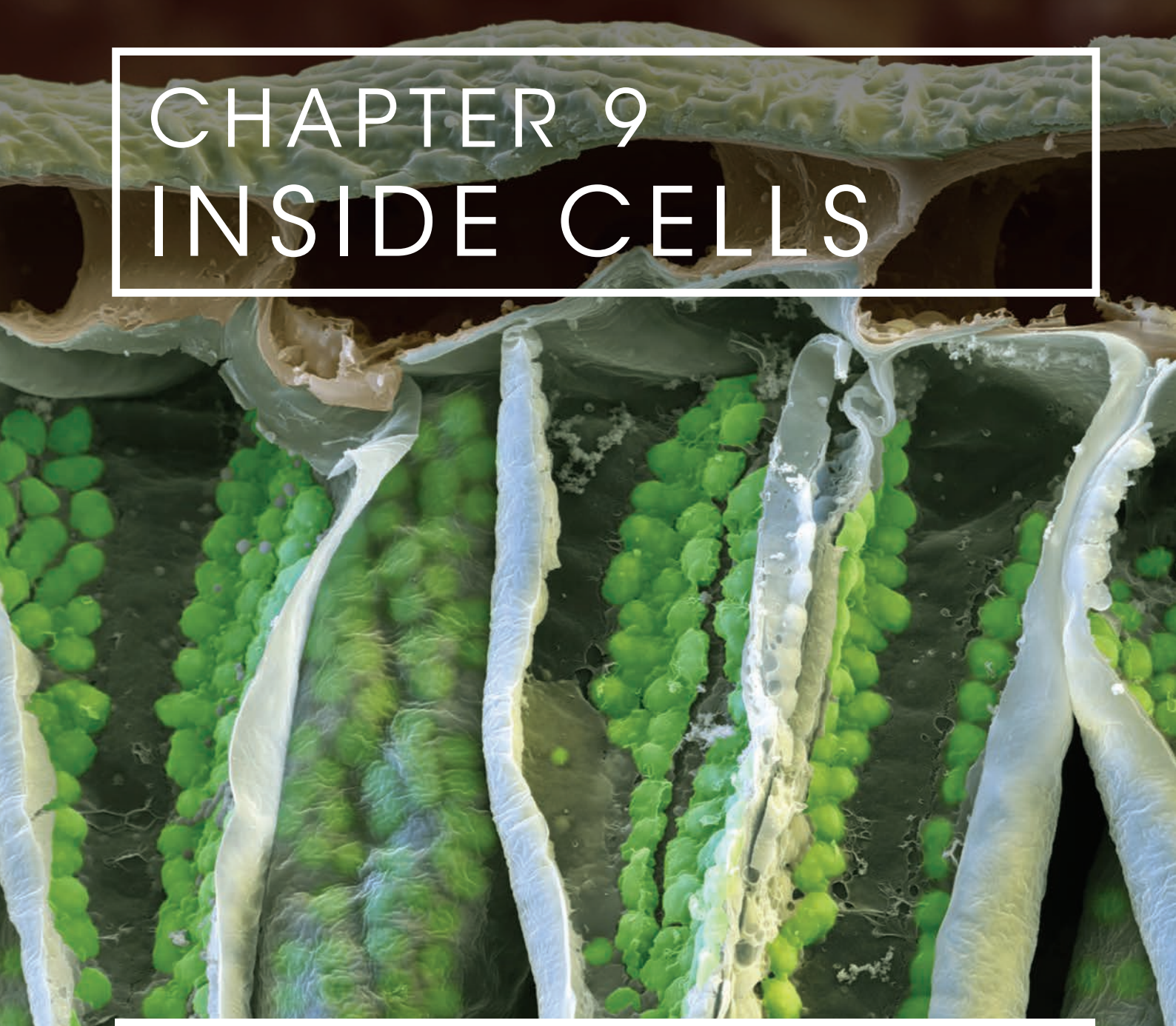
CHAPTER 9
INSIDE CELLS



Science Photo Library/Eye of Science

By the end of this chapter you will have covered the following material.

Science Understanding

- Biochemical processes in the cell are controlled by the nature and arrangement of internal membranes, the presence of specific enzymes, and environmental factors (ACSBL050)
- Enzymes have specific functions, which can be affected by factors including temperature, pH, the presence of inhibitors, and the concentrations of reactants and products (ACSBL051)
- Photosynthesis is a biochemical process that in plant cells occurs in the chloroplast and that uses light energy to synthesise organic compounds; the overall process can be represented as a balanced chemical equation (ACSBL052)
- Cellular respiration is a biochemical process that occurs in different locations in the cytosol and mitochondria and metabolises organic compounds, aerobically or anaerobically, to release useable energy in the form of ATP; the overall process can be represented as a balanced chemical equation (ACSBL053)

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Figure 9.1 ▶ Ocean dwellers such as this comb jelly can produce bioluminescent light as a result of the enzyme luciferase, which converts chemical energy to light energy.

All organisms rely on biochemical pathways to live. Each of these essential pathways relies on specific enzymes to function. Besides requiring these molecules to live, some organisms rely on enzymes to glow. Bioluminescent species of fish, jellies, fungi and insects can produce 'cold light' in a chemical reaction involving the enzyme luciferase. In the presence of oxygen and energy packets of **adenosine triphosphate (ATP)** produced in cells, luciferase oxidases luciferin to produce light. In the black of night or deep in the ocean, bioluminescent abilities help to find both mates and prey.

Biochemical processes are essential for life

Most animals die in a few days without water and in a few minutes without oxygen. Some bacteria die in the presence of oxygen. Every living cell of every part of an organism needs matter and a source of energy to keep it alive. Each kind of organism has its own way of making this happen but there are processes that are common to all.

Unicellular organisms take in materials (inputs) from their external environment and use these materials inside their single cell. The outputs or **products** of these activities are biomolecules and inorganic wastes. The biomolecules form the structures that carry out tasks for the organism. For instance, intracellular structures such as ribosomes synthesise proteins. Biomolecules such as carbohydrates provide a cell with the energy to perform these tasks. The waste products produced by working cells must be removed.

Internal cellular membranes control biochemical processes

A factory is a building that uses raw materials and energy to manufacture certain products that are then transported to other locations. Within the factory, different activities are happening. The management area plans the development and directs operations. The assembly room builds the products and the warehouse area of the factory stores the products ready for transport. This is analogous to the functioning of a cell.

Similar to a factory, compartmentalising a cell by having membrane-bound organelles creates specialised environments for specific functions. A large number of activities can occur at the same time in a very limited space and under different conditions. For example, chemical reactions in lysosomes break down compounds brought into the cell by using strong digestive enzymes in an acidic environment. Enclosing them in a membrane prevents them destroying the cell. Specific chemical reactions can occur using only a handful of enzymes that can be concentrated and recycled in membrane-bound areas. Membrane-bound structures can concentrate reactants and store products.

Some organelles, such as mitochondria, increase their internal surface area by the folding and stacking of internal membranes. These folded membranes (cristae) are studded with enzymes needed for cellular respiration. Enzymes are arranged in order of the steps involved in cellular respiration. The internal membranes of chloroplasts are also studded with enzymes necessary to keep photosynthetic reactions continuing.

Specific enzymes control biochemical pathways

Towards the end of the 19th century, the German chemist Eduard Buchner was experimenting to find a way of preventing yeast extracts from going bad. In one trial he added sugar to yeast extract and, rather than preventing change, he found that the sugar was fermented and converted to alcohol. Louis Pasteur had already demonstrated that yeast was responsible for the fermentation of sugar but Buchner took the research further. He showed that the juice extracted from living yeast cells was responsible for fermentation, not the yeast cells themselves. To describe the active ingredient in the juice that caused the fermentation he coined the term enzyme, from the Greek word 'zyme' meaning leavened. This is now the collective term for the thousands of organic protein molecules extracted from cells and found to act as organic **catalysts**.

▼ **Figure 9.2**
Thermal imaging reveals the heat emitted from objects. Living organisms with a high metabolism tend to give off more heat, which is why mammals such as sheep glow more brightly than trees and grass.

Getty Images/Cultura Science/Joseph Giacomin

The sum of the thousands of chemical reactions that occur constantly in each living cell is known as **cellular metabolism**. The rate of cellular metabolism varies among organisms. The metabolic reactions that occur in cells do not take place randomly; all are controlled and regulated to maintain cell functions and to meet the energy needs of the cell. These reactions need to occur at a rate that allows the cell to function. What is it that controls the type and duration of these reactions? Each step in the pathway is controlled by an enzyme, the protein described by Buchner that speeds up the rate of chemical reactions without undergoing any change itself.

See Chapter 12 for more information about extracellular enzymes.

Enzymes are one of the most important groups of proteins. Without enzymes, the reactions that occur in living organisms would be so slow as to hardly proceed at all; this would be incompatible with the maintenance of life.

Enzymes do more than merely speed up the reactions; they also control them. Over 1000 different reactions can take place in an individual cell. The functional organisation that this demands is achieved by a specific enzyme in a particular place within the cell acting as a catalyst for each individual reaction.

There are as many enzymes in living organisms as there are types of chemical reactions. They are divided into two broad groups: intracellular and extracellular. Intracellular enzymes occur inside cells, where they speed up and control metabolic reactions. Extracellular enzymes are produced by cells but achieve their effects outside the cell. They include digestive enzymes, which break down food in the small intestines.

Chemical reactions in cells occur in a series of enzyme regulated steps called biochemical pathways.

