosphoenol pyruvate (the *substrate*) is able to transfer a phosphate group directly to ADP. There is no ATP synthase involved and no  $P_i$ . The process is still catalysed by an enzyme, it is just not ATP synthase.

Figure 5.6 shows how substrate level phosphorylation works. As already mentioned, this process only produces a relatively small amount of the ATP produced in aerobic respiration – in fact it produces about 10% of the total ATP produced in aerobic respiration.

As about 90% of the ATP produced in aerobic respiration is produced by ATP synthase, many of the reactions of this process are geared to producing the hydrogen ions that will spin the rotor of the ATP synthase molecule.

Many different organic molecules can be respired – they are called **respiratory substrates**. However, glucose is the most commonly respired substrate and so we will begin by looking at how this molecule is respired.

## How are hydrogen ions transferred from glucose to ATP synthase?

Two molecules are important in this transfer process:

- Nicotinamide Adenine Dinucleotide (NAD)
- Flavine Adenine Dinucleotide (FAD)

Both are coenzymes and are capable of accepting hydrogen ions. When this happens, we say that the molecules have been **reduced**. We write the reduced forms of the molecules as NADH and FADH or NAD(reduced) and FAD(reduced).

These molecules can release their hydrogen ions and become **oxidised** again. The hydrogen ions can then be used to turn the rotor of ATP synthase.

## What are the stages of aerobic respiration of glucose?

There are four stages in the aerobic respiration of glucose. These are:

- glycolysis
- the link reaction
- Krebs cycle
- electron transport and chemiosmosis

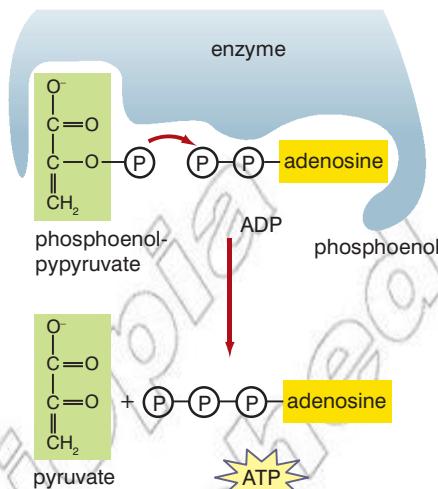


Figure 5.6 Substrate level phosphorylation

### KEY WORDS

**substrate level phosphorylation** when another molecule (substrate) is able to transfer a phosphate group directly to ADP

**respiratory substrates**

organic molecules that can be respired

**reduce** decrease the oxidation state of a substance

**oxidise** increase the oxidation state of a substance

### Activity 5.1

Make a simple model of ATP which you can use to demonstrate how it is converted to ADP and back again by the removal or addition of a phosphate group.

## DID YOU KNOW?

### Oxidation and reduction

Reduction is the opposite of oxidation, in which particles accept oxygen, lose hydrogen or lose electrons. In reduction, a particle loses oxygen, gains hydrogen or gains electrons. When a particle of compound A is oxidised by (say) losing electrons, the electrons have to go somewhere. A particle of compound B accepts the electrons and is reduced. The two processes always happen together and the reactions in which they are involved are called redox (reduction and oxidation) reactions. Figure 5.7 shows this.

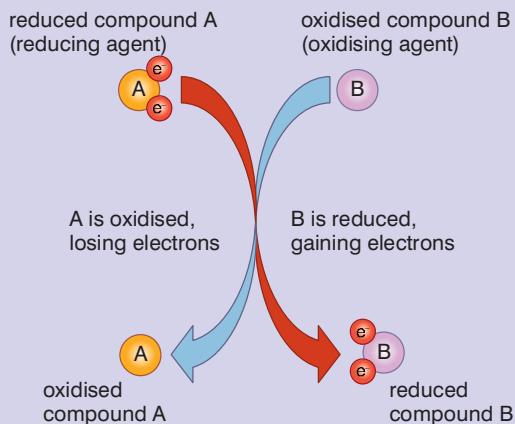


Figure 5.7 A redox reaction

### Activity 5.2

Draw and label a simple diagram of a mitochondrion which shows where the various parts of cellular respiration takes place and demonstrates the importance of the mitochondrial membranes.

The first stage, glycolysis, takes place in the cytoplasm. It does not take place inside the mitochondria because:

- the glucose molecule cannot diffuse through the mitochondrial membranes (it is a medium-sized molecule and is not lipid soluble), and
- there are no carrier proteins to transport the glucose molecule across the membranes.

Glycolysis (literally 'glucose splitting') results in glucose being converted into a smaller molecule containing only three carbon atoms – pyruvate. Pyruvate can enter the mitochondria and so all the other stages take place inside the mitochondrion. Figure 5.8 shows where the stages of aerobic respiration take place.

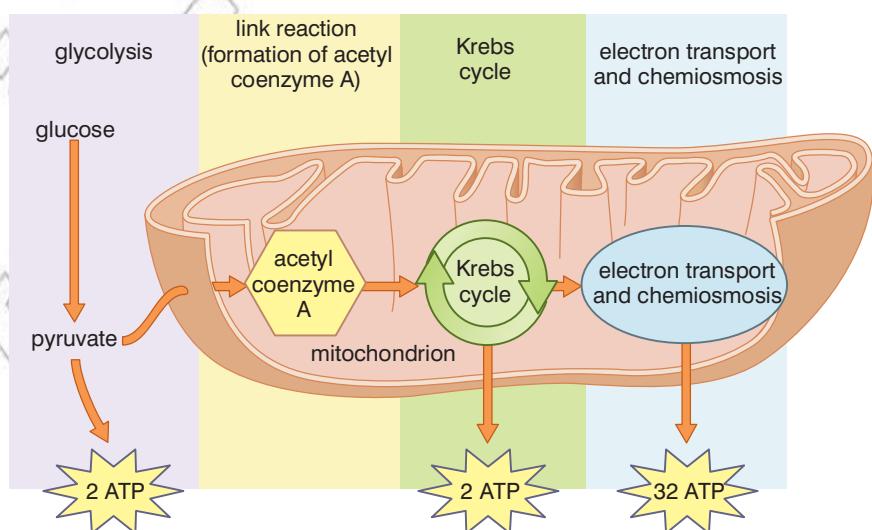


Figure 5.8 The stages of aerobic respiration

In the link reaction, pyruvate is then converted into a two-carbon compound that enters into a cycle of reactions – the Krebs cycle (named after Sir Hans Krebs who discovered the reactions involved). Both these stages take place in the fluid **matrix** of a mitochondrion.

In all three stages (glycolysis, the link reaction and Krebs cycle), hydrogen atoms are transferred to NAD to produce reduced NAD. The Krebs cycle also produces reduced FAD. These molecules later release their hydrogen atoms as protons (hydrogen ions) and electrons in the final stage of aerobic respiration. The electrons pass along a series of molecules called an **electron transport chain**. The protons are used in the chemiosmotic synthesis of ATP as they spin the rotor of the ATP synthase enzyme located in the inner membrane of the mitochondrion. Eventually, the protons (hydrogen ions) and electrons will combine with oxygen to form water.

Without the oxygen, this cannot happen as there is nothing at the end of the electron transport chain to accept the electrons. The electron transport chain grinds to a halt and so does the production of ATP by ATP synthase. Because it is oxygen-dependent, this method of production of ATP is called **oxidative phosphorylation**.

The link reaction, Krebs cycle and the reactions of the electron transport chain all depend on the presence of oxygen. None of these occurs in anaerobic respiration. Glycolysis can take place in the absence of oxygen and is the only energy-releasing process in anaerobic respiration.

### What happens in glycolysis?

The reactions of glycolysis take place in the cytoplasm. The following reactions take place in glycolysis:

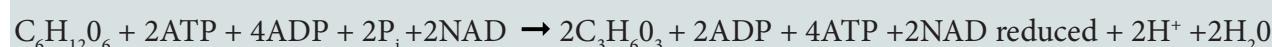
- two molecules of ATP are used to 'phosphorylate' each molecule of glucose. This makes the glucose more reactive
- in the phosphorylation process, it is converted to another six-carbon sugar (fructose 1,6-bisphosphate)
- the fructose 1,6-bisphosphate is split into two molecules of the three-carbon sugar glyceraldehyde-3 phosphate (GP)
- each molecule of GP is then converted into pyruvate, with the production of two molecules of ATP (by substrate level phosphorylation) and one molecule of reduced NAD

The main reactions of glycolysis are shown in figure 5.9. Note:

- the figures in brackets give the number of carbon atoms in that molecule, so (6C) means six carbon atoms per molecule
- two molecules of pyruvate are produced from one molecule of glucose

At the end of glycolysis, there is a net gain of two ATP molecules per molecule of glucose (two molecules are used initially and then four are produced). Two molecules of reduced NAD are also produced (per molecule of glucose). The molecules of pyruvate pass into the mitochondria through carrier molecules in the mitochondrial membrane.

A summary of the overall reaction of glycolysis:



### KEY WORDS

**matrix** fluid in the mitochondrion in which the reactions of the Krebs cycle take place

**electron transport chain** a series of molecules along which electrons travel

**oxidative phosphorylation** oxygen-dependent production of ATP

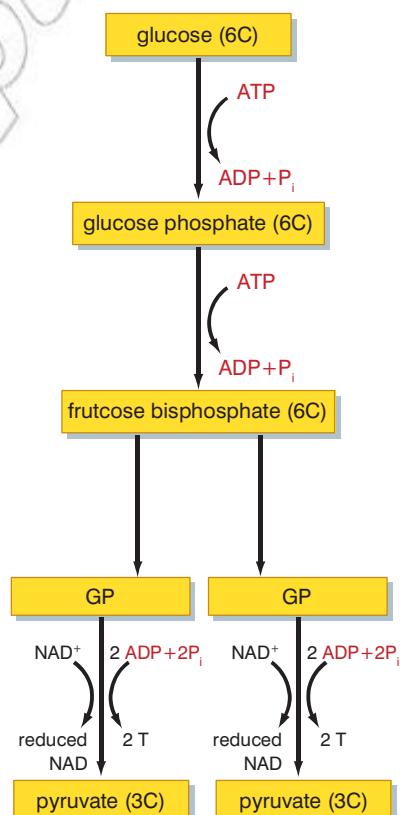


Figure 5.9 The main stages of glycolysis

### Two ideas to keep in mind

1. The idea of net gain of ATP is like the profit a business makes. It invests money in materials, advertising and staff. It sells its product and the extra money is profit – net gain. Glycolysis ‘invests’ two molecules of ATP to make the glucose reactive, then, later, produces four molecules of ATP – a net gain of two molecules of ATP.
2. There are two molecules of pyruvate made from each molecule of glucose. So all the gains of ATP and reduced NAD and reduced FAD that accrue from each pyruvate must be doubled to give the gain from each molecule of glucose.

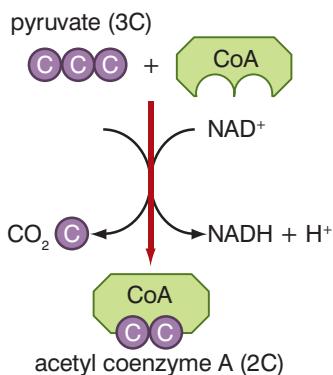


Figure 5.10 The link reaction

Both of these stages of respiration take place in the fluid matrix of the mitochondrion.

In the link reaction, a molecule of pyruvate reacts with a molecule of **coenzyme A** (CoA) to form a molecule of **acetyl coenzyme A** (acetyl CoA). In the reaction:

- hydrogen is lost and reduced NAD is formed; removing hydrogen from a molecule is **dehydrogenation**
- a carbon atom is lost to form carbon dioxide; removing carbon from a molecule is **decarboxylation**.

The acetyl coenzyme A then reacts with a C4 molecule (a molecule containing four carbon atoms) called oxaloacetate. In the reaction, acetyl CoA breaks down into:

- a two-carbon ‘acetyl’ group, which reacts with the C4 compound oxaloacetate to form a C6 compound, and
- the original coenzyme A molecule, which is reused in further reactions with other molecules of pyruvate.

This is the first reaction of the Krebs cycle.

### What happens in the Krebs cycle?

- the two-carbon group from acetyl coenzyme A reacts with the four-carbon compound **oxaloacetate** to form a six-carbon compound called **citrate**
- citrate then loses a carbon atom (is decarboxylated) to form a five-carbon compound and CO<sub>2</sub> is produced
- the five-carbon compound is then further decarboxylated to form a four-carbon compound and CO<sub>2</sub> is again produced; a molecule of ATP is also produced by substrate level phosphorylation
- the four-carbon compound undergoes several molecular transformations to regenerate the original four-carbon compound (oxaloacetate) and the cycle is complete and can begin again with oxaloacetate reacting with another molecule of acetyl CoA

### KEY WORDS

**coenzyme A** coenzyme derived from pantothenic acid needed for respiration

**acetyl coenzyme A** produced by the reaction of coenzyme A with a molecule of pyruvate

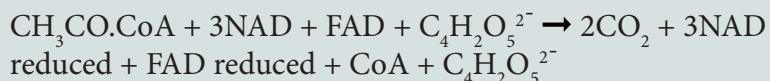
**dehydrogenation** removing hydrogen from a molecule

**decarboxylation** removing carbon from a molecule

- in several reactions in the cycle, reduced NAD is produced and, in just one reaction, reduced FAD is produced

The reactions of the Krebs cycle are summarised in figure 5.12.

A summary of the overall reaction of the Krebs cycle:



## KEY WORDS

**oxaloacetate** an ester of oxaloacetic acid

**citrate** an ester of citric acid

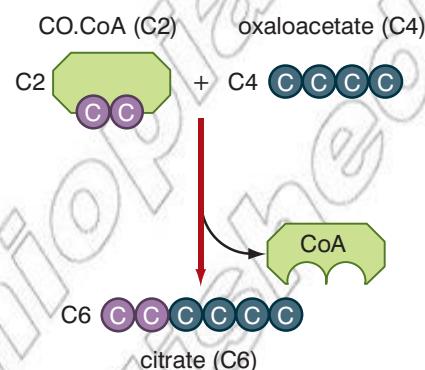


Figure 5.11 The first reaction of the Krebs cycle

## What happens in the electron transport chain and chemiosmosis?

The electron transport chain and chemiosmosis together make up the process of oxidative phosphorylation.

Whereas the reactions of the link reaction and Krebs cycle take place in the fluid matrix of the mitochondrion, the reactions of the electron transport chain and chemiosmosis take place on the inner mitochondrial membrane. Figure 5.13 shows an electron-micrograph of a mitochondrion.

On the cristae, the following events take place:

- the hydrogen atoms carried by reduced NAD and reduced FAD are released and split into protons (hydrogen ions) and electrons
- the electrons pass along a series of electron carriers that form the transport chain; they lose energy as they pass from one carrier to the next
- three of the electron carriers are proton pumps that move protons from the matrix of the mitochondrion to the inter-membrane space
- as the electrons are transferred through these three proton pumps, the energy they lose powers the pumps which move the protons into the inter-membrane space
- electrons from reduced NAD make this happen at all three pumps

The molecules that act as electron carriers in the electron transport chain are:

- reduced NAD dehydrogenase (also a proton pump)
- ubiquinone (also a proton pump), and
- a number of carriers called cytochromes (these are proteins that contain iron); two of them form a complex that acts as the third proton pump.