Biochemical processes involve energy inputs and outputs

The main metabolic pathways that transfer energy through living systems are photosynthesis and cellular respiration. These reactions transform energy in order to keep an organism alive.

Consider the case of starch breaking down into sugar. This is a type of chemical reaction where a complex molecule breaks down into a simpler one. Reactions such as this are termed **catabolic reactions**. When amino acids are joined together to form proteins, small molecules

WOW
Ribozymes

Certain RNA molecules called ribozymes are the exception to the 'all enzymes are protein' rule. They are able to catalyse some cell reactions. The substrates for ribozymes are also RNA molecules. Because of their enzymatic properties and their ability to catalyse their own synthesis, ribozymes are considered by some to be the first evolutionary step towards life.

build up into larger molecules. These are **anabolic reactions**. You may have heard the term anabolic before with reference to anabolic steroids and body building. Both anabolic and catabolic reactions apply to building up and breaking down of structures or molecules.

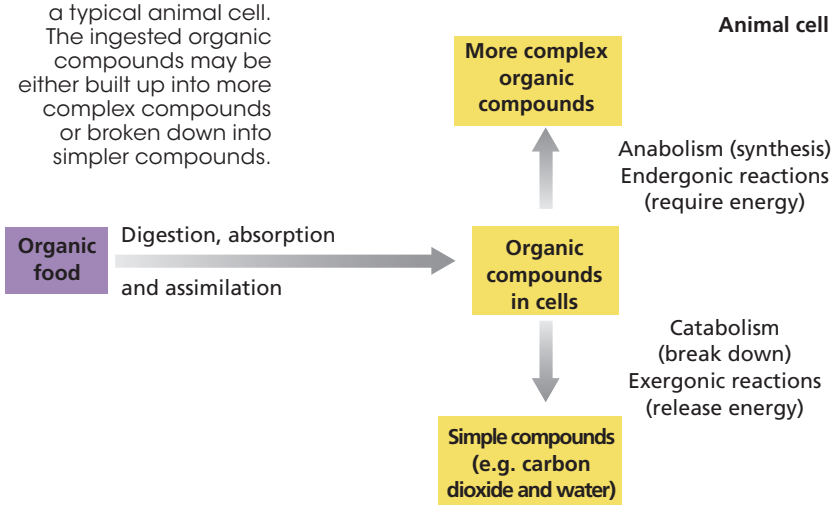
Now consider the energy changes of chemical reactions. **Exergonic reactions** ('exo' = out) are those that release energy. They are sometimes referred to as 'downhill' reactions. When molecular bonds are broken, energy is released. It makes sense that when a large molecule is broken down to smaller molecules, a large amount of energy is released. The opposite type of reaction, when molecular bonds are formed, is called an **endergonic reaction** ('endo' = in). This is sometimes referred to as an 'uphill' reaction.

For example, when starch breaks down to sugar, energy is released. Catabolic reactions always release energy so they are always exergonic. In another example, when proteins are built up from amino acids, energy is used. These types of anabolic reactions are always endergonic. Cells use the available energy released from catabolic reactions to fuel anabolic reactions (Figure 9.3).

The energy released from catabolic reactions, such as cellular respiration, is 'shuttled' into anabolic reactions, particularly those that recharge molecular 'cell batteries'. To understand how this works we need to understand the role of biological reactions in making and breaking molecules.

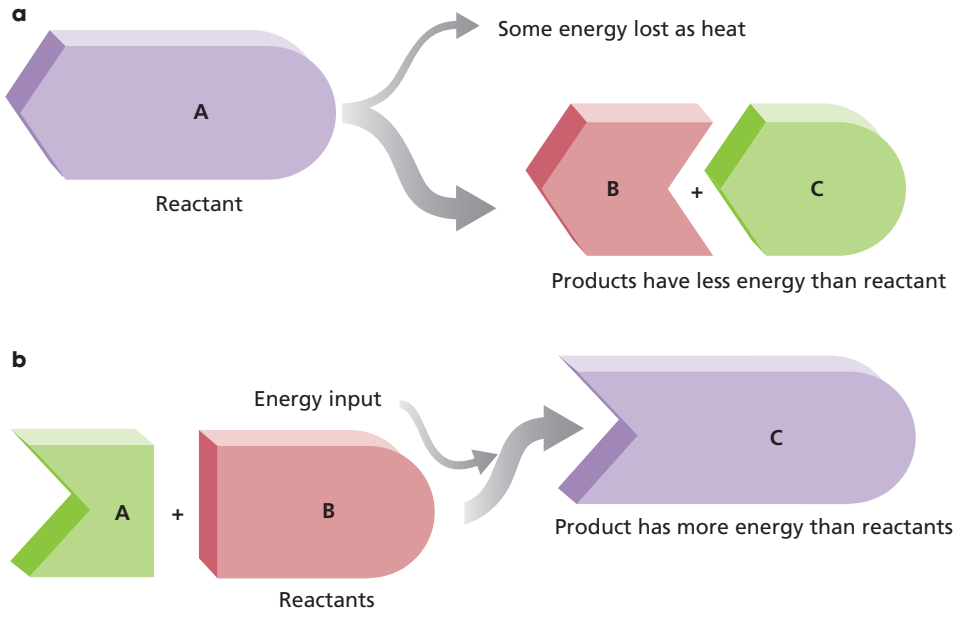
A biochemical process or reaction occurs when the chemical bonds of inputs or reactants are broken and the atoms recombine to form a new substance or substances: the output or products. In endergonic reactions, a total net amount of energy is absorbed and locked up in the bonds of the products, which have more stored energy than the reactants. In exergonic reactions, a total net amount of energy is released from the bonds of the reactants and the products have less energy than the reactants (Figure 9.4).

Figure 9.3 ▼
The relationship between catabolic and anabolic reactions in a typical animal cell. The ingested organic compounds may be either built up into more complex compounds or broken down into simpler compounds.



Roberts, M., Reiss, M. & Monger, G. (1993) *Biology: Principles and Processes*. Thomas Nelson. New edition released as *Advanced Biology* (Nelson Thornes, 2000). © Micheal Roberts, Michael Reiss and Grace Monger 1993, 2000. Reprinted by permission of Oxford University Press.

Figure 9.4 ▶
a) The reactants of exergonic reactions have more energy than the products. b) The reactants of endergonic reactions have less energy than the products.



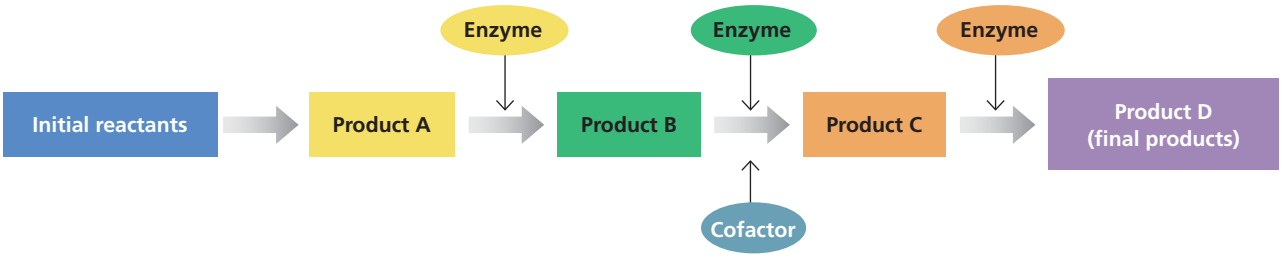
- Anabolic reactions use energy to build up molecules in an endergonic reaction.
- Catabolic reactions release energy when molecules are broken down in an exergonic reaction.

Atoms and molecules in a cell do not stay still; they are constantly in motion and colliding. For reactants A and B below, a reaction will only occur if they receive enough energy for collisions between them to give rise to the products C and D. The amount of energy needed to strain and break the reactants' bonds is called the **activation energy**. When enough activation energy is available to break the chemical bonds of the reactants, new bonds form between the atoms, thus generating one or more products.

$$\underset{\text{reactants}}{A + B} \longleftrightarrow \underset{\text{products}}{C + D}$$

For cells to continue functioning, enough energy must be provided to maintain the process of generating products from reactants. Cells must control the rate of energy released so they do not burn up. All chemical reactions are reversible under certain conditions. It is important that products are removed from a cell so that they do not build up and thus slow down vital metabolic reactions. To achieve this, chemical reactions in cells occur in a series of regulated steps collectively called biochemical pathways. The product of one step becomes the reactant for the next step (Figure 9.5). In this way, a product from one reaction is continually removed by being the reactant for the next reaction.

▼ **Figure 9.5**
A biochemical pathway. The products or outputs of the first step become the reactants or inputs in the next step until the final products are reached. Each step is regulated by a specific enzyme. Cofactors may be involved.



Cells have a number of ways of removing the final product from solution so that a biochemical pathway keeps operating in the required direction. In a plant cell, the final product of photosynthesis is glucose. Glucose, a soluble substance, is converted into an insoluble polysaccharide starch, which is stored by the plant. Thus, the plant is able to continue to produce and store glucose. In cellular respiration, the products of carbon dioxide diffuse from cells and are expelled into the atmosphere. Each step in the biochemical pathways of photosynthesis and cellular respiration is controlled and regulated by enzymes, with energy being supplied or released.

Cofactors are described on page 222.