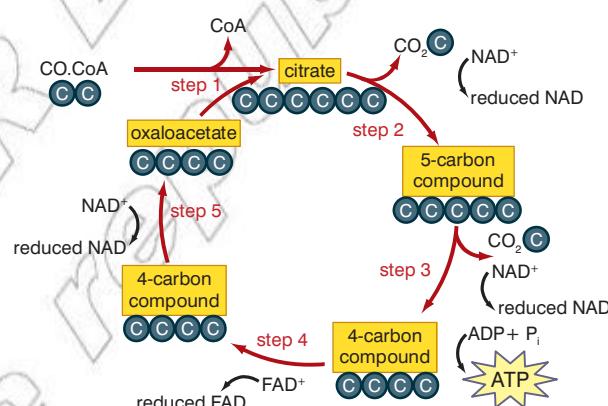


Figure 5.12 The main stages of the Krebs cycle

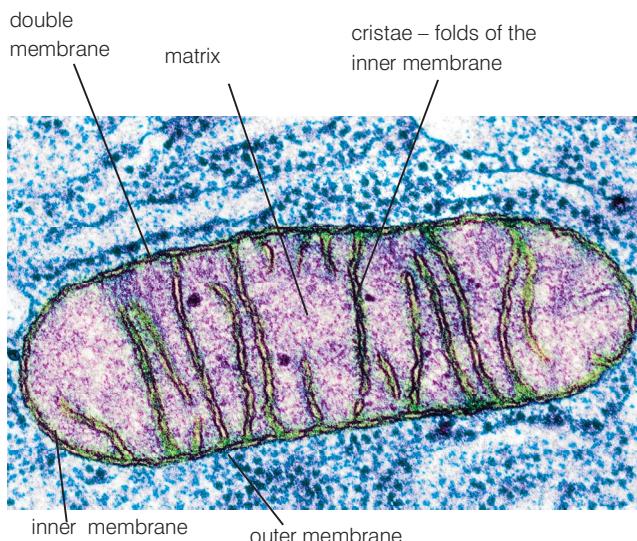
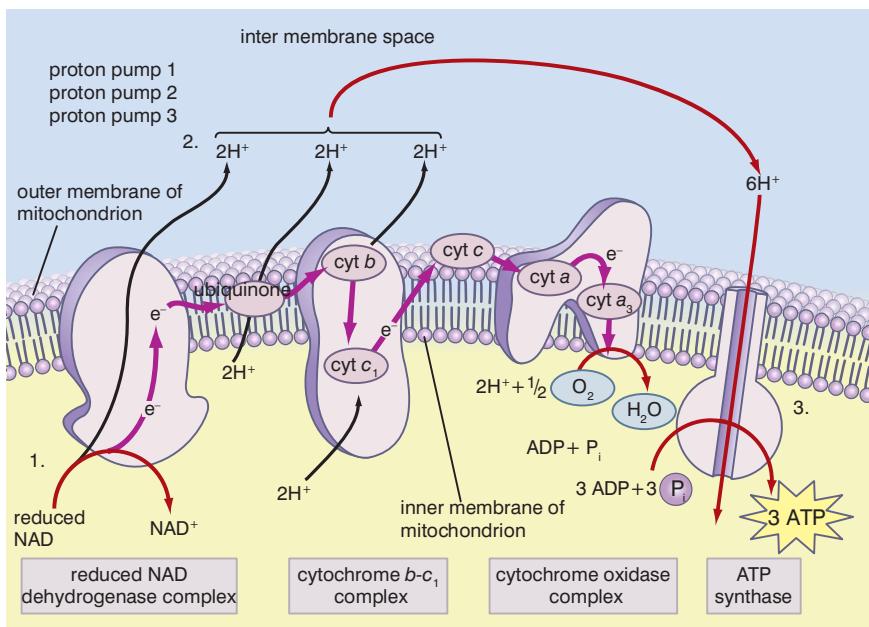


Figure 5.13 An electron-micrograph of a mitochondrion

The arrangement of these molecules is shown in figure 5.14.



**Figure 5.14** The carrier molecules in the electron transport chain on the inner membrane of a mitochondrion

At the end of the electron transport chain, the electrons combine with protons and with oxygen to form molecules of water. Because of this, oxygen is known as the **terminal electron acceptor**.

Whereas reduced NAD is dehydrogenated by the NAD dehydrogenase complex, reduced FAD is dehydrogenated by ubiquinone. So electrons from reduced FAD only operate two of the three proton pumps.

Because of the action of the proton pumps, protons accumulate in the inter-membrane space creating a higher concentration there than in the matrix (on the other side of the membrane). This proton gradient results in protons diffusing through the ATP synthase molecule (down the concentration gradient) making the synthase rotor 'spin' and produce ATP from ADP and  $P_i$ . The diffusion of hydrogen ions through the ATP synthase is chemiosmosis.

The oxidation of one molecule of reduced NAD results in six protons passing through ATP synthase and so leads to the synthesis of three molecules of ATP.

The oxidation of one molecule of reduced FAD results in four protons passing through ATP synthase and so leads to the synthesis of just two molecules of ATP.

By adding up the number of molecules of ATP produced, the model of aerobic respiration we have discussed predicts that there will be a net yield of 38 molecules of ATP per molecule of glucose.

## KEY WORD

## terminal electron acceptor

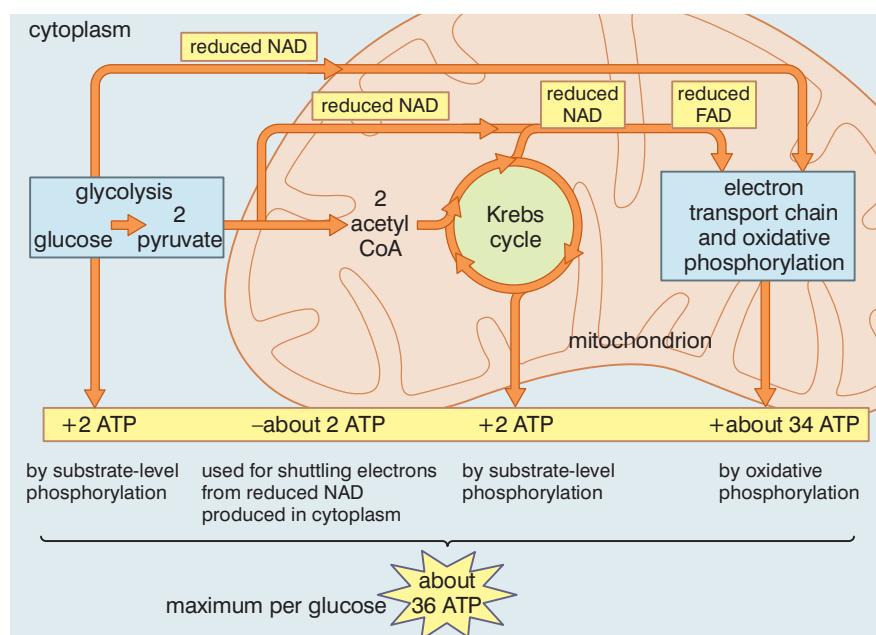
*the final molecule at the end of the electron transport chain to accept an electron*

A summary of the overall reaction of the electron transport system:

6 reduced NAD (from Krebs' cycle) + 2 reduced NAD (from glycolysis) + 2 reduced FAD (from Krebs' cycle) + 30 ADP + 30 Pi - 2ATP (used in proton pumps)  $\rightarrow$  36 ATP + 8 NAD + 2FAD

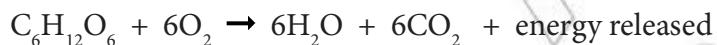
In practice, this is not achieved because some energy (the equivalent of just over two molecules of ATP) is used to drive the proton pumps. The actual yield is about 36 molecules of ATP per molecule of glucose.

Figure 5.15 summarises the production of ATP in aerobic respiration.



**Figure 5.15** The production of ATP during the aerobic respiration of glucose

The summary equation for aerobic respiration is:



### Respirometers

Respirometers come in several different forms, but they all work on the principle that oxygen is used in aerobic respiration and carbon dioxide is produced.

The overall summary equation for the aerobic respiration of glucose is:



This equation predicts that the volume of oxygen used ( $6\text{O}_2$ ) is equal to the volume of carbon dioxide produced ( $6\text{CO}_2$ ). This is the basis of how respirometers work.

Figure 5.16 overleaf shows a basic respirometer. For every molecule of oxygen the organism uses, a molecule of carbon dioxide will be produced, but, the carbon dioxide will be absorbed by the potassium hydroxide (KOH). So, over time, there will be a reduction in volume inside the respirometer.

### Activity 5.3

Make a large annotated wall chart showing glycolysis and Krebs cycle and how they are linked together. Make sure you show the different compounds and where ATP is formed. This should be as accurate as possible so it can form the basis of your revision of this complex topic.

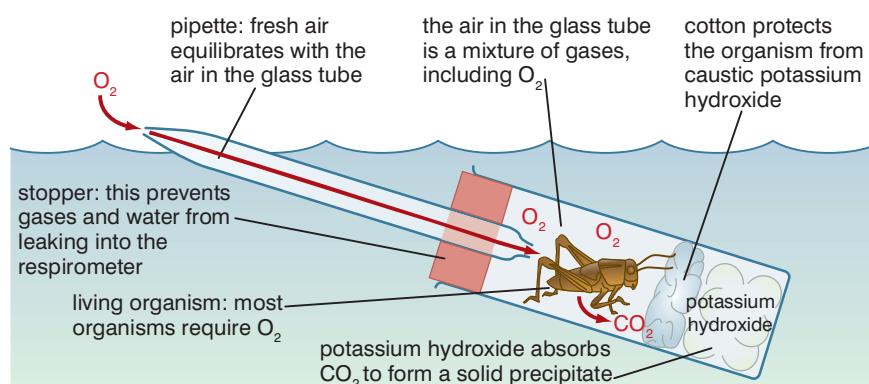


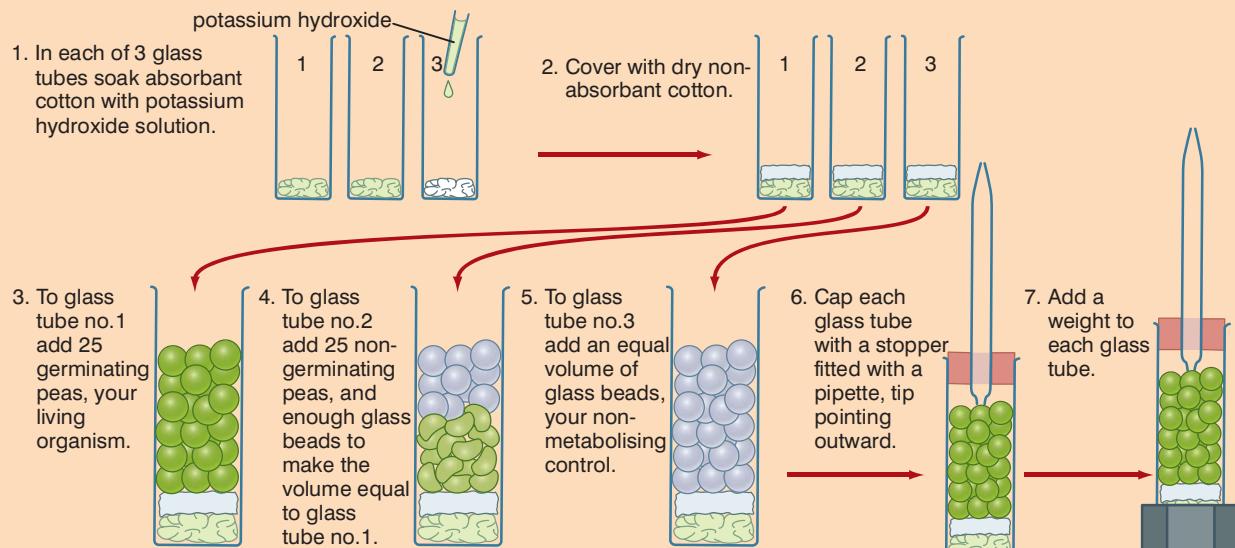
Figure 5.16 A basic respirometer

Figure 5.16 shows the respirometer placed under water. As the volume inside the respirometer decreases, water will enter the pipette. The volume of water entering is equal to the volume of oxygen being used up. We can use this to measure the rate of respiration by measuring how much oxygen is used in a set period of time (say 10 minutes) then working out a rate per minute.

### Activity 5.4: Measuring the rate of respiration of pea seeds

#### You will need

- 3 respirometers, set up as in figure 5.17
- 3 water baths



#### Method

1. Place the three respirometers in a water bath at 20 °C with the tips of the pipettes out of the water, resting on a sling of tape. Leave them for five minutes to equilibrate.
2. Lower the tips of each pipette into the water and immediately:

Figure 5.17 How to assemble a respirometer

- take a reading from each
- start a timer
- 3. Take a reading from each respirometer every two minutes for 20 minutes.
- 4. Repeat the investigation at 30 °C and at 40 °C.

The volume of oxygen used is the same as the volume of water that has entered the pipette.

There are several aspects of this experiment you should consider:

- Why did we use tubes containing non-germinating peas and glass beads as well as the tube with the germinating peas?
- Why does the water move into the pipette during the investigation?
- What was the purpose of leaving the tubes for five minutes before starting each investigation?
- How could you investigate temperatures lower than 20 °C?

A different design of respirometer removes the need to set up three at the same time. This is shown in Figure 5.19.

In this design, the tap on tube A is left open for five minutes at the start of the investigation and the levels of the coloured oil in the U-tube are equalised with the syringe.

When the investigation starts, the tap is closed and the coloured oil moves towards the organisms (tube B). The distance it moves per minute is a measure of the rate of respiration.

Once one investigation is complete, the tap can be reopened and the levels reset using the syringe ready for a repeat or another investigation at a different temperature. In this design of respirometer, tube A acts as a control tube and so another set of apparatus with glass beads or non-germinating seeds is not necessary. Figure 5.20 shows how this respirometer can be used to investigate the rate of respiration at different temperatures.

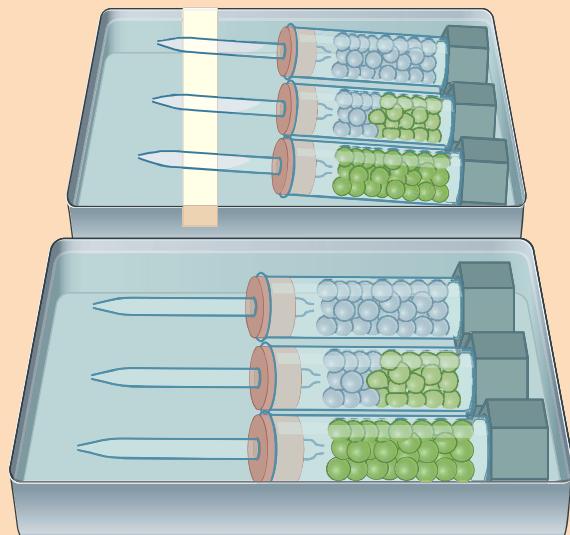


Figure 5.18 Carrying out the experiment

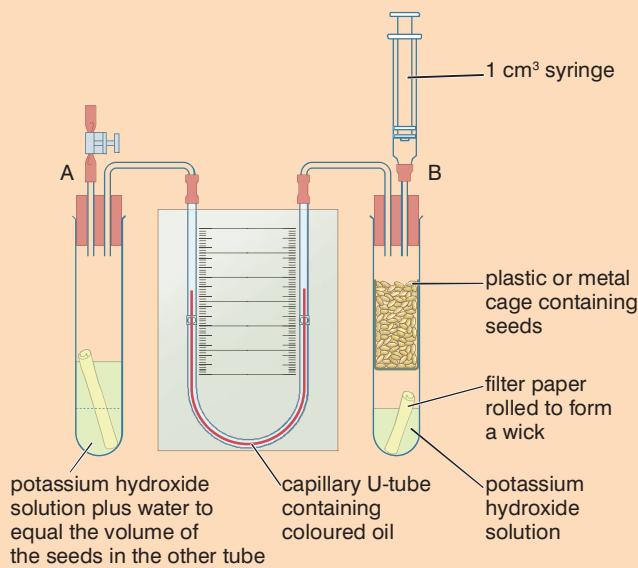


Figure 5.19 A more sophisticated respirometer

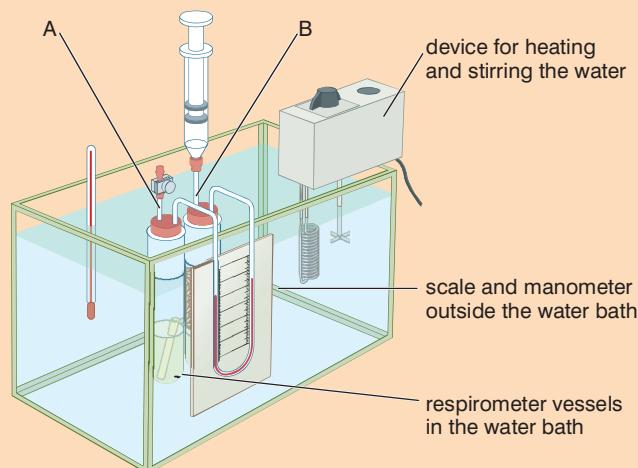


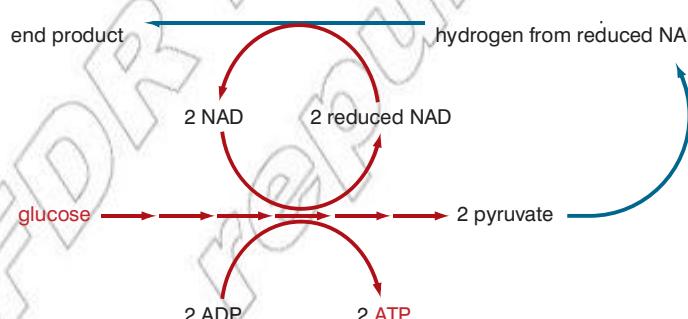
Figure 5.20 Using the respirometer in a water bath

## What happens in the anaerobic pathway?

If there is no oxygen present, the final reaction of oxidative phosphorylation, where electrons and protons react with oxygen to form water, cannot take place. As a result, the electron transport chain comes to a halt. No protons are pumped and the action of ATP synthase also stops.