WOW

Getting rid of pesticides

Australian scientists at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), in partnership with other organisations, have developed an enzyme-based product that can rapidly degrade unwanted pesticide residues in agricultural soil and water. Enzymes from naturally occurring soil bacteria were extracted and their effectiveness was enhanced using advanced molecular biology techniques.

QUESTION SET 9.1

Remembering

1 Define 'cellular metabolism'.
2 Define 'biochemical pathway'.
3 Compare intracellular and extracellular enzymes.

Understanding

4 a Explain why chemical reactions in cells proceed in a series of steps called a biochemical pathway.
  b Predict what would happen to the reaction if the final product was not removed.
5 Compare anabolic and catabolic reactions and give an example of each.
6 Compare exergonic and endergonic reactions and give an example of each.
7 Describe the relationship between:
  a anabolic and endergonic reactions.
  b catabolic and exergonic reactions.
8 Explain how the structure of a mitochondrion increases enzyme activity.
9 'Endergonic reactions and exergonic reactions are interdependent.' Explain this statement.

Applying

10 When sugar is added to yeast extract in a sealed test tube, the temperature increases.
  a Is the reaction occurring likely to be endergonic or exergonic? Explain your answer.
  b Predict what type of biomolecule is causing the chemical reaction to occur.
  c Compare the size of molecules before and after the reaction.

Properties of enzymes affect their specific functions

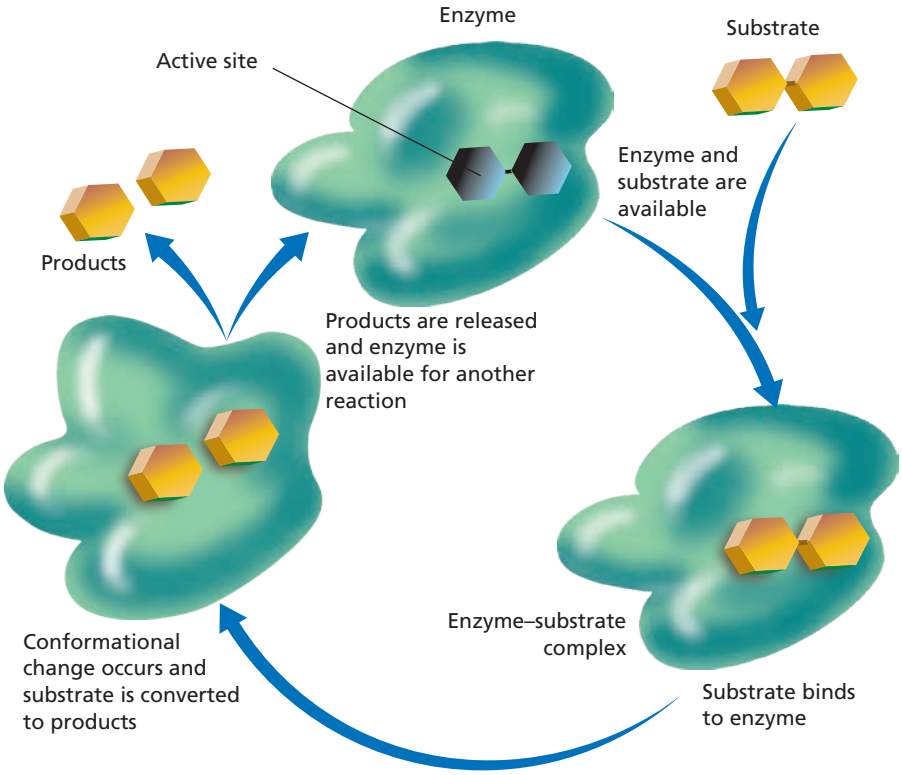
Enzymes are types of proteins and, like all proteins, they have a specific shape. It is this shape that allows it to bind with a specific reactant (**substrate**). They are generally named according to the substrate they catalyse, or the reaction, and they usually end in '–ase': sucrase, lipase, maltase. We can explain the properties of enzymes by suggesting that, when an enzyme-controlled reaction takes place, the enzyme and substrate molecules become joined together for a short time to form an **enzyme–substrate complex**. The substrate is converted to the end product by the catalytic action of the enzyme. The enzyme is unchanged by the reaction and can be used again. This means enzymes are often only needed in small quantities within a cell.

$$\text{enzyme} + \text{substrate(s)} \rightarrow \text{enzyme–substrate complex} \rightarrow \text{enzyme} + \text{end product(s)}$$

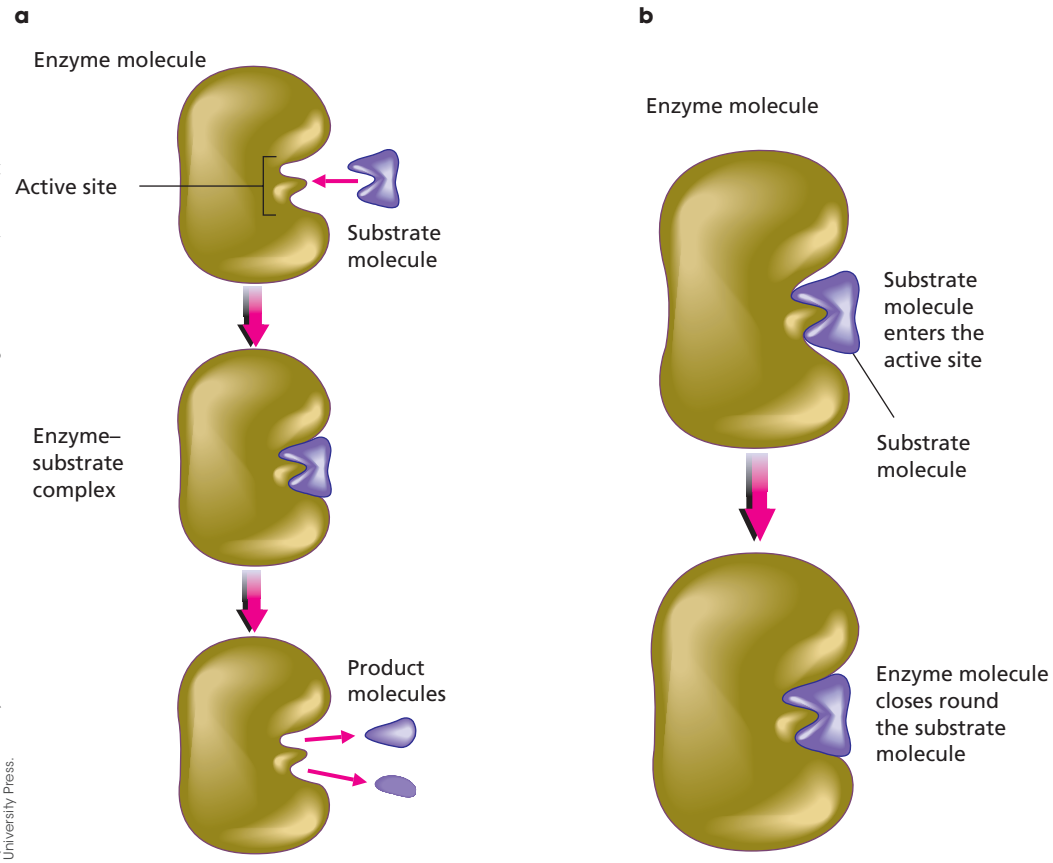
It is thought that each enzyme has a precise place on its surface to which the substrate can become attached. This is called the **active site** (Figure 9.6). All enzymes are substrate-specific. For instance, sucrase can only catalyse the substrate sucrose. There are two models for how an enzyme–substrate complex is formed at the active site.

One model of enzyme action is known as the **lock-and-key model** (Figure 9.7a). The folding of an enzyme protein forms a fixed groove or pocket-shaped active site. This groove can accommodate one or more particular substrate molecules. The active site is highly specific for a particular substrate. The substrate must be of a compatible shape for binding to occur.

The other model is known as the **induced-fit model** of enzyme action (Figure 9.7b). This model is more widely accepted. In this case, the enzyme shape is not fixed. The bonds that form between an enzyme and its substrate are thought to modify the shape of the enzyme so that the substrate can be fully accommodated by the enzyme. In this situation, the bonds within the substrate molecule are stretched and bent by the molecular interactions with the amino acid groups that line the active site. As a result of these stresses on the substrate, the activation energy required to kick-start the reaction is dramatically lowered and new product molecules are formed at a faster rate.



◀ Figure 9.6
Enzymes are highly specific molecules. A small part of the enzyme, called the active site, has the correct shape, or conformation, to bind with a specific reactant (substrate). The conformational change that results prepares the substrate for reaction.



◀ Figure 9.7
a) With the lock-and-key model, the substrate fits into a specific active site on the surface of the enzyme, where the reaction takes place. b) The induced-fit model. The substrate molecule enters the enzyme's active site, causing the enzyme molecule to change its shape so that the two molecules fit together more closely.

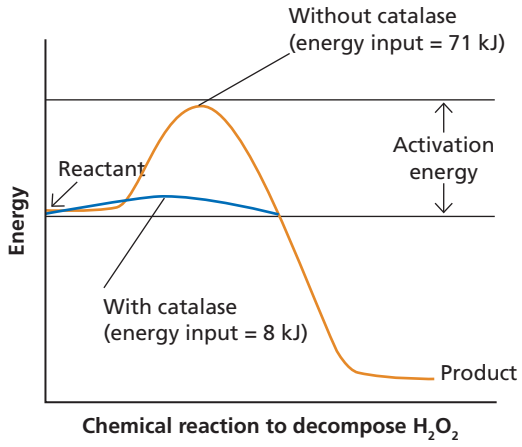


Figure 9.8 ▲ Catalase reduces the activation energy needed to break down hydrogen peroxide. Enzymes are powerful because they reduce the activation energy for chemical reactions.

Enzymes can work rapidly

One of the fastest enzymes is catalase. This enzyme is found in several organs and tissues, including the liver, where its job is to speed the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Hydrogen peroxide is a toxic by-product of metabolism, so it is essential that the cell removes it as fast as possible. Hydrogen peroxide has a high activation energy, which means that the energy input required before it will break down into oxygen and water is high. The simple addition of some ferric ions (Fe^{3+}) into a solution of hydrogen peroxide increases the rate of decomposition by a factor of 30 000. The ferric ions act as a catalyst and lower the activation energy required for hydrogen peroxide to decompose. In living cells, the enzyme catalase does a similar job. The decomposition of hydrogen peroxide in the presence of catalase can proceed up to 100 million times faster than without it! The action of enzymes in reducing the activation energy of reactions is represented in Figure 9.8.

Enzymes are not destroyed or altered by reactions

As enzymes are not destroyed or altered by the reactions they catalyse, they can be used again. The product molecules are not specific to the active site of an enzyme, so when they are released the active site becomes available for another substrate molecule. This is not to say that a given molecule of an enzyme can be used indefinitely, because the action of an enzyme depends critically on its shape, which is readily affected by changes such as temperature and acidity.

Enzymes can work in either direction

Enzyme-controlled reactions can work in either direction because metabolic reactions are generally reversible. The direction in which the reaction proceeds at any given time depends on the relative amounts of substrates and products present.

If there are a lot of reactants compared with products, an enzyme-controlled reaction will go from reactants to products until equilibrium between them is reached. One direction may be more favourable than the other, but the unfavourable direction of the reaction can occur using a different enzyme-controlled reaction. These types of reactions can also be referred to as equilibrium reactions.

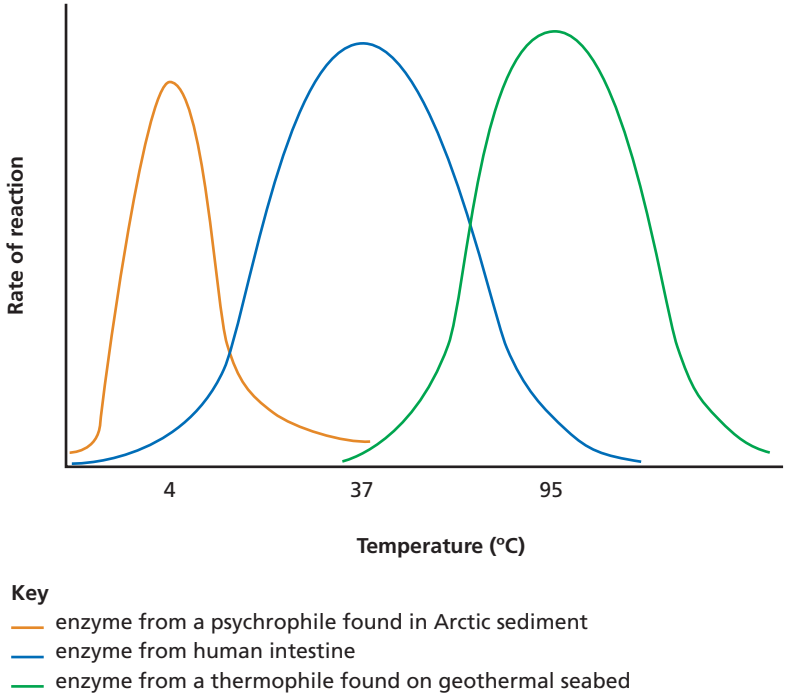
Factors that affect enzyme activity

Enzymes are involved in the processing of inputs both inside and outside cells. The intracellular and extracellular environments that enzymes work in are regulated to ensure that enzymes perform in a manner suitable to the cell's needs. Enzymes are sensitive to changes in temperature, pH, substrate and product concentrations, and other substances that may compete with a substrate for an active site.

Enzymes are affected by temperature

Enzymes are affected by temperature and have an optimal range in which they operate. The temperatures that enzymes work best in are the temperatures of the environment they can be found in. For example, enzymes operating in the human body work best at temperatures

of around 37°C (Figure 9.9), which is the relatively constant core temperature of the body. The enzymes of **psychrophiles** (or cryophiles), micro-organisms that live in near-freezing environments such as the wind-blasted rocks of snow-covered mountain summits, can operate at very low temperatures. This may be caused by the loss of some of the bonds that keep the protein rigidly folded. Having a more flexible structure means that enzymes require less energy to work. The micro-organism *Pyrodictium* exists in geothermal-heated areas of the sea floor. It is a **thermophile** and its enzymes operate best at temperatures of around 95–105°C.



◀ **Figure 9.9**
The temperature range for three different enzymes. Activity gradually increases until the optimum temperature for enzyme activity is reached. As temperature continues to increase, enzymes become denatured so the reaction rate decreases.

Enzymes from another thermophile, *Thermus aquaticus*, are utilised in biotechnology. The enzyme, *Taq* polymerase, is used in a technique called the polymerase chain reaction, which is used to make millions of copies of DNA, because it operates at the required reaction temperature of 70°C and is not **denatured** at elevated temperatures of 90°C.

So how does a change in temperature affect enzyme activity? As the temperature increases, molecules become more active and collide more often. This increase in collisions increases the opportunity for a substrate to bump into its enzyme so that it binds at the active site. The rate of reaction therefore increases. However, if the temperature gets too high, the protein loses its functional shape and the substrate can no longer bind with the active site. This change in shape is called denaturation. If the shape has changed enough to break the bonds between the connecting units of amino acids, proteins cannot return to their original shape when conditions revert to normal. In this case, the protein is destroyed.

This has repercussions for us that can be both dangerous and useful. If our body temperature rises too much during an infection, critical enzymes in our brain could denature, leading to seizures and possible death. On the other hand, we are able to chew and digest meat more easily after cooking. Raw meat is difficult to chew because of fibrous proteins contained in the muscle cells. By heating the meat, proteins are denatured, making it easier to chew and digest.

Consider the boiling of a lobster. Living lobsters obtain their blue-purple colour from the pigment astaxanthin. These pigment molecules are an orange colour until they bind to a protein in the lobster shell, which alters their shape and light-absorbing properties. This protein denatures when exposed to high cooking temperatures. As a result, the pigment molecules can no longer bind to the protein so the pigment returns to its free form and the lobster appears orange (Figure 9.10). Cooling the lobster after cooking does not make it blue-purple again because the effects of denaturing proteins are irreversible. It is important to note that enzymes are not denatured at low temperatures. Like all chemical reactions, a lower temperature means a lower rate of activity and therefore a lower rate of reaction.

Unit 3 of the biology course covers more information about biotechnology and genetic techniques, including the applications of polymerase chain reactions.

DENATURATION

How are enzymes denatured? Work through the material presented, click on the question mark boxes and view the animations.

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Figure 9.10 ▶
The change in colour from a) blue-purple to b) orange when lobster is cooked is caused by a pigment-binding protein in the shell, which denatures on cooking. As the lobster stays orange when it cools, it is apparent that the action of denaturing proteins is not reversible.

Enzymes are affected by pH

The pH of the solution surrounding enzymes, whether it is acidic, basic or neutral, can have a profound effect on the structure and activity of the active site of an enzyme and its interactions with a substrate. Each enzyme has an optimum pH at which it works (Figure 9.11). Some enzymes can work in a broad range of pH environments, while others are very sensitive and will only work in a narrow pH range. Most enzymes work most effectively around a neutral pH of 7. Again, the optimal pH of an enzyme relates to the environment in which it is found. Some work in environments of extreme pH, such as the enzyme pepsin, which operates in acidic gastric juices. It has an optimal pH of 1.5. Catalase, which works in the neutral environment of cells in the liver, has an optimal pH of 7. Alkaline phosphatase, which is found in the relatively alkaline environment of the bone, has an optimal pH of 9.5.

Some enzymes change shape in response to changes in pH. In some cases, such as excessive acidity or alkalinity, the active site shape can alter so much that the enzyme becomes denatured and can no longer catalyse a reaction, or the substrate may change shape so it no longer fits into an active site.

Enzymes work best within a limited temperature and pH range.