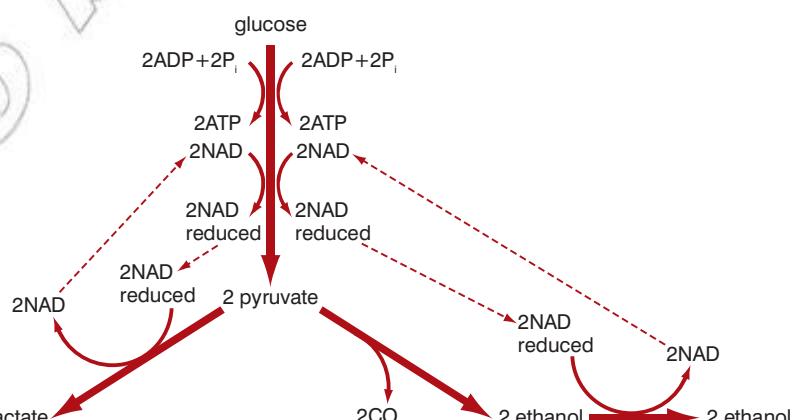
There is a further ‘knock-on’ effect. If the electron transport chain does not function, NAD is not regenerated from reduced NAD and FAD is not regenerated from reduced FAD. Very quickly, the Krebs cycle and the link reaction come to a halt as both NAD and FAD are required in their oxidised forms for the Krebs cycle to function. NAD is also required in the link reaction and so this comes to a halt also.

However, glycolysis *can* continue even though it also requires NAD. This is because the reduced NAD formed during glycolysis can be regenerated under anaerobic conditions by converting the pyruvate into another product in a reduction reaction. Reduced NAD supplies the hydrogen for this reduction and becomes oxidised itself. It is therefore regenerated and can be used again in glycolysis.



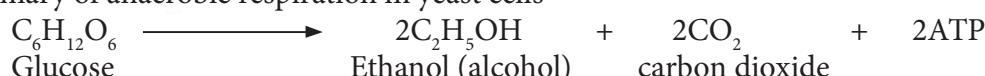
**Figure 5.21** How NAD is regenerated in fermentation

Different organisms produce different fermentation end products. Animal cells produce lactate (lactic acid) when they ferment glucose. Yeast cells produce ethanol (ethyl alcohol). But both only produce two molecules of ATP per molecule of glucose.

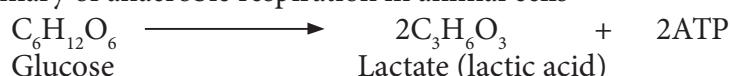


**Figure 5.22** The fermentation processes in animal cells and yeast

## A summary of anaerobic respiration in yeast cells



## A summary of anaerobic respiration in animal cells



## KEY IDEA

## Lactate formation during exercise

During exercise, the energy demand of muscle cells increases greatly. More glucose is respired to meet the demand. However, sometimes, aerobic respiration is insufficient to meet this energy demand. Fermentation of glucose supplies the extra energy. But it also forms lactate and as this accumulates, it leads to muscle fatigue. Also, fermentation only yields 2 molecules of ATP per molecule of glucose whereas aerobic respiration yields 38. However, fermentation is a much faster process and can produce a lot of ATP quickly, over a short period of time. The ATP used in sprints and short-distance runs is nearly all generated anaerobically.

But, due to muscle fatigue, this cannot be sustained. Longer races must be run slower to allow aerobic respiration to produce the ATP at its slower rate.

Lactate, once formed, can be used to regenerate glucose or be metabolised as an energy source by the liver. Figure 5.23 shows how the Cori cycle makes this happen.

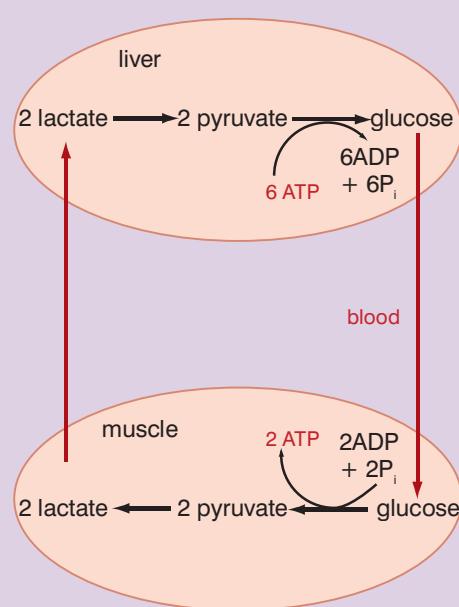


Figure 5.23 The Cori cycle

Other organisms produce other fermentation products, many of which are made use of in different industries. Figure 5.24 shows some of these.

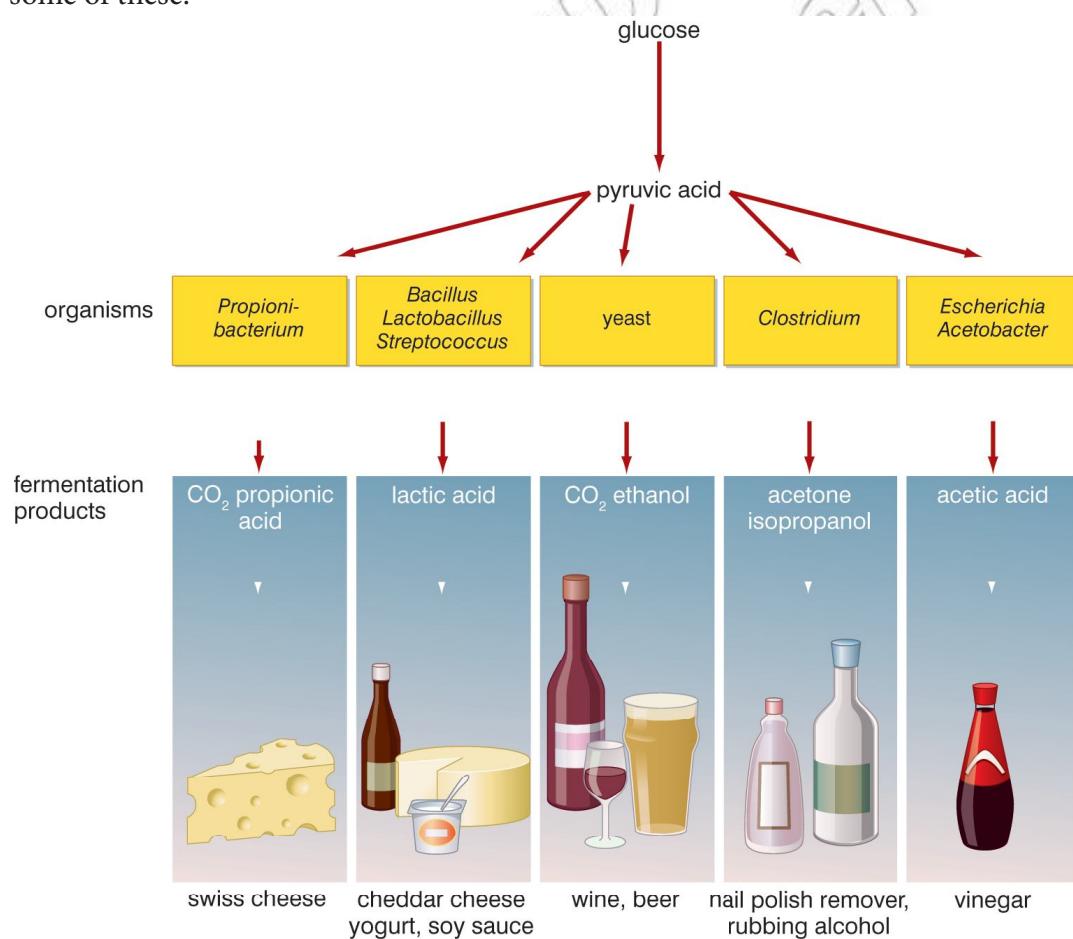


Figure 5.24 Some uses of fermentation in industry

### Activity 5.5: Investigating the rate of fermentation in yeast

There are many different ways of carrying out this investigation, ranging from those using only basic equipment to sophisticated electronically monitored fermenters. Figure 5.25 shows just about the simplest way of investigating this. The test tube containing the yeast and glucose can be held in a water bath at the desired temperature and the number of bubbles collected per minute recorded. However, rate of bubbling is not the most accurate way of measuring rate of respiration. Are you sure that all the bubbles are the same volume? The method is improved if the test tube of water is replaced by a gas syringe.

Using this basic equipment, can you devise experiments to investigate:

- the effect of temperature on the rate of fermentation
- the effect of different substrates (different sugars) on the rate of fermentation
- the effect of substrate concentration on the rate of fermentation

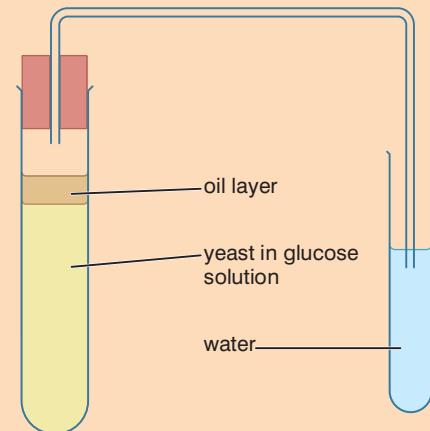
In your plans, you should make clear:

- the independent variable
- the dependent variable
- other variables that you intend to control as well as:
  - why you need to control them, and
  - how you intend to control them.

More sophisticated fermenters (such as that shown in figure 5.26) control all the conditions inside the fermenter and monitor the changes in the concentration of oxygen, carbon dioxide and ethanol. Other sensors could also monitor the concentration of the sugar being fermented.

Figure 5.27 shows the output from one such fermenter.

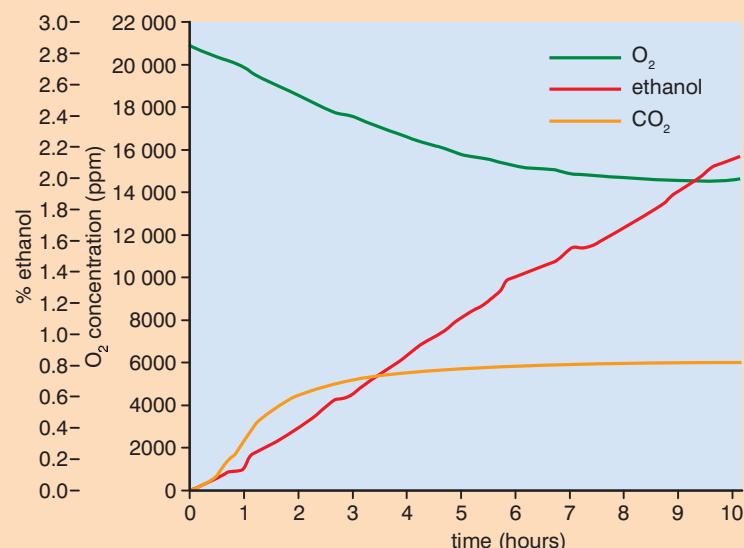
Can you explain the changes in the concentrations of the various substances as fermentation proceeds?



**Figure 5.25** A simple way of investigating the rate of fermentation in yeast



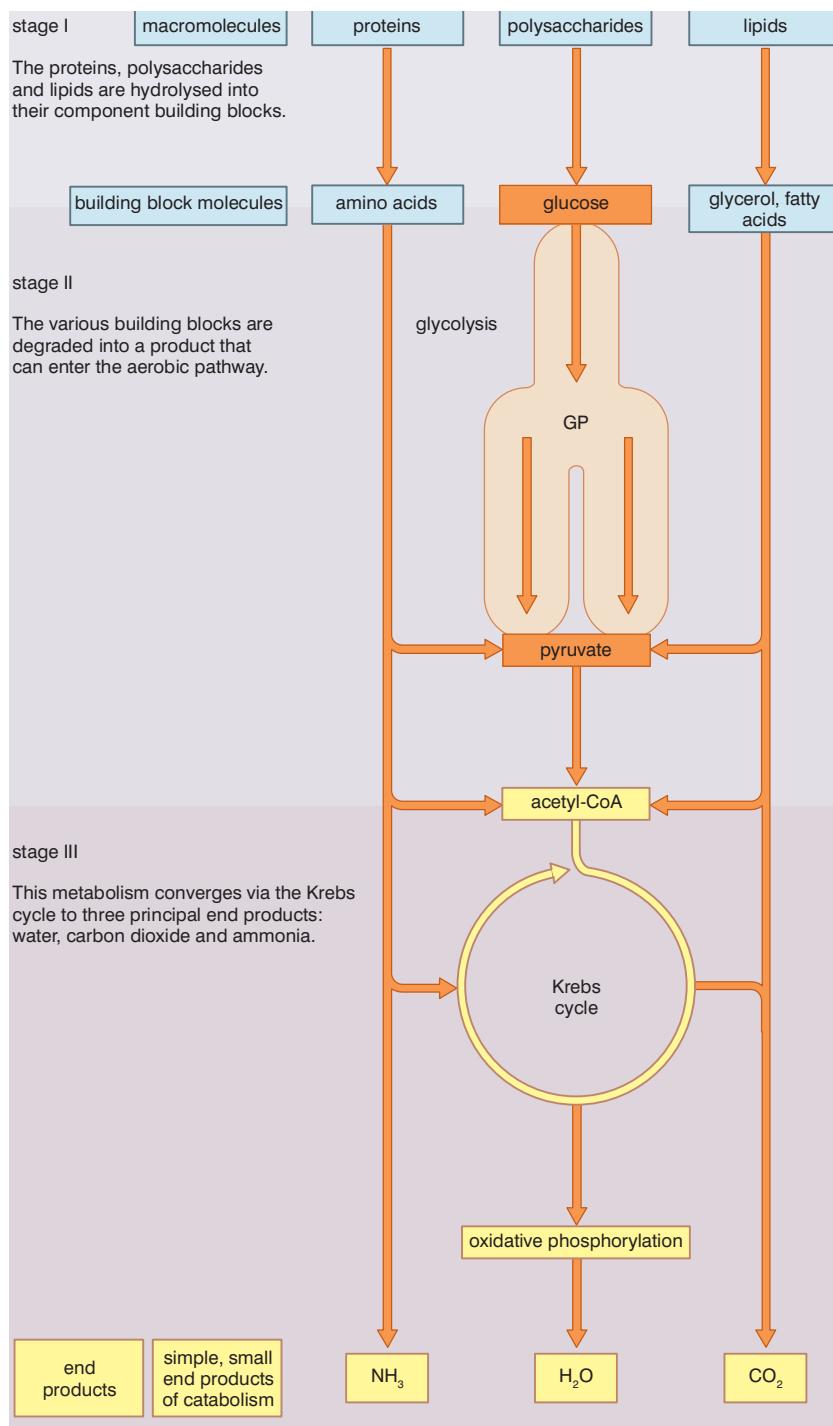
**Figure 5.26** A fermenter that monitors changes electronically



**Figure 5.27** Output from a fermenter

## What substances can be used as energy sources?

We have so far concentrated on the respiration and fermentation of glucose. But lipids and proteins can also be used as respiratory substrates. Figure 5.28 shows how lipids and proteins are converted into substances that can enter the aerobic respiration pathway at some point. The metabolism of proteins, lipids and carbohydrates 'converges' on the Krebs cycle.