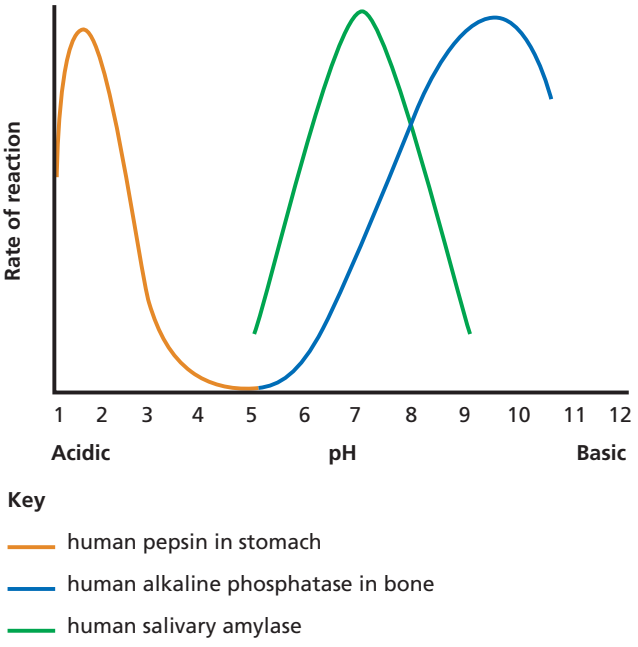


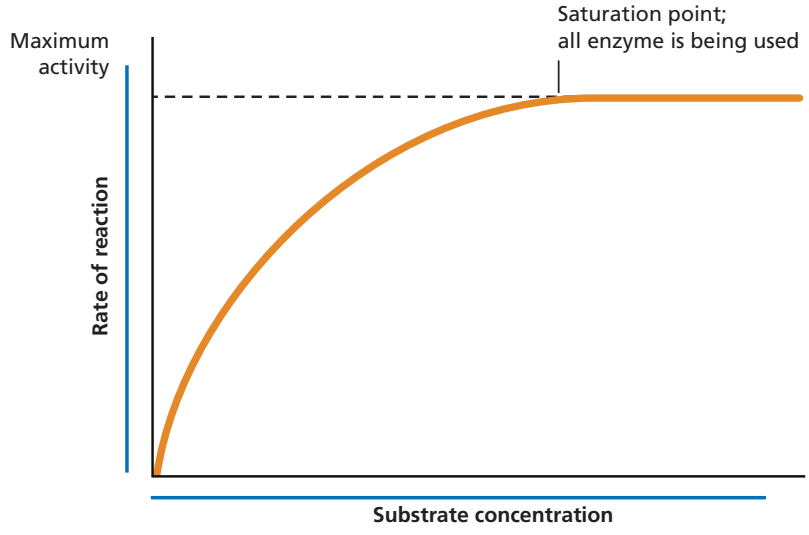
Figure 9.11 ▶
The optimum pH range for three different enzymes. The enzyme pepsin digests proteins in the acidic juices of the stomach, the enzyme alkaline phosphatase catalyses reactions in the relatively alkaline environment of the bone and the enzyme salivary amylase digests carbohydrates in the mouth at a neutral pH.

Substrate and enzyme concentration can affect enzyme activity

The amount of substrate or enzyme present in a reaction mix can limit the amount of product produced. Increased amounts of substrate will result in more products being made until all the enzyme molecules are working at their maximum capacity (Figure 9.12).

When the amount of enzyme in a system is increased, the amount of product increases exponentially. This keeps on happening until the product starts to inhibit enzyme action or the substrate is depleted. The rate of reaction is proportional to the enzyme concentration, provided there is excess substrate present.

Enzyme concentrations are regulated in response to the needs of a cell. This regulation is achieved by controlling the production of the protein, breaking down the enzyme or by activating the enzyme in response to a stimulus. For example, pepsinogen is an inactive form of the enzyme pepsin. When it enters the acidic environment of the stomach, it is activated to catalyse the digestion of proteins.



◀ **Figure 9.12**
The effect of increases in substrate concentration on the rate of an enzyme-catalysed reaction. At saturation, further increases in substrate concentration do not increase the rate of the reaction.

Enzymes are affected by inhibitors

Some enzymes have two or more active binding sites. These enzymes can move between their active and inactive state when inhibitor or activator molecules bind with them. The activity of almost every enzyme in a cell is regulated by feedback inhibition, in which the product of a reaction can inhibit enzyme activity. If a large amount of product is present in the cell, it will act as an inhibitor by binding to a site on the enzyme, other than the active site, thus slowing the rate of reaction. When the inhibitor binds to the enzyme, the active site of the enzyme changes shape so that it no longer has an affinity for its substrate. If the product is removed, inhibition will be reduced and the product will be produced again. This helps cells keep the concentration of products within a certain range. In this case, the inhibitor does not bind with the enzyme's active site so it is said to be a **non-competitive inhibitor**.

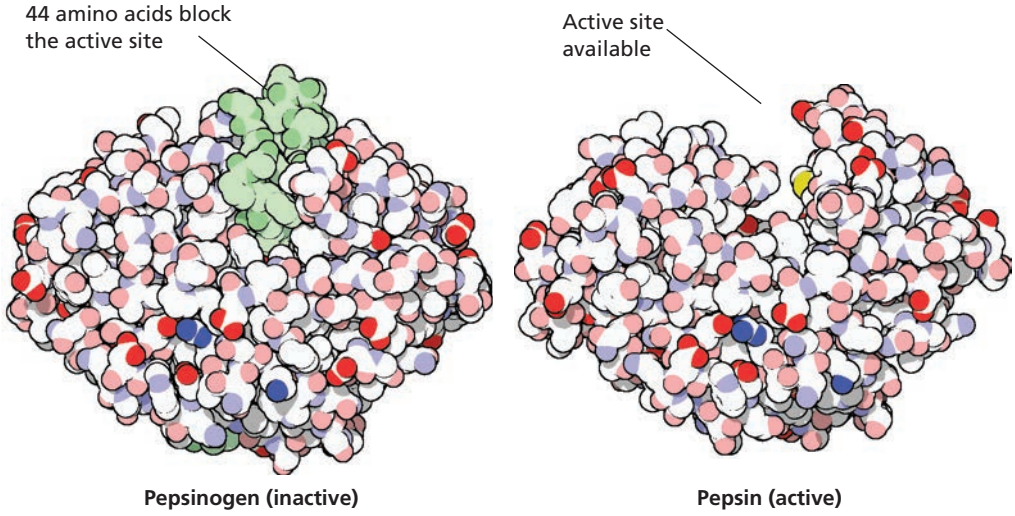


Figure 9.13 ▶
In the stomach, protein chains bind in the deep active site groove of pepsin so they are digested. Part of the polypeptide (shown in green) is removed from pepsinogen, the inactive form of pepsin. This then exposes the active site groove where proteins will bind.

ENZYME INHIBITION

View the animation of types of enzyme inhibitors.

Other inhibitors compete with the substrate for space in the active site and are said to be **competitive inhibitors**. For example, arsenic is an irreversible inhibitor that cannot be detected by the senses. Arsenate molecules resemble the phosphate substances used by cells for energy and signalling, and compete with them to bind to an enzyme's active site. Once arsenates are bound to an active site, the normal substrate is permanently excluded. Over time, less and less of the active enzyme remains to catalyse the reactions that produce energy for the cell. Thus, arsenic is called a chronic poison.

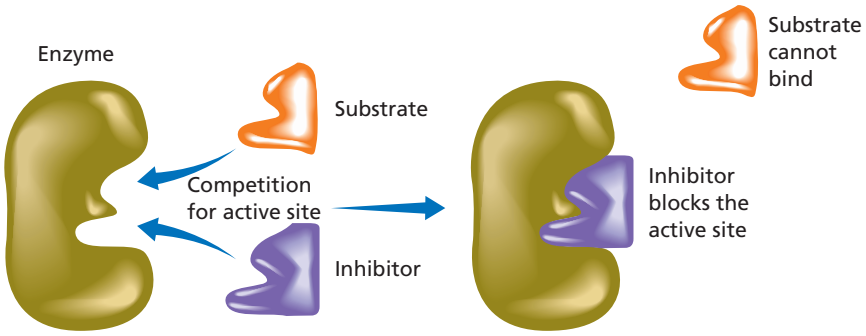


Figure 9.14 ▶
A competitive enzyme inhibitor blocks the active site of an enzyme so that the substrate can no longer fit in. Some inhibitors (poisons) bind irreversibly so the enzyme can no longer perform its specific function.

Enzyme activity is affected by cofactors and coenzymes

Some enzymes are inactive until they bind with other molecules or ions that change their conformation. This alters the shape and the charge of the enzyme's active site so that it can capture substrate molecules and catalyse reactions more efficiently. Two classes of substances bind to enzymes, or to the substrate, to activate the enzyme: **cofactors** and **coenzymes**. Cofactors are small inorganic substances, such as zinc ions and magnesium ions. Coenzymes are non-protein organic substances that are required for enzyme activity, and are relatively small molecules compared to the enzyme. Many are made by organisms from dietary vitamins and act as carriers of substances to and from reactions that are catalysed by enzymes. Coenzymes play a major role in metabolic pathways.

The cause of beri-beri, a disease that causes brain damage and affects short-term memory, is a deficiency of the vitamin thiamine, also known as vitamin B_1. Thiamine is converted to the coenzyme thiamine pyrophosphate. This aids in the breakdown of glucose during cellular respiration. Without thiamine, there is an insufficient amount of energy available. Beri-beri affects the brain because the brain depends on the breakdown of glucose for energy.

EXPERIMENT 9.1

THE EFFECT OF TEMPERATURE ON ENZYME ACTIVITY

Chemical reactions, such as those involved in photosynthesis and respiration, take place in living cells. Such reactions are critical to the continued life of the cell. It is also very important that these reactions take place at adequate rates to supply the cells with their needs as demanded by their activities. Enzymes have a vital role in these reactions. They speed up chemical reactions that occur in cells by decreasing the amount of activation energy required. Enzymes are proteins and are therefore affected by the same factors that affect all proteins. All proteins are sensitive to heat, and enzymes are no exception. The temperature at which an enzyme works most effectively is called its optimum temperature.

The enzymes studied in this activity are examples of intracellular enzymes, that is, they speed up and control metabolism within cells.

Aim

To test the effect that temperature has on enzyme function

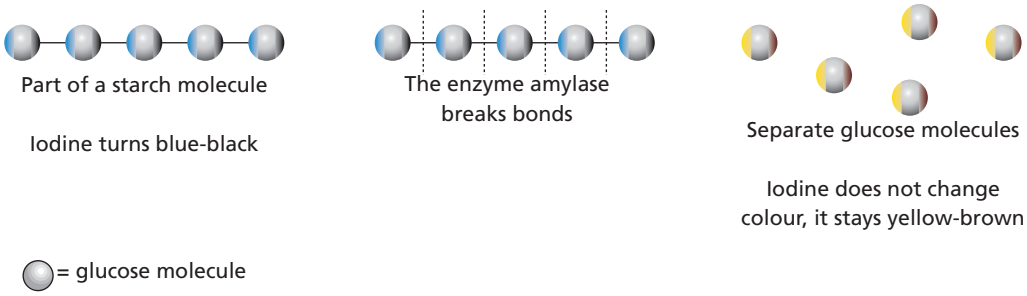
Materials

- six test tubes
- large beakers to hold test tubes
- clock or timer
- permanent marker
- thermometer
- 2% amylase solution in a dropper bottle
- 5% starch solution in a dropper bottle
- distilled water in a dropper bottle
- iodine solution
- two pipettes: 5 mL and 1 mL
- eight Pasteur pipettes
- toothpicks
- white tile or spotting tile
- glass rods
- test-tube rack
- Bunsen burner or hot plate
- ice water
- four water baths for the class

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Iodine can stain skin and clothing.	Take care to avoid spilling iodine on skin and clothing.
Hot water baths can burn.	Do not touch the sides of the water bath or the water within it.

Procedure

Amylase is an enzyme that breaks down starch molecules into separate glucose molecules. Iodine is a stain that turns blue-black in the presence of starch, but stays yellow-brown when starch is not present. Therefore, the colour of iodine is an indicator of how much starch is present.



▲ **Figure 9.15**
Amylase breaks starch down to glucose.

1 Each group of students will study an allocated temperature (see Table 9.1).

Table 9.1 Temperatures to be studied

	Group 1	Group 2	Group 3	Group 4	Group 5
Test tubes	1, 2, 3 A, B, C	1, 2, 3 A, B, C	1, 2, 3 A, B, C	1, 2, 3 A, B, C	1, 2, 3 A, B, C
Temperature of water bath	20°C	40°C	60°C	80°C	Ice water

2 Label six test tubes as indicated in Table 9.2 according to your group's temperature allocation.

3 To test tubes 1–3, add 5 mL of distilled water.

4 To test tubes A–C, add 5 mL of amylase solution.

5 Groups 1–4 are to place their test tubes in a beaker and place their beakers in their assigned water baths for 10 minutes. Group 5 maintains ice water in the beaker for 10 minutes.

6 While you are waiting, put a drop of amylase solution onto a white tile. Now add a drop of iodine to it and observe any colour change. Repeat this step using distilled water and then starch solution instead of amylase. Observe and record the final colour for each combination.

7 After 10 minutes, remove the test tubes from the water bath, place in the test-tube rack and allow the solutions to cool or warm to room temperature for 5 minutes.

8 Add 10 mL of starch solution to each test tube and mix, using a separate pipette for each test tube.

9 Use the pipette to remove a drop of each starch solution from each test tube. Place each drop on a clean white tile and add one drop of iodine to each. Mix with a clean toothpick.

10 Observe and record the colour in Table 9.2.

11 Repeat steps 8 and 9 every 5 minutes for each tube, for 20 minutes in total.

Results

1 Record any colour changes you observed when mixing:
- **a** amylase + iodine.
- **b** distilled water + iodine.
- **c** starch + iodine.

2 Record your group's results by copying and completing Table 9.2.

Table 9.2 Results of amylase activity at _____ temp.

Test tube	0 min.	5 min.	10 min.	15 min.	20 min.
1					
2					
3					
A					
B					
C					

3 Compare the rates of reaction at different temperatures.

Discussion

1 Using the class results, explain why some groups found different results for starch presence compared with other groups. Did any group find that the presence of starch changed over the testing time? Explain.

2 Explain how the colour produced after adding iodine indicates the activity of the enzyme. Explain why the colour changes throughout the testing time in some test tubes.
3 Explain why you tested the distilled water and the amylase with iodine before the solutions were placed in the water bath.
4 Explain why you had three identical test tubes for each condition you were testing.
5 Outline why tubes 1–3 were set up as controls.
6 Predict the optimum temperature for amylase activity. Justify your answer using the class results.
7 Are there any temperatures where amylase does not appear to function at all? Explain why this might occur.

Conclusion

1 Use the class results to propose a conclusion about whether temperature is an important factor in determining amylase activity. Explain your conclusion.

Taking it further

1 Design an experiment that investigates the effect of pH on enzyme activity. If possible, carry out the experiment.
2 As a result of a fever, a person's temperature may rise above the normal level. Discuss how this could affect cellular activity and in turn the entire body.

QUESTION SET 9.2

Remembering

1 Summarise the features of an enzyme.
2 Describe what happens to an enzyme after it has catalysed a reaction.
3 Outline how enzymes affect the activation energy required by reactants for a reaction to occur.
4 Outline two factors that can affect the activity of an enzyme.

Understanding

5 List the main properties of enzymes. Relate each property to the lock-and-key model.
6 Explain what has happened to an enzyme when it becomes denatured.
7 Describe how the amount of product produced in a reaction can affect an enzyme's activity.
8 Distinguish between a non-competitive inhibitor and a competitive inhibitor.
9 Discuss why enzymes are important for the maintenance of life.
10 Explain why there are thousands of different types of enzymes in the human body.

Applying

11 Explain the 'induced-fit model' of enzyme action. Describe how this is different to the 'lock-and-key model'.
12 Explain why doctors get worried if their patient develops a temperature in excess of 42°C.
13 Consider the enzyme-mediated reaction occurring early in cellular respiration:

$$\text{glucose 6-phosphate} \leftrightarrow \text{fructose 6-phosphate}$$

a Name this type of reaction.
b Predict which direction the reaction would proceed if there were high amounts of glucose 6-phosphate.
c Predict the conditions under which the reaction would proceed in the opposite direction.
d Suggest advantages in having enzyme-controlled reactions that can work in both directions.

Energy is transferred between reactions

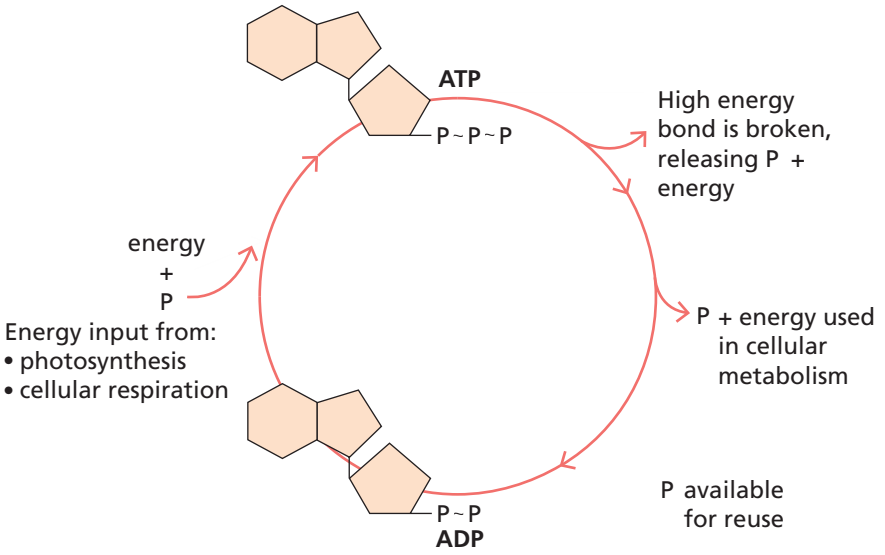
Cells use the chemical energy released from exergonic reactions to fuel endergonic reactions. The two reactions happen simultaneously in cells. In this process, some energy is lost as heat, which escapes from cells into the surrounding environment. As these reactions do not always occur in the same place within the cell, energy has to be transferred between reactions. This transfer is achieved by a molecule called ATP. ATP is readily moved around the cell.

ATP is an energy-carrier in all living cells. It couples energy-releasing reactions with energy-requiring ones. ATP is a nucleotide containing adenosine attached to a sugar group (ribose), which is bound to a chain of three phosphate groups.

ATP is a well-designed, renewable energy source. When a cell requires energy to drive an endergonic reaction, the high-energy chemical bonds attaching the last phosphate group to ATP are broken, thus releasing stored energy. This energy is now available to fuel a cellular reaction. The remaining molecule now has only two phosphate groups and is called **adenosine diphosphate (ADP)**. This reaction is sped up by the enzyme ATPase.

Free energy obtained from an exergonic reaction can also be used to add a phosphate group to ADP, converting it to ATP. The ATP–ADP cycle is the cell's way of shuttling energy between reactions (Figure 9.16). It provides the cell with an efficient linking or coupling of energy-yielding processes to energy-requiring processes within the cell by conserving, transferring and releasing energy.

Figure 9.16 ▶
The ATP–ADP cycle is the cell's way of renewing its supply of immediate energy.