### Activity 5.6

Plan a simple demonstration of anaerobic respiration in the muscles. Several students work together. You are going to investigate how quickly anaerobic respiration sets in by repeating a simple action until the muscles begin to ache. This action could be stepping on and off a step or box, and repeatedly lifting a book from the surface of the desk to the shoulder. All start the action together and time how long it takes for the muscle aching which indicates a build up of lactic acid to develop. Explain exactly what is happening in the muscles and discuss what individuals can do to change their physiology and maintain aerobic respiration in their muscles for as long as possible.

**Figure 5.28** The metabolic pathways by which carbohydrates, lipids and proteins are respired

**Activity 5.7**

The Krebs cycle was worked out by Hans Krebs. He was awarded a Nobel Prize for his work. Find out as much as you can about Krebs and how he came to discover the chemistry of aerobic respiration in the cell. You can look in encyclopaedias, in other text books and online, e.g.

[http://www.nobel-winners.com/Medicine/hans\\_adolf\\_krebs.html](http://www.nobel-winners.com/Medicine/hans_adolf_krebs.html)

[http://nobelprize.org/nobel\\_prizes/medicine/laureates/1953/krebs-bio.html](http://nobelprize.org/nobel_prizes/medicine/laureates/1953/krebs-bio.html)

[http://en.wikipedia.org/wiki/Hans\\_Adolf\\_Krebs](http://en.wikipedia.org/wiki/Hans_Adolf_Krebs)

**Review questions**

Choose the correct answer from A to D.

1. The ATP molecule is sometimes described as:
  - a phosphorylated nitrogenous base
  - a phosphorylated nucleotide
  - a glycosated nucleotide
  - a glycosated nitrogenous base
2. ATP is formed from:
  - AMP and  $P_i$
  - ADP and AMP
  - ADP and  $P_i$
  - AMP and A
3. Examples of processes requiring ATP include:
  - simple diffusion and active transport
  - active transport and facilitated diffusion
  - conduction of nerve impulses and osmosis
  - active transport and protein synthesis
4. The ATP synthase molecule produces ATP when:
  - electrons turn the rotor to activate sites in the catalytic knob
  - hydrogen ions spin the catalytic knob
  - electrons spin the catalytic knob
  - hydrogen ions turn the rotor to activate sites in the catalytic knob
5. ATP is an ideal energy transfer molecule in cells because it:
  - releases energy in small amounts
  - releases energy quickly
  - can move freely in, but not escape from, the cell
  - all of the above
6. Which of the following does not take place during the Krebs cycle?
  - oxidative phosphorylation
  - substrate-level phosphorylation
  - electron transport
  - the link reaction
7. In fermentation:
  - oxidative phosphorylation does not take place
  - substrate-level phosphorylation does take place
  - NAD is reduced in glycolysis
  - all of the above

8. Which of the following statements about mitochondria is NOT true?
- A the carrier molecules of the electron transfer chain are found on the inner mitochondrial membranes
  - B the reactions of the Krebs cycle take place inside the mitochondria
  - C all of the ATP needed by the cell is made in the mitochondria
  - D much of the ATP needed by the cell is made in the mitochondria
9. In the electron transport chain, electrons are passed:
- A from the lumen of the mitochondrion to the inter-membrane space
  - B from the inter-membrane space to the lumen of the mitochondrion
  - C through ATP synthase
  - D along a series of electron carriers
10. Oxidative phosphorylation includes:
- A the electron transport chain and chemiosmosis
  - B the electron transport chain and the Krebs cycle
  - C the Krebs cycle and chemiosmosis
  - D none of these
11. In the Krebs cycle:
- A some ATP is made by oxidative phosphorylation
  - B the four-carbon compound oxaloacetate is regenerated
  - C ATP is used
  - D the six-carbon compound citrate is split into two three-carbon compounds
12. When compared with aerobic respiration, fermentation of glucose by yeast:
- A yields less ATP per molecule of glucose
  - B produces lactate
  - C produces more  $\text{CO}_2$
  - D none of the above
13. Which of the following statements about aerobic respiration is correct?
- A Glycolysis takes place in the matrix of the mitochondrion.
  - B Carrier molecules of the electron transport chain exist on the outer membrane of the mitochondrion.
  - C A high concentration of hydrogen ions builds up in the matrix of the mitochondrion.
  - D The Krebs cycle takes place in the matrix of the mitochondrion.

**Activity 5.8**

Work as a whole class with your teacher. Before you begin to study photosynthesis, brainstorm everything you know about photosynthesis from your studies in the lower grades. Your teacher will put all your ideas together into a big spider diagram and keep it until the end of this topic. Then you can look back and see how much you have learned.

14. In a respirometer...
- the amount of oxygen used by the organism is replaced with an equal amount of carbon dioxide
  - the carbon dioxide given off is absorbed by potassium hydroxide
  - the breathing rate of an organism is measured
  - we measure the uptake of oxygen by an organism
15. Which of the following occur in both aerobic respiration and fermentation in mammals:
- substrate-level phosphorylation
  - chemiosmosis
  - link reaction
  - decarboxylation

## 5.2 How do plants harness light energy in photosynthesis?

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

- Draw, label and describe a chloroplast.
- Locate where light-dependent and -independent processes occur in the chloroplast.
- Name the products of the light-dependent and -independent processes.
- Explain how the structure of a photosystem is related to its function.
- Explain what is meant by a photosynthetic unit.
- Describe how glucose is synthesised in the light-independent reactions of photosynthesis.
- Describe the factors that affect the rate of photosynthesis and explain why they affect the rate.
- Separate photosynthetic pigments by paper chromatography.
- Explain photorespiration and how it is related to higher temperatures.
- Distinguish between C<sub>3</sub> and C<sub>4</sub> plants and give at least three examples of each.
- Appreciate the importance of C<sub>4</sub> plants in Ethiopia.
- Describe the CAM photosynthetic pathway and explain why this brings added benefits to plants living in desert conditions.

## Photosynthesis

In photosynthesis, light energy is used in a series of reactions that lead to the synthesis of a range of organic molecules. The energy that entered the system as light is now held in the organic molecules produced. It is now chemical energy. When energy is changed from one form to another, we say it has been **transduced**. This takes place in a series of reactions called the **light-dependent reactions**. Light energy is absorbed by special **photosensitive pigments** such as **chlorophyll** in the chloroplasts. The light-dependent reactions take place in the membranes of the **thylakoids** in the chloroplasts. The liquid stroma is the site of the light-independent reactions, in which carbohydrates are synthesised. Chemical reactions like these take place most effectively in solution, rather than if some were fixed in membranes.

### How is the structure of a chloroplast suited to its function?

The chlorophyll and other photosensitive pigment molecules are arranged in special **photosystems** that are linked to electron transport chains (ETCs). The molecules of the photosystems and the electron transport chains are fixed in the membranes of the thylakoids. This makes the process much more efficient than if they were just floating around in a solution.

There are two different photosystems, each sensitive to light of a different wavelength and linked to a different electron transport chain. These are called **photosystem I** and **photosystem II**.

### KEY WORDS

**transduced** conversion of energy from one form to another

**light-dependent reactions** reactions of photosynthesis dependent on light

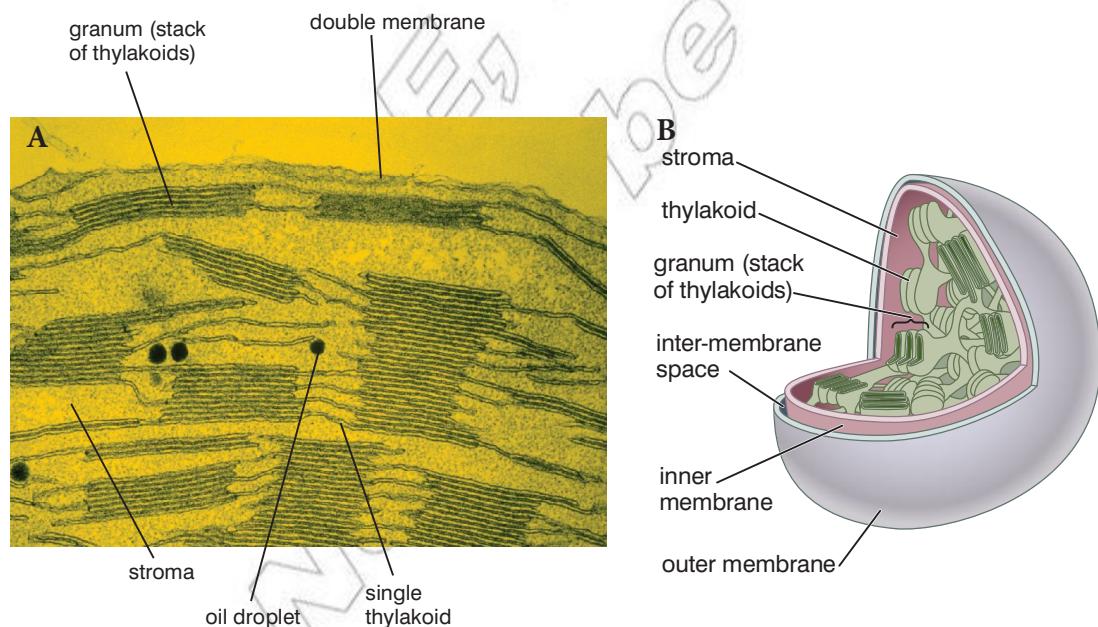
**photosensitive pigments** pigments having a response to light

**chlorophyll** green pigment that absorbs blue and red light

**thylakoids** flattened sacs inside a chloroplast on which light-dependent reactions of photosynthesis take place

**photosystems** biochemical mechanism by which chlorophyll absorbs light energy

**photosystem I** photosystem in photosynthetic light reactions. Discovered before **photosystem II**



**Figure 5.29** The structure of a chloroplast: **A** A transmission electron micrograph of a section through a chloroplast; **B** A three-dimensional representation of the structure of a chloroplast

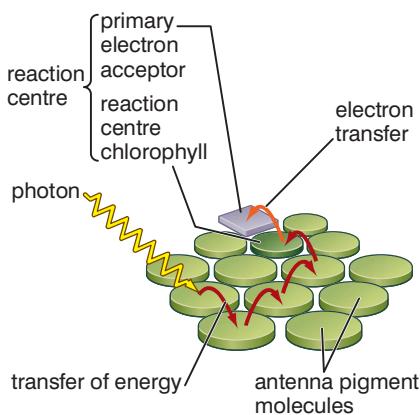


Figure 5.30 The structure of a photosystem

### Activity 5.9

Draw and label the structure of a chloroplast, showing where the light-dependent and the light-independent reactions take place.

Compare this diagram to the one you made earlier of a chloroplast and describe the similarities and differences between these two organelles.

### What is the structure of a photosystem?

A photosystem consists of a number of pigment molecules all clustered around one particular chlorophyll molecule called the reaction centre molecule. This cluster of pigment molecules is called an antenna complex. Only the reaction centre molecule is positioned next to the electron transport chain. Energy absorbed by other molecules in the photosystem is transferred to the **reaction centre molecule**, where the light-dependent reactions begin. Different pigment molecules in the **antenna complex** can absorb different wavelengths of light, making the whole system more efficient. The pigments in the antenna complex include chlorophyll a, chlorophyll b and carotenoids. The reaction centre molecule is always chlorophyll a. The range of wavelengths each molecule absorbs is its **absorption spectrum**. Figure 5.31A shows the absorption spectrum of chlorophyll a, chlorophyll b and carotenoids. Figure 5.31B shows the **action spectrum** for different wavelengths of light. This shows how effective photosynthesis is at each wavelength.

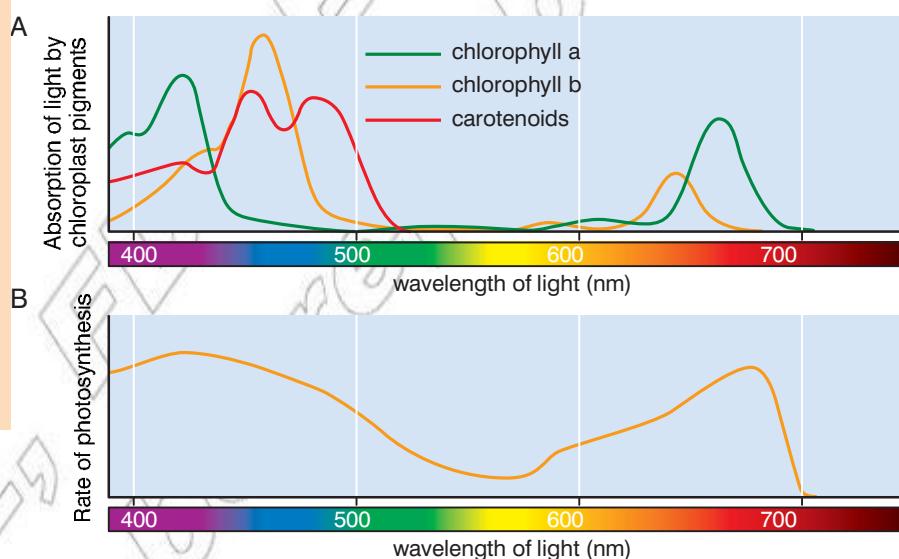


Figure 5.31A The absorption spectrum of chlorophyll a, chlorophyll b and carotenoids. Notice how between them they absorb most wavelengths of visible light – except 500 nm to 600 nm – green! Plants are green because these wavelengths are reflected, not absorbed; B The action spectrum for different wavelengths of light. Notice the dip in the 'green' region of the spectrum

### KEY WORDS

**reaction centre molecule** where light-dependent reactions begin

**antenna complex** an array of protein and chlorophyll light-harvesting molecules embedded in the thylakoid membrane

**absorption spectrum** the range of wavelengths a molecule absorbs

**action spectrum** the photosynthesis effectiveness of each wavelength

### What happens in the light-dependent reactions?

The light-dependent reactions use light energy to 'drive' the synthesis of two molecules that will, in turn, drive the light-independent reactions. These two molecules are:

- ATP – this provides the energy for the reactions, and
- reduced NADP – this provides the hydrogen ions for a key reduction reaction.

NADP is very similar to NAD that is used in respiration and it has the same function – transporting hydrogen ions.

The main events in the light-dependent reactions are summarised in figure 5.32.

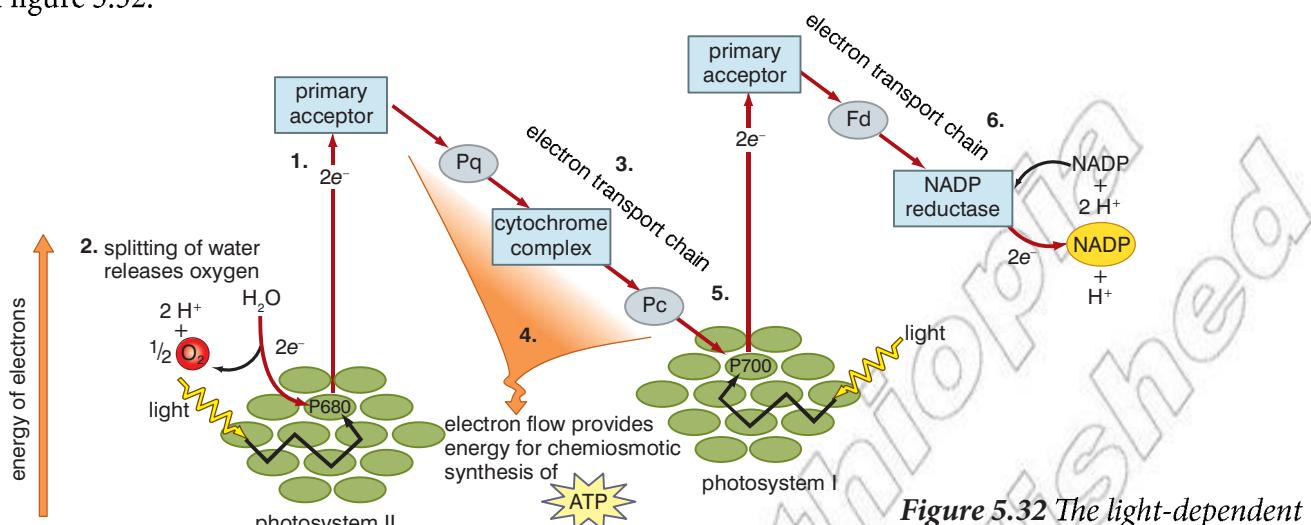


Figure 5.32 The light-dependent reactions of photosynthesis

### Photosystem I and photosystem II

- Electrons (e<sup>-</sup>) in chlorophyll molecules in photosystem II are excited by the energy in photons of light – they become more energetic. Because of the extra energy, they escape from the chlorophyll and pass to an electron acceptor (the **primary electron acceptor**).
- The conditions created in the chloroplast cause the following reaction to occur:
$$2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$$
This light-dependent splitting of water is called **photolysis**. The electrons replace those lost from the chlorophyll molecule.
- The primary electron acceptor passes the electrons to the next molecule in an electron transport chain (plastoquinone or 'Pq'). The electrons then pass along a series of cytochromes (similar to those in the mitochondrial electron transport chain) and finally to plastocyanin (Pc) – the last carrier in the chain. The electrons lose energy as they are passed from one carrier to the next.
- One of the molecules in the cytochromes complex is a proton (hydrogen ion) pump. As electrons are transferred to and then transferred from this molecule, the energy they lose powers the pump which moves protons from the stroma of the chloroplast to the space inside the thylakoid. This leads to an accumulation of protons inside the thylakoid, which drives the chemiosmotic synthesis of ATP.
- Electrons in chlorophyll molecules in photosystem I are excited (as this photosystem absorbs photons of light) and escape from the molecule. They are replaced by the electrons that have passed down the electron transport chain from photosystem II.
- The electrons then pass along a second electron transport chain involving ferredoxin (Fd) and NADP reductase. At the end

### DID YOU KNOW?

The chlorophyll a molecule in photosystem II is most active with light of wavelength of 680 nm (P680); that in photosystem I is most active with light of a wavelength of 700 nm (P700).

### KEY WORDS

- primary electron acceptor**  
the first molecule to accept the excited electron displaced from a chlorophyll molecule
- photolysis** light-dependent splitting of water

**KEY WORDS**

**photosynthetic unit** an arrangement of molecules capable of carrying out all the reactions in the light-dependent stage of photosynthesis.

**non-cyclic photophosphorylation** the formation of ATP via photosystem II

**cyclic photophosphorylation** use of only photosystem I to generate ATP

of this electron transport chain, they can react with protons (hydrogen ions) and NADP in the stroma of the chloroplast to form reduced NADP.

Figure 5.32 is part graph and part flow chart showing how the reactions take place and in what sequence. But it doesn't show how the molecules are arranged in relation to each other to form what is called a **photosynthetic unit**. Figure 5.33 shows this arrangement.

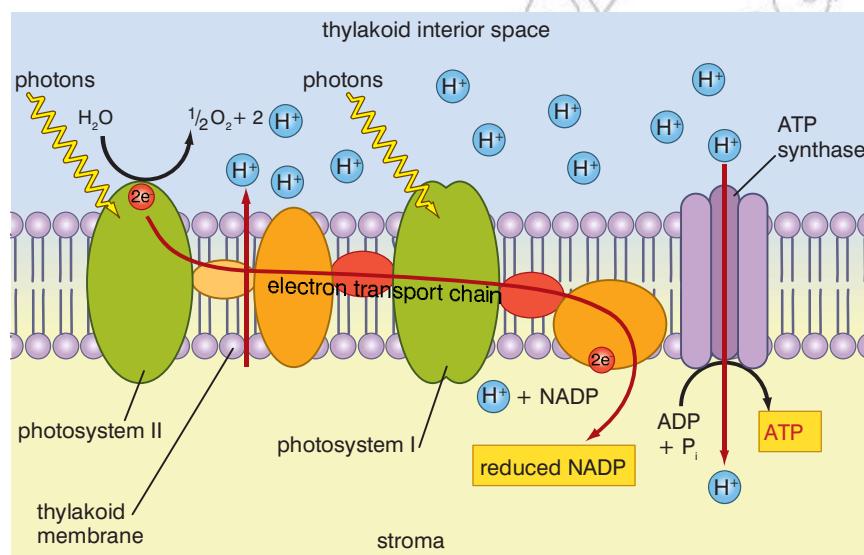


Figure 5.33 How the molecules are arranged in a photosynthetic unit

A photosynthetic unit is a unit of pigments, electron carriers and ATP synthase that is capable of carrying out all the reactions in the light-dependent stage of photosynthesis. The formation of ATP in the way described above is called **non-cyclic photophosphorylation**. This is because:

- the phosphorylation (formation of ATP) is light-dependent
- the electrons lost from the chlorophyll are not recycled in any way

Plants sometimes generate ATP by **cyclic photophosphorylation**. In cyclic photophosphorylation, only photosystem I is used. No oxygen and no reduced NADP are formed. Figure 5.34 shows this system. Here, you can see that electrons lost from the chlorophyll molecule are returned to it. Hence the name 'cyclic'. This process usually only happens when sugars cannot be synthesised for some reason – such as lack of carbon dioxide.

In cyclic and non-cyclic photophosphorylation, ATP is produced because:

- there is an accumulation of protons (hydrogen ions) in the interior of a thylakoid
- this creates a concentration gradient between the thylakoid interior and the stroma of the chloroplast
- protons move down this concentration gradient, through ATP synthase, causing the rotor to spin, just as in mitochondria during respiration

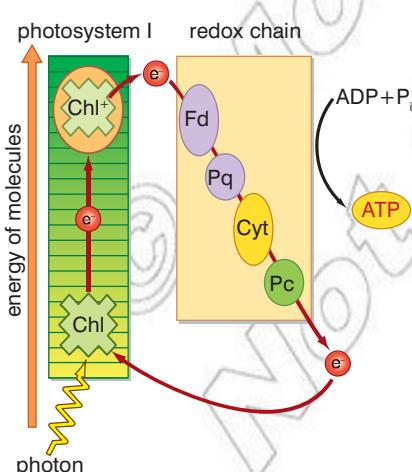


Figure 5.34 Cyclic photophosphorylation

### A summary of the light-dependent reactions

Light energy is used to excite electrons which then:

- cause the transfer of protons to the inside of the thylakoid membrane as they pass along the first electron transport chain; this eventually leads to the formation of ATP, and
- react with hydrogen ions and NADP at the end of the second electron transport chain to form reduced NADP; this reaction could only happen because of the extra energy possessed by the electrons.

The ATP and reduced NADP are used to drive the synthesis of carbohydrates in the light-independent reactions of photosynthesis.

### Activity 5.10: Separate the photosynthetic pigments in spinach leaves

#### You will need:

- spinach (or other) leaves
- 80% acetone
- filter funnel, beaker, measuring cylinder, glass jar with a tight cork
- no.1 filter paper, petroleum ether, acetone, hook, micropipette
- pestle and mortar
- calcium carbonate

#### Method

- Take 50 g of fresh spinach leaves in a pestle and mortar. Grind them with 20 ml of 80% acetone.
- Add a pinch of calcium carbonate and again crush.
- Filter the extract. The deep-green-coloured filtrate contains the photosynthetic pigments (chlorophylls, carotenoids and xanthophylls).
- Take a glass jar (about 45 cm high) with a tight cork fitted in it. The cork should have a hole in the centre.
- Mix 25 cm<sup>3</sup> petroleum ether and 3 cm<sup>3</sup> acetone. Pour the solvent into the jar and allow the jar to become saturated.
- Cut a strip of filter paper of the size which will fit in the jar.
- Mark a pencil line about 3 cm from one end.
- Place a small circular spot of pigment extract on the pencil line.
- Allow the spot to dry and add another spot in the same place.
- Repeat stages 8 and 9 several times until you have a concentrated spot – but do not let the spot ‘spread’ too far whilst you are preparing it.
- Now hang the strip inside the jar (you could tape it to the base of the cork) and close the cork. DO NOT ALLOW THE SPOT TO DIP INTO THE SOLVENT.
- Allow the chromatogram to run until the solvent has nearly reached the top of the filter paper. DO NOT LET THE SOLVENT RUN TO THE TOP OF THE FILTER PAPER.

You should see something like the distribution of pigments shown in figure 5.35.

Chlorophyll a  
Chlorophyll b  
Xanthophyll  
Carotene

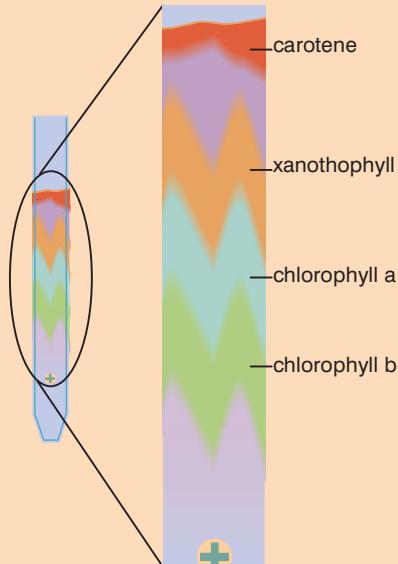


Figure 5.35 A chromatogram of the pigments in spinach leaves

## KEY WORDS

**ribulose bisphosphate** a five-carbon compound in the stroma

## DID YOU KNOW?

## About Rubisco

The full name for the enzyme is ribulose bisphosphate carboxylase oxygenase. Notice that there are two '-ases' in the name. Rubisco can catalyse the addition of  $\text{CO}_2$  or  $\text{O}_2$  to ribulose bisphosphate. It is unusual for an enzyme to be able to catalyse two reactions involving different substrates. We shall see the importance of this when we study photorespiration later.

## How is carbohydrate synthesised in the light-independent reactions?

The light-independent reactions of photosynthesis occur in the stroma of the chloroplasts. They comprise a complex cycle of reactions that involves the addition of carbon dioxide to a pre-existing five-carbon molecule (a molecule containing five carbon atoms) within the chloroplast. The resulting molecules are modified to regenerate the original molecule whilst, at the same time, synthesising glucose. The sequence of reactions was discovered by Melvin Calvin, an American biologist. Because of his work, the light-independent reactions are also referred to as the Calvin cycle.

In the 1950s Melvin Calvin experimented with unicellular algae called *Chlorella* by exposing them to radioactive carbon dioxide.

After different periods of time, the algae were killed and the chemicals in the algae that contained radioactive carbon (which must have come from the carbon dioxide) were identified using two-dimensional chromatography.

As time passes more compounds contain the radioactive carbon. By refining the experiment and using shorter and shorter intervals, Calvin identified the first stable compound to be formed as a compound containing three carbon atoms called glycerate phosphate (GP).

The main stages of the light-independent reactions are:

- carbon dioxide reacts with **ribulose bisphosphate (RuBP)** – a five-carbon compound in the stroma; the reaction is catalysed by the enzyme **Rubisco**.
- two molecules of the three-carbon compound GP are formed from this reaction as figure 5.36 shows
- each molecule of GP is converted to TP (triose phosphate – another three-carbon compound); this is a reduction reaction using hydrogen ions from reduced NADP and energy from ATP
- some of the TP formed is used to regenerate the RuBP (ATP is again required) whilst some is used to form glucose and other useful organic compounds

Figure 5.37 summarises the light-independent reactions of photosynthesis. It shows how three 'turns of the cycle' result in an output of one molecule of TP. Six turns of the cycle would give an output of two molecules of TP – enough to make one molecule of glucose.

TP can also be converted to lipids, amino acids and from these into nucleotides and all the other organic molecules found in plants. TP is the basis for the synthesis of all organic molecules.

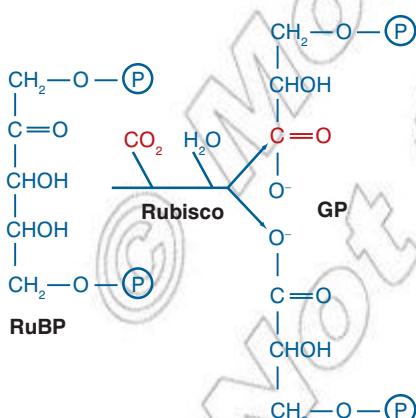


Figure 5.36 The action of Rubisco

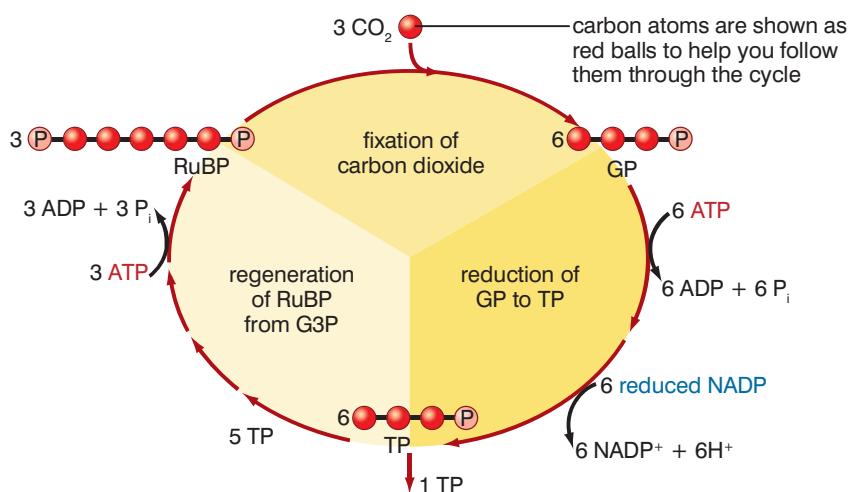


Figure 5.37 The light-independent reactions

### DID YOU KNOW?

#### How the light-dependent and light-independent reactions are related

During the light-independent reactions, reduced NADP is reoxidised to NADP and ATP is hydrolysed to ADP and  $\text{P}_i$ . These are then reused in the light-dependent reactions to regenerate ATP and reduced NADP to be used again in the light-independent reactions ... and so on. Figure 5.38 summarises the relationship between the light-dependent reactions and the light-independent reactions.