Photosynthesis

If a pot plant growing in soil at a suitable temperature is put in light and watered, the plant's mass increases over time. The rate of photosynthesis in the plant is greater than the rate of cellular respiration. If the plant is enclosed in an airtight container, we could measure the amounts of oxygen and carbon dioxide in the air. While the photosynthetic rate is greater than the cellular respiration rate, the amount of oxygen would be increasing and the amount of carbon dioxide decreasing. If the plant was kept in the dark, the plant

Shutterstock.com/Andy Dean Photography

◀ **Figure 9.17**
Plants exposed to light increase in mass. Pumpkin plants would not be able to grow large fruit in the dark.

is undergoing cellular respiration only and we would find the amount of carbon dioxide would increase and oxygen would decrease in the surrounding container. The plant would gradually lose mass. This knowledge is not obvious by simply looking at a plant growing in the garden.

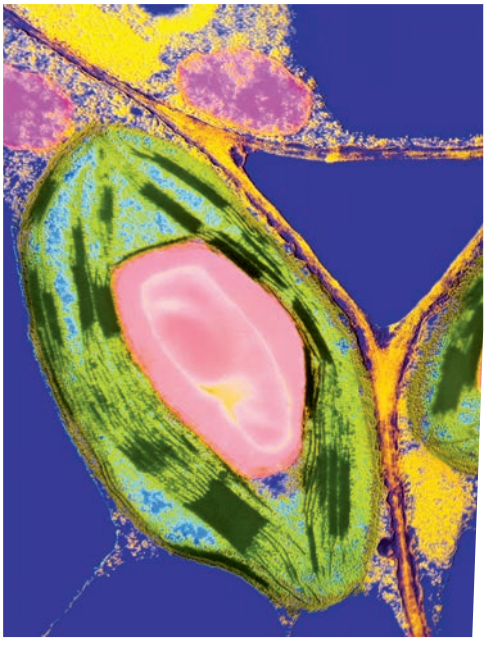
Photosynthesis is a series of steps

Photosynthesis is the process by which plants utilise light energy and use it to break down water and carbon dioxide molecules, and build them up into oxygen, glucose and water molecules. It can be summarised by the following equation.

However, this equation shows only the initial reactants and the final products. It does not tell the whole story. Photosynthesis actually occurs as a series of steps in a biochemical pathway, each catalysed by specific enzymes. It requires light as an energy source that is captured by the pigment chlorophyll.

Chloroplasts

Chloroplasts are the site of photosynthesis. Chloroplasts have an outer and an inner membrane. The **stroma** is enclosed by the inner membrane. This is a gel-like matrix rich in enzymes. Suspended in the stroma is a membrane system, the **thylakoid membranes**. These are flat, sac-like structures that are called **grana** when grouped together into stacks.



Getty Images/SPL/Dr Jeremy Burgess

◀ **Figure 9.18**
False-colour transmission electron micrograph of a green chloroplast from a leaf of the plant *Plectranthus scutellarioides*. The green, thread-like strands are thylakoid membranes. They pack together tightly to form grana. The large pink region is a starch grain, where the products of photosynthesis are temporarily stored after they have been produced in the light-independent reactions that take place in the stroma (magnification x5000).

See Figure 7.18 in Chapter 7 for a generalised sketch of a chloroplast. Chloroplasts have their own genetic material and ribosomes. See also Chapter 7 pages 169–70 for information on the evolutionary origin of chloroplasts.

The photosynthetic reaction is divided into two distinct stages: the **light-dependent stage** and the **light-independent stage**. Each stage is confined to specific sites within the chloroplast.

The light-dependent stage

Light energy is absorbed by different pigments within the thylakoid membranes. These pigments include chlorophylls (green), carotenoids (orange) and xanthophylls (yellow). Chlorophylls absorb the wavelengths of blue and red light, and they reflect the green wavelengths, which is why plant parts having an abundance of chlorophyll molecules appear green to us. All green algae and plants have chlorophylls as their major photosynthetic pigments.

When a chlorophyll molecule in the thylakoid membrane absorbs light energy, electrons within the molecule become energised. The energy is used to split water molecules (H_2O) into hydrogen ions (H^+) and oxygen gas (O_2), a by-product of photosynthesis. ATP molecules are formed in this stage.

Photosynthesis:

carbon dioxide + water → glucose + oxygen + water

$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$

The light-independent stage

The light-independent reactions occur in the stroma of the chloroplast. In this reaction, glucose molecules are produced from carbon dioxide. This reaction requires a supply of carbon dioxide gas (CO_2), hydrogen ions (H^+) and chemical energy in the form of ATP. ATP molecules formed in the light-dependent stage provide the chemical energy for the conversion of carbon dioxide to glucose molecules.

Many chemical reactions are involved in both the light-dependent and light-independent stages of photosynthesis.

Sucrose, starch and cellulose are polymers of glucose molecules produced in photosynthesis via other biochemical pathways. During daylight hours, chloroplasts convert the newly formed glucose molecule to sucrose or starch. Of all plant carbohydrates, sucrose is the most easily transported. Starch is the most common storage form. It is stored briefly in the stroma during the day. At night, cells convert starch to sucrose for export to other cells in leaves, stems and roots that lack chloroplasts.

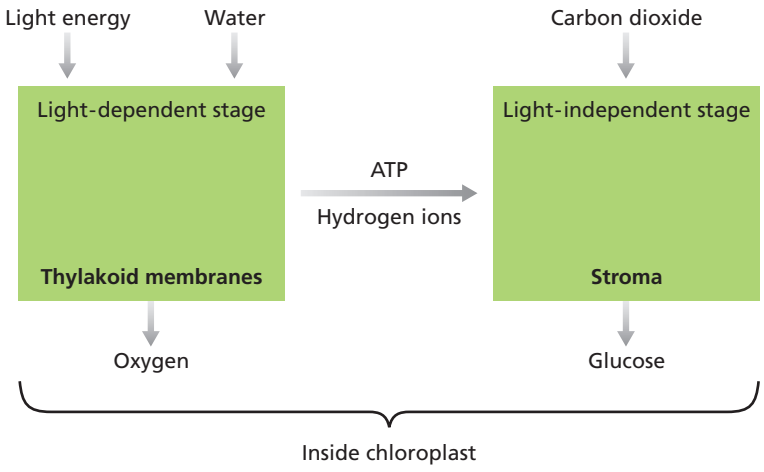


Figure 9.19 ▶ Photosynthesis is a series of reactions occurring in two stages: the light-dependent and the light-independent stages.

Scientific literacy: New sources of fuel

Global carbon emissions are rising steadily and if our planet is to avoid catastrophic warming, we must work rapidly to replace fossil fuels. Can photosynthesis help?

Plant power has already been harnessed for biofuel. US distilleries produce more than 50 billion litres of bioethanol annually, mainly from fermented corn. Most is blended with conventional petrol and used to power vehicles.

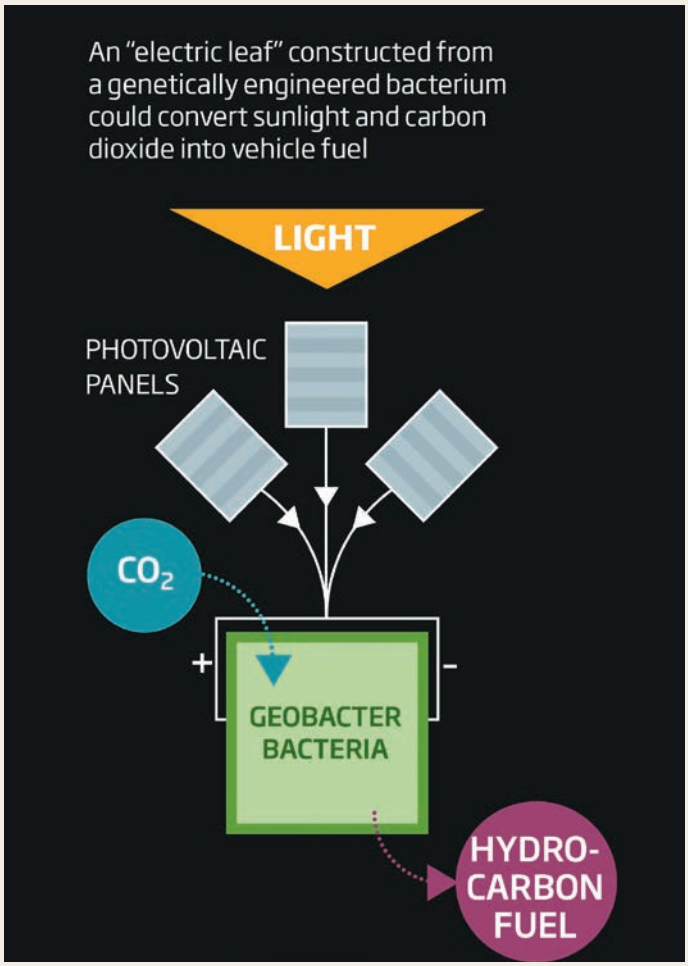
Yet questions remain over the sustainability of this biofuel. The conversion of solar energy into bioethanol is very inefficient, meaning huge areas of land are needed if production is to be scaled up.

Another way that photosynthesis can offer us fuels is if we can mimic the way in which plants and algae use light to split water, to generate H_2 as well as O_2. Scientists already do this in the lab – photovoltaic cells connected to a pair of platinum electrodes immersed in water will generate bubbles of H_2 fuel. However, this technique would be prohibitively expensive on a large scale because of the high cost of platinum. The challenge is to mass-produce electrodes at lower cost.