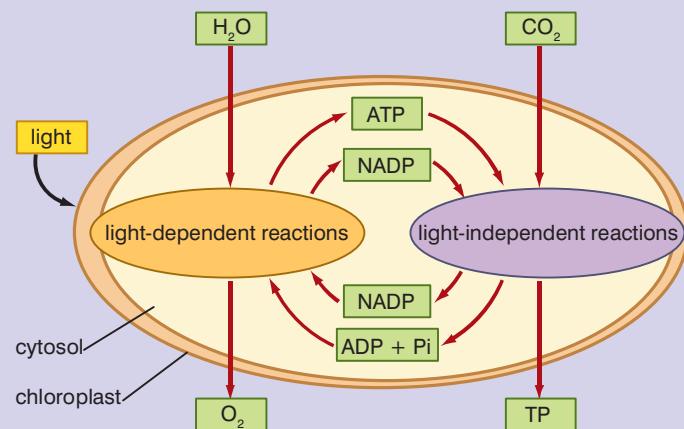


Figure 5.38 The relationship between the light-dependent and light-independent reactions

### What factors affect the rate of photosynthesis?

Photosynthesis is dependent on a number of factors. The main ones, and their effects, are shown in the table below.

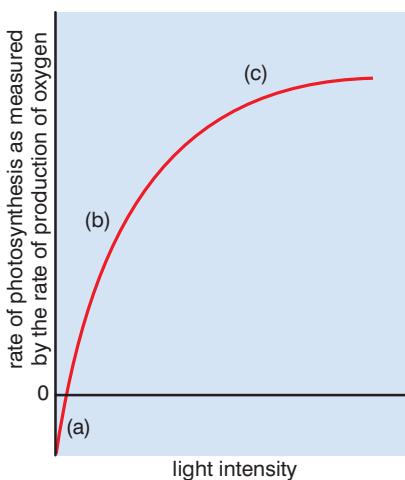
Table 5.1 Factors affecting the rate of photosynthesis

Factor	Effect on photosynthesis
Light intensity	Low light intensity can limit the light-dependent reactions by reducing the number of electrons in chlorophyll molecules that are photo-excited.
Carbon dioxide concentration	Can limit the light-independent reactions by influencing the rate of the initial reaction with RuBP.
Temperature	Can limit the rate of enzyme action, for example, ATP synthase (light-dependent reactions) and Rubisco (light-independent reactions).

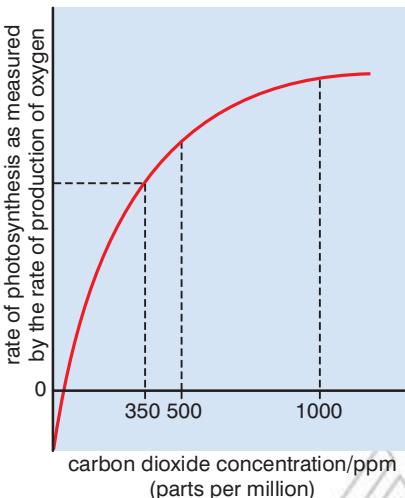
### DID YOU KNOW?

#### About the optimum temperatures of enzymes controlling photosynthesis

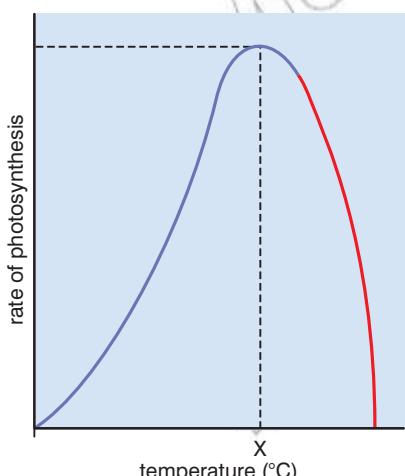
The actual optimum temperature for the enzymes of photosynthesis varies with the geographical location. The enzymes of plants that live within the Arctic Circle have a much lower optimum than those of plants found in the tropics.



**Figure 5.39** The effect of light intensity on the rate of photosynthesis (measured by the rate of production of oxygen)



**Figure 5.40** The effect of carbon dioxide on the rate of photosynthesis



**Figure 5.41** The effect of temperature on the rate of photosynthesis

In table 5.1, you can see that there is a reference to the factors 'limiting' the rate of photosynthesis when their presence is in short supply. But which factor actually limits the rate of photosynthesis? The answer to this question could well be different on different days. On a cold, bright day in an Arctic country, temperature is likely to hold back the rate of photosynthesis. On a warm, cloudy day in summer, light intensity is likely to limit the rate. On a warm, sunny day in summer, it could well be the concentration of carbon dioxide. In general terms we can say that:

The rate of photosynthesis is limited by the factor that is present in a limiting quantity.

This is known as the **principle of limiting factors**.

### What is the effect of light intensity on the rate of photosynthesis?

This is shown as a graph in figure 5.39. The graph is divided into three regions:

- very low light intensities – respiration is still occurring and is taking in oxygen faster than photosynthesis is producing it
- medium light intensities – photosynthesis is producing more oxygen than respiration uses, the rate of photosynthesis increases with increasing light intensity
- very high light intensities – the rate of photosynthesis is beginning to level out, even though the light intensity is still increasing; some other factor is probably limiting the rate

### What is the effect of the concentration of carbon dioxide on the rate of photosynthesis?

Again, it is convenient to show this as a graph (figure 5.40). The graph is similar to that in figure 5.39. At very low concentrations of carbon dioxide, little photosynthesis takes place, although respiration is still using up oxygen. As the carbon dioxide concentration increases, so does the rate of photosynthesis. Again, however, it begins to level off at higher concentrations. This may be due to some other factor, or it could be due to the saturation of Rubisco.

### How does temperature affect the rate of photosynthesis?

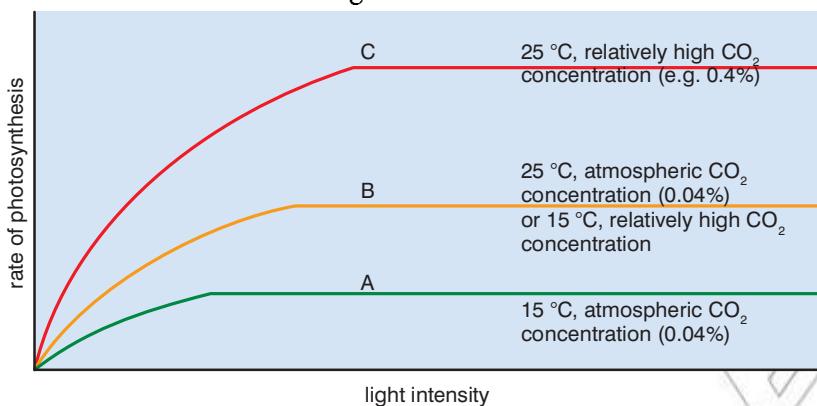
Many of the reactions in both the light-dependent stage and the light-independent stage are controlled by enzymes, which are affected by temperature. Once the temperature exceeds the optimum, the enzyme denatures and the rate of photosynthesis decreases rapidly.

### How can all the factors interact to influence the rate of photosynthesis?

Increasing the light intensity should increase the rate at which ATP and reduced NADP are produced in the light-dependent reactions and, as a result, increase the rate at which the Calvin cycle can take place. However, the rate at which the Calvin cycle can 'turn' could be limited by:

- a low temperature (limiting the rate at which enzymes such as Rubisco can operate)
- a low concentration of carbon dioxide

This limits the rate at which reduced NADP and ATP can be used, which, in turn, limits the amount of NADP and ADP + P<sub>i</sub> that can be reused by the light-dependent reactions. The whole process is therefore limited, even though the light intensity continues to increase. This is shown in figure 5.42.



In the region of the graphs where light is non-limiting (horizontal lines), the factors that are limiting are:

- A – both temperature and carbon dioxide; increasing either produces an increase in the rate of photosynthesis to level B
- B – temperature or carbon dioxide concentration (the factor that hasn't been increased from A); increasing the temperature increases the rate to level C

As well as the major factors discussed above, a number of other factors influence the rate of photosynthesis. These include:

- the wavelength of the light; photosynthesis takes place faster in 'red' and 'blue' wavelengths than in other wavelengths because these wavelengths are absorbed more efficiently than others; leaves are green because green wavelengths are reflected
- the amount of chlorophyll present

### KEY WORD

**principle of limiting factors**  
*limitation by a factor that is present in a limiting quantity*

**Figure 5.42** The effect of several factors on the rate of photosynthesis



**Figure 5.43** Crop plants being grown in a polytunnel

Just enclosing the plants in a greenhouse or polytunnel will increase the temperature (because of the 'greenhouse effect') without any extra heating costs. However, this will only happen during daylight hours. At night, the greenhouse will cool down and growth processes other than photosynthesis will also slow down.

However, before investing in any equipment

to maintain increased temperatures and carbon dioxide concentrations, the grower needs to be aware of the likely gains. He/she needs to ask what will be the extra yield:

- from increasing the concentration of carbon dioxide?
- from increasing the temperature?

And will the extra cost of this be offset by extra profits?

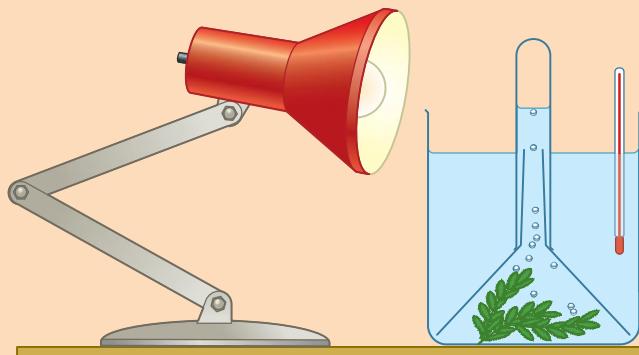
### Activity 5.11: Investigating the rate of photosynthesis

The rate of photosynthesis can be measured in some aquatic plants by collecting the oxygen given off in a certain period of time. The diagram below shows a simple apparatus for collecting the oxygen produced by *Elodea* – a pond weed.

The lamp is to make sure that the plant is illuminated constantly for 24 hours. Carbon dioxide is supplied by dissolving sodium hydrogen carbonate in the water. In solution, the sodium hydrogen carbonate releases carbon dioxide over a period of time.

You can use this simple apparatus to plan investigations into:

- the effect of temperature on the rate of photosynthesis
- the effect of carbon dioxide concentration on the rate of photosynthesis
- the effect of light intensity on the rate of photosynthesis



**Figure 5.44** Investigating the effect of light intensity on the rate of photosynthesis

In your plans, you must make clear:

- how you will change the independent variable
- how you will measure the dependent variable
- how you will control other variables that might influence your results
- the steps you will take to ensure that your results are as reliable as possible

Are there any other ways of photosynthesising?

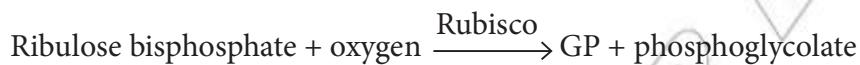


### C3 photosynthesis and photorespiration

What we have just described is the method of photosynthesis that takes place in plants living in temperate environments, such as those found in Europe. It is called C3 photosynthesis – because the first compound formed in the light-independent reactions of the Calvin cycle is GP, which contains three carbon atoms. C3 plants have leaves that are adapted to this method of photosynthesis. These leaves are generally broad, to catch as much sunlight as possible.

The cells that contain most chloroplasts (the palisade cells) are nearest the upper surface of the leaf (to absorb as much light as possible). The stomata are mainly on the lower surface, to minimise water loss. During the day, the stomata are open for most of the time to allow the entry of carbon dioxide, but they can be closed if the water loss is too great on a hot day. The spongy mesophyll has air spaces that allow easy diffusion of carbon dioxide and oxygen between the palisade layer and the stomata. Figure 5.45 shows the structure of the leaf of a C3 plant.

However, plants in the tropics have a problem. Here, it can be very hot and the leaves close their stomata to minimise water loss. When C3 plants do this, the concentration of carbon dioxide in the leaves falls and the enzyme Rubisco starts to behave in an unusual way. In the low concentrations of carbon dioxide, Rubisco binds with oxygen, not carbon dioxide. This means that RuBP is oxidised to one molecule of GP (not two) and a molecule of phosphoglycolate. In addition, carbon dioxide is produced in the process. The process is called **photorespiration** because it involves oxidation of carbon.



The one molecule of GP formed in photorespiration can re-enter the Calvin cycle, but the phosphoglycolate must be converted into GP for use in the Calvin cycle by a complex series of reactions. These reactions (involving a chloroplast, an organelle called a peroxisome and a mitochondrion) are summarised in figure 5.46

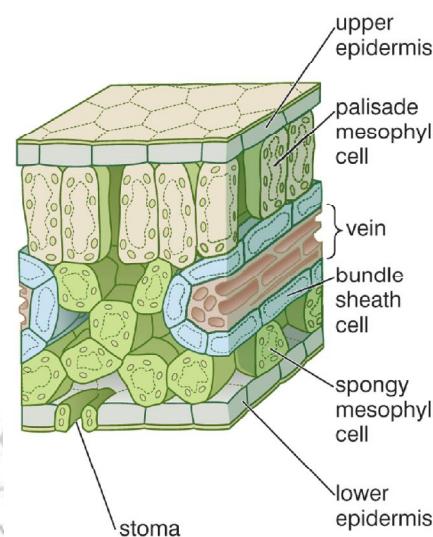


Figure 5.45 A leaf from a C3 plant

#### KEY WORD

**photorespiration process**  
involving the oxidation of carbon

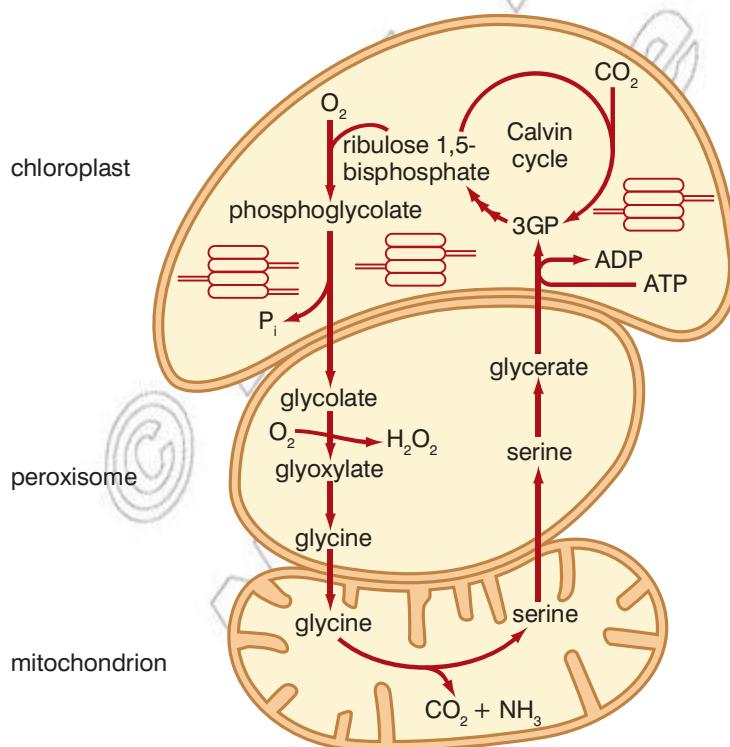


Figure 5.46 The reactions of photorespiration

**KEY WORD**

**C4 photosynthesis** *light-dependent reactions are the same as in C3 photosynthesis but the first compound formed in the light-independent reactions contains four carbons, not three*

It is not necessary to try to remember all these reactions. Instead, think of the two phases of photorespiration:

1. Rubisco catalyses a reaction between oxygen and RuBP to form one molecule of GP (not two) and one molecule of phosphoglycolate.
2. The phosphoglycolate is converted to GP in reactions in the chloroplast, peroxisome and mitochondrion.

Photorespiration reduces the efficiency of photosynthesis for several reasons, including:

- the carbon is oxidised, which is the reverse of photosynthesis – the reduction of carbon to carbohydrate
- the ribulose bisphosphate must be resynthesised and the phosphoglycolate removed
- ATP is used in the resynthesis of RuBP.

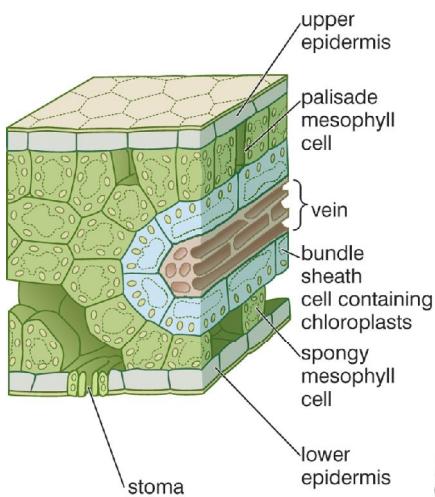
### C4 photosynthesis

To get round the problem of photorespiration reducing the efficiency of photosynthesis, plants that grow in tropical areas like Ethiopia (such as maize, crabgrass, sorghum and sugar cane) have evolved a different photosynthetic pathway called **C4 photosynthesis**.

As the name suggests, the first compound formed in the light-independent reactions is a C4 compound (contains four carbon atoms) not GP (a C3 compound). The light-dependent reactions are the same as in the C3 plants, but there is a difference in how glucose is synthesised in the light-independent reactions. First, look at the structure of the leaf of a C4 plant in figure 5.47. The structure is essentially similar to that of a C3 plant, but there is one important difference. The cells of the bundle sheath contain chloroplasts, which they don't in C3 plants. Having no thylakoids means that the light-dependent reactions cannot occur here and so oxygen is not produced in these chloroplasts. This helps to prevent photorespiration and allows the Calvin cycle to take place in these cells.

The light-dependent reactions in the C4 pathway also involve a set of reactions not found in C3 plants. These reactions take place in the mesophyll cells, which have chloroplasts with thylakoids and so can carry out the light-dependent reactions. However, they do not have the enzymes to catalyse the reactions of the Calvin cycle. Instead, the following reactions take place:

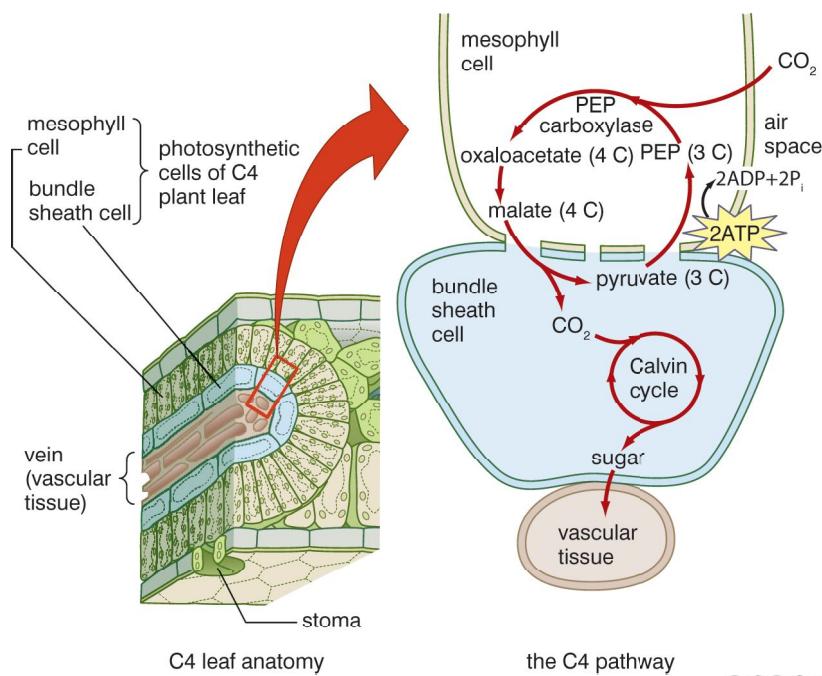
1. Carbon dioxide reacts with a C3 compound called PEP to form the C4 compound oxaloacetate. This is catalysed by the enzyme PEP carboxylase.
2. Oxaloacetate is converted into another C4 compound (malate), which then passes from the mesophyll cell into a bundle sheath cell.



**Figure 5.47** The structure of a leaf from a C4 plant

### Activity 5.12

List as many C4 plants that grow in Ethiopia as you can – you can use your textbook and the library, ask your teacher and use the internet if it is available to help you find as many as possible.



**Figure 5.48** The light-independent reactions of the C4 pathway of photosynthesis

3. In the bundle sheath cell, malate is converted to pyruvate with the release of a molecule of carbon dioxide, which starts the reactions of the Calvin cycle by binding with RuBP.
4. The pyruvate is converted back to PEP; this reaction requires ATP. These reactions are summarised in figure 5.48.

Overall, the C4 cycle uses two more molecules of ATP to deliver a molecule of carbon dioxide to Rubisco than does the C3 cycle. During active photosynthesis in the tropics, this is not a problem, as the high light intensity generates much ATP from the light-dependent reactions.

C4 photosynthesis is most efficient in conditions of:

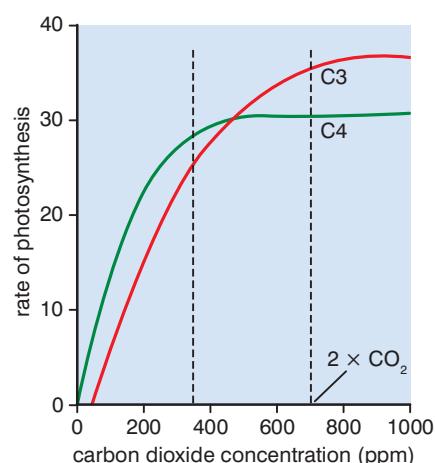
- low carbon dioxide concentration
- high light intensity
- high temperature

Figure 5.49 compares the efficiency of C3 and C4 photosynthesis under different concentrations of carbon dioxide.

### DID YOU KNOW?

#### Why C4 plants experience a low concentration of carbon dioxide

This is not because the composition of air in tropical regions is any different from that in other regions. It is because C4 plants (grasses, maize) often grow very close together and so compete for the carbon dioxide in the air, reducing its concentration.



**Figure 5.49** The efficiency of C3 and C4 photosynthesis at different carbon dioxide concentrations

## DID YOU KNOW?

## Cacti do it yet another way!

In the extreme heat of deserts, having stomata open during the day is a sure path to desiccation and death for the plants. But if they don't open their stomata, how will they get the carbon dioxide they need for photosynthesis? The answer is obvious really – open them at night when temperatures fall.

Cacti use what is essentially the same set of reactions as C4 plants, but they separate the two stages not by carrying them out in different cells, but by carrying them out at different times. The CAM photosynthesis cycle is as follows:

1. At night, the plants open their stomata to allow in  $\text{CO}_2$ , which then reacts with PEP in mesophyll cells to form oxaloacetate, and then malate just as in the C4 pathway.
2. The malate is then stored in the vacuoles of these cells overnight.
3. During the day, the light-dependent reactions generate ATP and reduced NADP so that the Calvin cycle can continue.
4. Malate is released from the vacuoles and is broken down to glyceral, releasing carbon dioxide for the reactions of the Calvin cycle.

Figure 5.50 compares the C4 pathway and the CAM pathway.

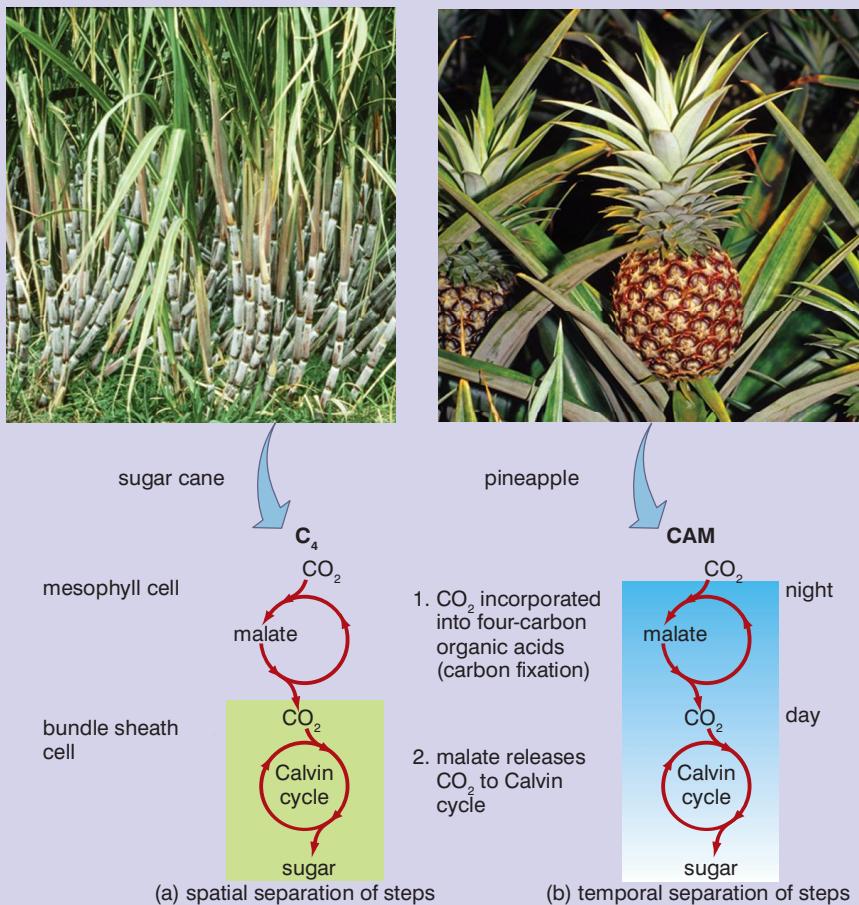


Figure 5.50 The C4 and CAM photosynthetic pathways

Table 5.2 compares several aspects of the two processes.

**Table 5.2 A comparison of C3 and C4 photosynthesis**

Feature	C3	C4
Bundle sheath cells	Lack chloroplasts	Have chloroplasts with no thylakoids
Enzyme used to fix $\text{CO}_2$	Rubisco	Pepco (PEP carboxylase)
Optimum temperature	15–25 °C	30–40 °C
Optimum $\text{CO}_2$ concentration	700 ppm	400 ppm
Fixation of $\text{CO}_2$	Mesophyll cells	Mesophyll cells
Calvin cycle	Mesophyll cells	Bundle sheath cells

The crop plants that are grown in Ethiopia (such as sorghum and wheat) are all C4 plants and are, therefore, well adapted to photosynthesise efficiently in the hot, bright days found in this country. Crop plants that are grown in temperate areas (such as peas and carrots) would not photosynthesise as efficiently, because they are C3 plants. They would, therefore, not produce high yields.

### Activity 5.13: presentations on aspects of photosynthesis

In this activity, you will be divided into groups to prepare a presentation on some aspect of photosynthesis.

The main aspects that different groups will cover are:

- the light-harvesting complex of pigments
- the light dependent reactions
- the light independent reactions
- photorespiration
- C3 and C4 photosynthesis

Each group should:

- concentrate on the main features of their assigned task (it is important not to over-complicate your presentation)
- present these in a manner that will be easily recognised and easily understood by those members of the class who have not made a detailed study of your aspect of photosynthesis
- include visual material to break up any text that they present
- try to keep their presentation brief – keep to five minutes if possible

## Review questions

Choose the correct answer from A to D.

1. In the light-dependent reactions of photosynthesis:

- A NADP is reduced
- B ATP is produced
- C ADP is produced
- D Light energy excites chlorophyll electrons

Figure 5.51 shows the effect of light intensity on the rate of photosynthesis at different concentrations of carbon dioxide and at different temperatures. Questions 2 and 3 relate to this graph.

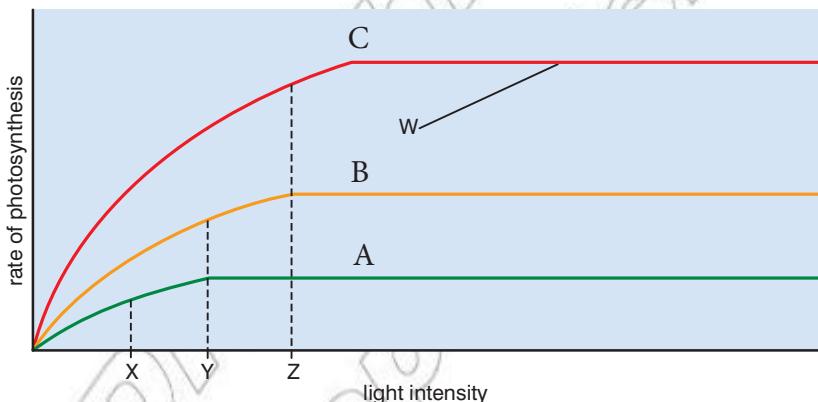
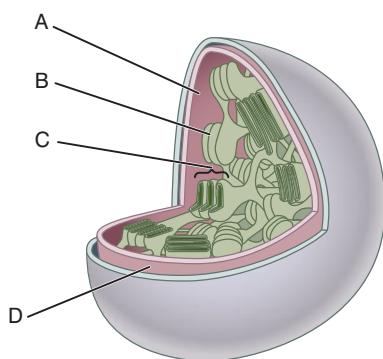


Figure 5.51

2. Line B could represent:
- A low carbon dioxide concentration and high temperature
  - B low carbon dioxide and low temperature
  - C high carbon dioxide and high temperature
  - D any of the above
3. Which region of the graph, W, X, Y or Z, represents conditions in which light intensity is not limiting the rate of photosynthesis?
- A W
  - B X
  - C Y
  - D Z
4. In the light-independent reactions of photosynthesis:
- A ATP is used to convert GP into TP
  - B reduced NADP is used to convert GP into TP
  - C ATP is produced
  - D carbohydrates are produced



**Figure 5.52** Structure of a chloroplast

Figure 5.42 shows the structure of a chloroplast. Questions 5 and 6 relate to this diagram.

5. During the light-dependent reactions of photosynthesis, ATP is produced in the regions labelled:
  - A A
  - B B
  - C C
  - D D
6. NADP moves from:
  - A A to B
  - B B to A
  - C B to C
  - D C to A
7. Cyclic photophosphorylation produces:
  - A oxygen and ATP
  - B reduced NADP and ATP
  - C oxygen only
  - D ATP only
8. In the light-independent reactions, reduced NADP is used to:
  - A oxidise GP to TP
  - B oxidise TP to GP
  - C reduce TP to GP
  - D reduce GP to TP
9. A photosynthetic unit can carry out:
  - A photolysis
  - B the synthesis of ATP
  - C the synthesis of reduced NADP
  - D all of the above
10. A photosystem consists of:
  - A a reaction centre molecule and an electron transport chain
  - B a reaction centre molecule and an antenna complex
  - C an accessory pigment and an antenna complex
  - D an accessory pigment and an electron transport chain

**Activity 5.14**

Make a big poster to show the Calvin cycle in photosynthesis. Have an inset area to show cyclic and non-cyclic photophosphorylation and how it fits in with the production of sugars.

11. In photorespiration:
  - A low oxygen concentrations cause Rubisco to form more GP than usual
  - B low oxygen concentrations cause Rubisco to form less GP than usual
  - C low carbon dioxide concentrations cause Rubisco to form less GP than usual
  - D low carbon dioxide concentrations cause Rubisco to form more GP than usual
12. In the C4 pathway:
  - A PEP carboxylase catalyses the reaction of carbon dioxide with RuBP in the mesophyll cells
  - B PEP carboxylase catalyses the reaction of carbon dioxide with RuBP in the bundle sheath cells
  - C PEP carboxylase catalyses the reaction of carbon dioxide with PEP in the mesophyll cells
  - D PEP carboxylase catalyses the reaction of carbon dioxide with PEP in the bundle sheath cells
13. C4 photosynthesis is more efficient than C3 photosynthesis in conditions of:
  - A high light intensity and high carbon dioxide concentrations
  - B low light intensity and high carbon dioxide concentrations
  - C high light intensity and low carbon dioxide concentrations
  - D low light intensity and low carbon dioxide concentrations
14. The chloroplasts in the bundle sheath cells of C4 plants are an adaptation to this pathway because:
  - A they contain no Calvin cycle enzymes
  - B they contain no thylakoids
  - C they have a large surface area
  - D they produce large amounts of oxygen
15. Which of the following statements about C4 and CAM photosynthesis is true?
  - A In CAM photosynthesis, the C4 stage and the Calvin cycle are separated in time.
  - B In CAM photosynthesis, the C4 stage and the Calvin cycle are separated in space.
  - C In C4 photosynthesis, the C4 stage and the Calvin cycle are separated in time.
  - D In C4 photosynthesis, the C4 stage and the Calvin cycle both occur in the same cell.

## Summary

In this unit you have learnt that:

- ATP is an ideal energy-storage molecule in a cell because:
  - energy is released from the molecule quickly, in a single-step hydrolysis reaction
  - energy is released in small amounts (that are closely matched to the amounts needed for cellular reactions)
  - the molecule is easily moved around within the cell but cannot leave the cell
- The main stages of aerobic respiration are: glycolysis, the link reaction, the Krebs cycle, the electron transport chain and the chemiosmotic synthesis of ATP.
- In glycolysis, glucose (C<sub>6</sub>) is converted to pyruvate (C<sub>3</sub>) with the net gain of two molecules of ATP and two molecules of reduced NAD.
- In the link reaction, pyruvate is converted to acetyl coenzyme A (C<sub>2</sub>) with the loss of carbon dioxide and the production of two molecules of reduced NAD.
- In the Krebs cycle, acetyl coenzyme A combines with oxaloacetate (C<sub>4</sub>) to form citrate (C<sub>6</sub>), which is then decarboxylated to a C<sub>5</sub> compound and then to a C<sub>4</sub> compound, which is then converted into oxaloacetate; the cycle produces six molecules of reduced NAD, 2 molecules of reduced FAD and two molecules of ATP (by substrate level phosphorylation).
- As electrons from reduced NAD and reduced FAD pass along the electron transport chain they lose energy, which is used to pump protons from the matrix to the inter-membrane space.
- Protons then pass down an electrochemical gradient back into the mitochondrion through molecules of ATP synthase; each proton that passes through the enzyme causes one molecule of ATP to be synthesised.
- In fermentation (the anaerobic pathway):
  - the reactions of the electron transport chain, Krebs cycle and the link reaction cannot occur as, without oxygen as the terminal electron acceptor, NAD and FAD cannot be regenerated from reduced NAD and reduced FAD
  - glycolysis still occurs in anaerobic conditions as the NAD needed can be regenerated from reduced NAD by reducing pyruvate to lactate (animal cells) or ethanol (plant cells and yeast cells)
- Chloroplasts are well adapted to carry out photosynthesis because:
  - the grana provide a large surface area for the arrangement of chlorophyll molecules and the associated electron

transport systems of the light-dependent reactions

- the stroma provides a fluid medium for the reactions of the light-independent reactions

- The light-dependent reactions produce ATP and reduced NADP that are needed in the light-independent reactions.
- In the light-independent reactions:
  - $\text{CO}_2$  combines with RuBP (C5) to form two molecules of GP
  - GP is reduced to TP; reduced NADP supplies the hydrogen ions and ATP supplies the energy; the NADP and ADP +  $\text{P}_i$  are recycled to the light-dependent reactions
  - some TP is used to synthesise useful carbohydrates (such as glucose)
  - most TP is used to regenerate the RuBP so that the cycle of reactions can begin again
- The rate of photosynthesis is influenced by light intensity, concentration of carbon dioxide and temperature.
- The factor present in the lowest quantity will limit the rate of photosynthesis.
- When carbon dioxide concentrations fall, photorespiration can occur because oxygen then outcompetes carbon dioxide for the active site of Rubisco.
- Photorespiration reduces the efficiency of photosynthesis because:
  - only one molecule of GP is produced from RuBP
  - phosphoglycolate is produced which must be reconverted to RuBP, using up ATP
- C4 photosynthesis has evolved in plants in the tropics as a way of preventing photorespiration.
- In this process, the reactions of the Calvin cycle only take place in chloroplasts in bundle sheath cells.
- The bundle sheath cells can carry out the reactions of the Calvin cycle efficiently because:
  - they have no grana, so produce no oxygen to compete with carbon dioxide for the active site of Rubisco
  - there is a high concentration of carbon dioxide due to the decomposition of malate
- C4 photosynthesis is more efficient than C3 photosynthesis in conditions of high light intensity, high temperature and low carbon dioxide concentrations.
- CAM photosynthesis is effective in desert plants because it separates the light-dependent and light-independent stages in time; the leaves only open their stomata to allow the light-independent reactions to take place during the night, saving precious water.

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Notes

## End of unit questions

- a) Describe how the structure of a chloroplast is suited to its function.  
b) Describe *two* ways in which chloroplasts and mitochondria are:  
(i) similar  
(ii) different
- a) Describe *three* ways in which the ATP molecule is suited to its function of energy carrier in a cell.  
b) Describe how ATP is formed by:  
(i) substrate-level phosphorylation  
(ii) chemiosmosis
- Figure 5.53 shows the structure of a chloroplast from a mesophyll cell of a C<sub>3</sub> plant.  
a) Name the parts labelled A, B, C and D.  
b) Describe how the chloroplasts from a bundle sheath cell of a C<sub>4</sub> plant would be different from this chloroplast. Explain the benefit to the plant of this difference.
- a) Make a drawing of apparatus you could use to measure the rate of fermentation of glucose by yeast.  
b) Describe how you could use your apparatus to investigate the effect of temperature on the rate of fermentation in yeast. You must make clear in your account:  
– how you will change the temperature  
– how you will measure the rate of fermentation  
– how you will control other factors that might influence the results
- Figure 5.54 shows the light-dependent reactions of photosynthesis. Explain what is happening at each of the stages 1–6.

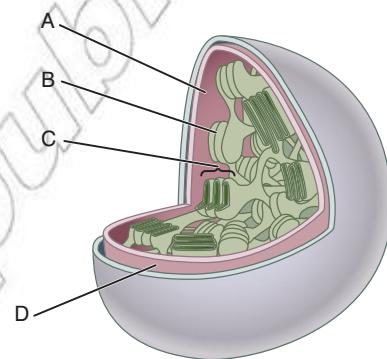


Figure 5.53

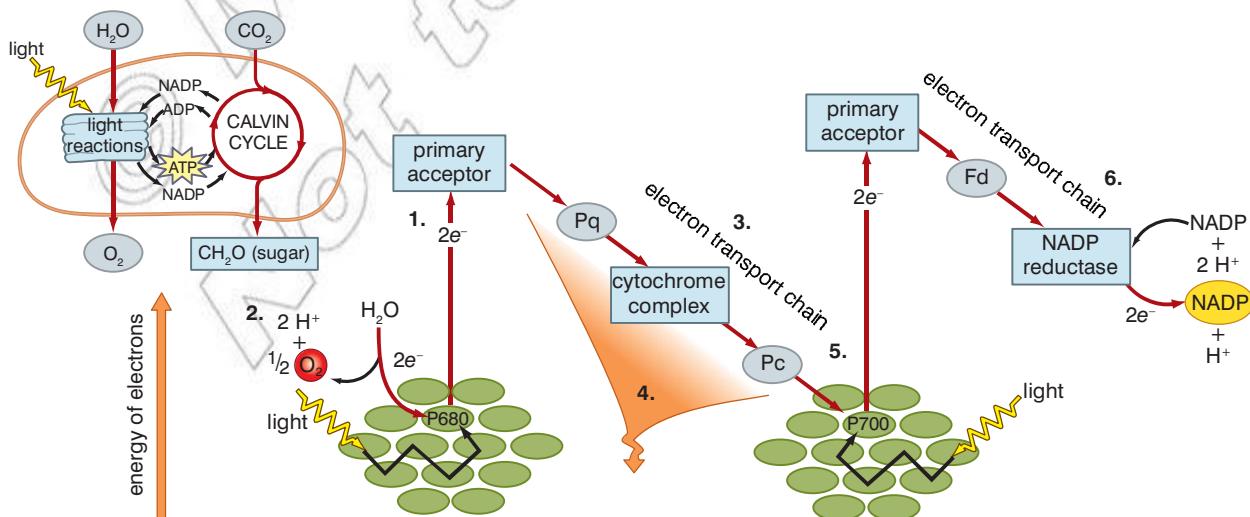


Figure 5.54

6. Figure 5.55 summarises the Krebs cycle.

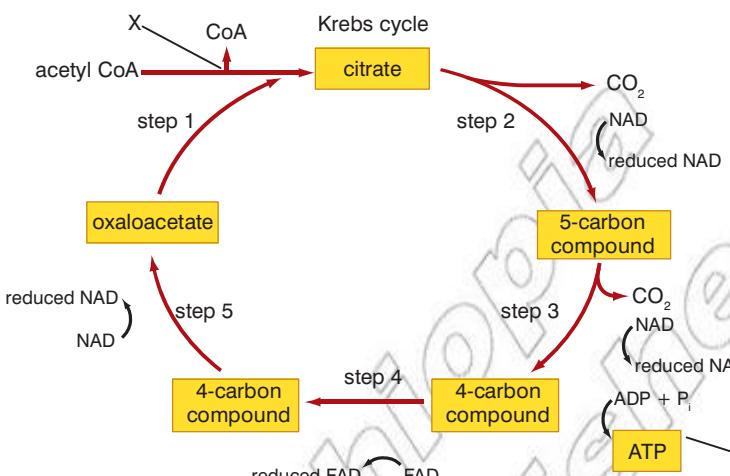


Figure 5.55

- a) (i) Name, and briefly describe, the processes labelled X and Y.  
(ii) Describe one other occasion during aerobic respiration where the process labelled Y takes place.
- b) In the absence of oxygen, the Krebs cycle cannot take place, even though its reactions do not use oxygen. Explain why.
- c) Reduced NAD is also produced during glycolysis. Explain what becomes of this reduced NAD in animal cells under:  
(i) aerobic conditions  
(ii) anaerobic conditions
7. The graph in figure 5.56 shows the influence of temperature, carbon dioxide and light intensity on the rate of photosynthesis.

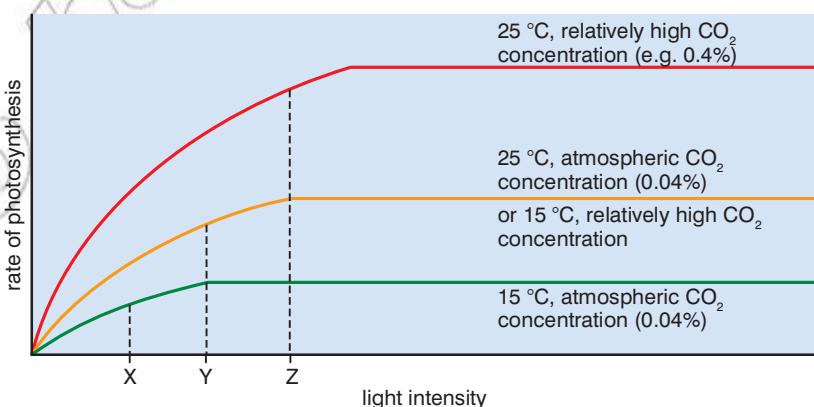


Figure 5.56

- a) In the regions labelled X, Y and Z, is light a limiting or a non-limiting factor? Give reasons for your answer.
- b) Describe and explain fully the difference between the three lines on the graph.

8. Figure 5.57 shows some of the reactions of the Calvin cycle.

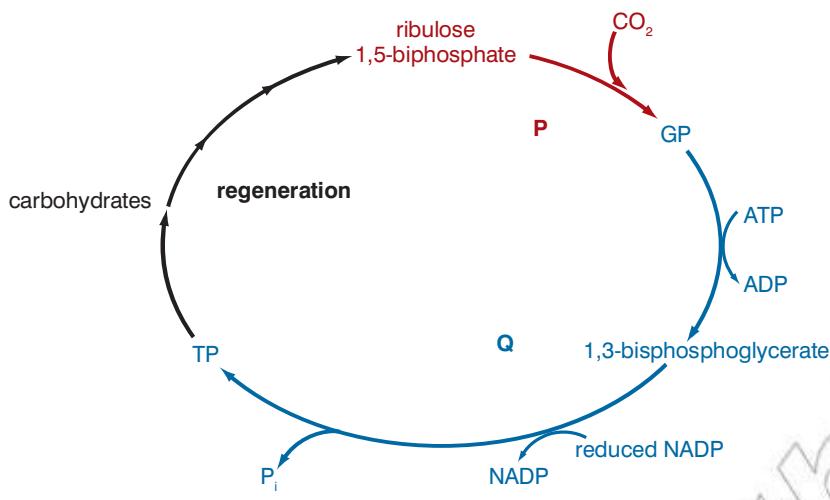


Figure 5.57

- Describe three possible fates of the TP formed in these reactions.
- Describe the processes occurring at P and at Q.
- The graph in figure 5.58 shows the changes in the levels of GP and RuBP in a chloroplast when the light source is removed.

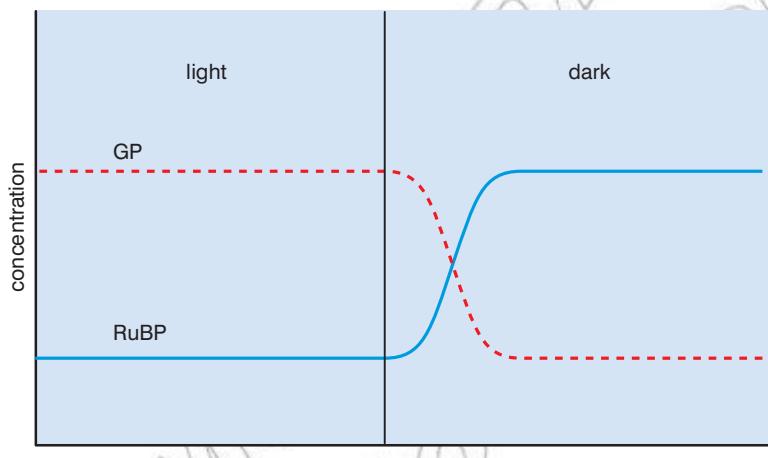


Figure 5.58

Use the graph to explain the changes in the levels of GP and RuBP when the light source is removed.

Copy the crossword puzzle below into your exercise book (or your teacher may give you a photocopy) and solve the numbered clues to complete it.



**Across**

3. Cycle of reactions that converts citrate to oxaloacetate (5, 5)
6. First stage of aerobic respiration (10)
9. Way in which ATP is produced by yeast when no oxygen is available (12)
10. Enzyme that catalyses the reaction between RuBP and carbon dioxide (7)
11. The main energy transfer molecule in a cell (3)
18. Smallest structure that can carry out all the reactions of the light-dependent stage of photosynthesis (14, 4)
20. Enzyme that catalyses the formation of ATP (3, 8)
21. Molecule that combines with  $P_i$  to produce ATP (3)
22. Nicotinamide adenine dinucleotide (3)

**Down**

1. Chain of molecules on cristae of mitochondria that moves electrons (8, 9, 5)
2. Reactions of photosynthesis that use ATP and reduced NADP to synthesise glucose (5, 11)
4. Process that releases energy from organic molecules (11)
5. Type of photosynthesis found in many tropical plants (2)
7. Stage of aerobic respiration in which pyruvate is converted to acetyl CoA (4, 8)
8. Stacks of thylakoids (5)
12. Process using light energy to drive the synthesis of carbohydrate (14)
13. Process in which RuBP combines with oxygen rather than carbon dioxide (16)
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