Still further off is the 'electric leaf' (Figure 9.20). This is a concept for a hybrid fuel generation system, using photovoltaic panels that supply electricity to living cells. These cells will be engineered to create not H_2, but energy-rich hydrocarbons.

A bacteria called *Geobacter* might provide the basis for the biological half of this double act. *Geobacter* isn't photosynthetic. Instead, it extracts electrons from minerals and uses them to power its metabolism.

This raises an intriguing question: could we modify *Geobacter* so it turns electrons into hydrocarbon fuel? The metabolic pathway required to synthesise hydrocarbons can be engineered into the bacteria *Escherichia coli*. In principle, the same thing could be done with *Geobacter*, creating a hybrid system that converts sunlight into a petrol substitute.



▲ **Figure 9.20**
An 'electric leaf' constructed from a genetically engineered bacterium could convert sunlight and carbon dioxide into vehicle fuel.

Cogdell, R. (2013) Instant expert 30: Photosynthesis, *New Scientist, 2902,* (2 February). © 2013 Reed Business Information–UK. All rights reserved. Distributed by Tribune Content Agency.

Questions

1 Explain how photosynthesis can help to replace fossil fuels.

2 **a** Predict the effect of biofuels replacing fossil fuels.
b Describe the arguments used by opponents of large-scale cultivation of biofuels.

3 Use Figure 9.20 to explain how an 'electric leaf' uses *Geobacter* to produce hydrocarbon fuel.

4 **a** Discuss why it is beneficial to engineer and enhance photosynthesis.
b What do you think is the most beneficial of these reasons? Explain your answer.

Case study

Combining Algal and Plant Photosynthesis (CAPP)

Plant scientists at the University of Cambridge in England have embarked on plans to improve crop yields by solving some of the limitations of photosynthesis.

Professor Howard Griffiths and Dr Julian Hibberd are two scientists at the University. Their CAPP (Combining Algal and Plant Photosynthesis) Project aims to address the growing demand for food and fuel by improving the process of photosynthesis. The ultimate aim is to introduce elements of algal photosynthetic systems into the model land plant *Arabidopsis thaliana*, to increase its photosynthetic efficiency. If successful, this may help pave the way for the production of 'super-efficient' crop plants in the future.

Professor Griffiths says, 'Plants really matter, and for the next generation, plant and microbial productivity will become the focus of key global issues: the basis for feeding an additional 2–3 billion mouths, to drive forward an economy currently trading on past sunlight, and maintain biodiversity in the face of climate change.'

The scientists are confident that now is a pivotal time for current progress in understanding photosynthesis to be harnessed with genetic techniques and traditional breeding resources to improve crop yields for the future. Nevertheless, the project is not a trivial undertaking, as Dr Hibberd explains: 'We're looking ahead to at least 15–20 years from now, to transform crop production in the decades when the potential yield of current crops has been exhaustively maximised.'

'For the next generation, plant and microbial productivity will become the focus of key global issues,' adds Professor Griffiths. 'It will be the basis for feeding an additional 2–3 billion mouths, for maintaining biodiversity in the face of climate change and for driving forward an economy currently trading on past sunlight.'

Information sourced from http://www.cam.ac.uk/research/news/turbocharging-a-new-green-revolution

Questions

1. Find out more about the plant *Arabidopsis thaliana*. Outline why it is widely used for studying plant science.
2. Consider a field trial to test the effectiveness of a modified crop plant.
   a. Describe the risks.
   b. Propose safeguards that would be required.
3. How willing would you be to eat a crop plant that has been modified by scientists? Give reasons for your decision.
4. How important is the development of super-efficient crops to you? Do you think it is as important to someone from a developing country? Explain your response.

QUESTION SET 9.3

Remembering

1. Name the products or outputs of the light-dependent reaction that are used as inputs in the light-independent reaction of photosynthesis.
2. Name the product of photosynthesis that contributes to the growth of plants.
3. Write a balanced equation for photosynthesis.

Understanding

4. Do all living plant cells carry out photosynthesis? Explain your answer.
5. Distinguish between the light-dependent and the light-independent stages of photosynthesis in terms of location, requirements and products.
6. Explain the relationship between ADP, ATP and ATPase.

Applying

7. Compare a battery to ATP. Describe how they are similar.

Cellular respiration

All organisms, with the exception of the Archaea, use glucose as the primary source of energy to drive cellular metabolism. The chemical bonds in glucose are broken, resulting in more stable products and the release of free energy in the form of ATP. This reaction, known as aerobic cellular respiration, can be summarised by the following equation.

Aerobic cellular respiration:

glucose + oxygen → carbon dioxide + water + adenosine triphosphate

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O +$ 36 ATP

The word 'aerobic' is used when oxygen is present. The equation above simply shows the initial reactants and the final products. There are approximately 20 reactions that occur in this biochemical pathway, each catalysed by specific enzymes. Most animals, plants, protists, fungi and bacteria are aerobes: they all require oxygen for cellular respiration. However, oxygen is not always present and many micro-organisms use other molecules instead. These organisms are called anaerobes and live in environments without oxygen. For all organisms, the breakdown of glucose to supply the cell with available energy, regardless of whether oxygen is present or not, starts with a biochemical pathway called **glycolysis**.

CELLULAR RESPIRATION

View the animation to assist your understanding of cellular respiration and compare this with photosynthesis.

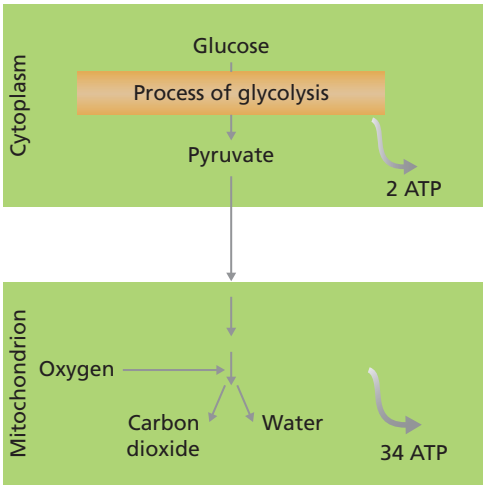
Glycolysis

Glycolysis takes place in the cytosol of cells. The biochemical pathway of glycolysis is made up of 10 reactions, with each step controlled by a specific enzyme. The initial reactant is glucose and the final product is two molecules of a compound called **pyruvate**. The glycolysis pathway also produces a net of two ATP molecules that may be used by the cell as a source of energy. These ATP molecules, which can be used by the cell immediately, may be sufficient for the needs of certain micro-organisms but is not sufficient for multicellular organisms. The fact that all organisms carry out glycolysis, either as their sole source of energy or as the first step in more elaborate pathways to gain sufficient ATP for their needs, points to glycolysis being one of the earliest reactions to produce energy for the cell. But what occurs after glycolysis in both prokaryotic and eukaryotic cells? This depends upon whether oxygen is present or absent.

Cellular respiration with oxygen

In eukaryotic cells that are supplied with oxygen, the two molecules of pyruvate formed in glycolysis enter the mitochondrion (Figure 9.21). Mitochondria are small, regularly shaped structures found scattered throughout the cell's cytosol. They are often described as the 'energy powerhouse' of the cell because large numbers of ATP molecules are produced in them.

After a series of steps, facilitated by various compounds within the mitochondrion, carbon dioxide molecules and water are produced. From all the reactions associated with aerobic cellular respiration, it is possible to produce a net 36 ATP molecules in one cycle.



◀ **Figure 9.21**
Glycolysis, the first stage of cellular respiration, occurs in the cytoplasm. The second stage of aerobic cellular respiration occurs in the mitochondria.

Cellular respiration without oxygen

The first energy-releasing pathways evolved around 3.8 billion years ago when there was very little free oxygen in the atmosphere. The process was essentially anaerobic in that it was able to run to completion without requiring oxygen. Many bacteria and protists still live in places where oxygen is absent or not always available. They produce ATP using anaerobic pathways. Such organisms have evolved biochemical pathways that allow glycolysis to continue in the cytosol by utilising molecules other than oxygen. Prokaryotes have evolved many anaerobic pathways, but eukaryotes commonly use two forms, which are referred to as **alcohol fermentation** and **lactic acid fermentation**.

Alcohol fermentation

Many micro-organisms, including yeast and some bacteria, carry out anaerobic cellular respiration. The products of alcohol fermentation are carbon dioxide and ethanol, an alcohol. The overall summary for alcoholic fermentation is given below.

Alcohol fermentation:

glucose → ethanol + carbon dioxide + adenosine triphosphate

$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 + 2\ ATP$

Shutterstock.com/colorvsbw

Figure 9.22 ▶ Alcohol fermentation has been used by industry to produce bread and wine. In bread, the alcohol is baked out.

Humans make use of these metabolic waste products in the production of wine, beer and bread (Figure 9.22). Plants, however, cannot make use of ethanol. It cannot be reconverted into carbohydrate, nor can it be broken down in the presence of oxygen. Furthermore, alcohol is toxic to cells and cannot be allowed to accumulate. Many plants (or parts of plants) can respire anaerobically for a short time, such as germinating seeds and roots living in water-logged soil, where there is little oxygen. However, before the concentration of ethanol reaches a certain level they must revert to aerobic respiration, otherwise they will be poisoned by the ethanol.

This is also true for yeast. Yeast is a classic example of an anaerobic organism that is used in the brewing and wine-making industries. Yet yeast grows much better in aerobic than in anaerobic conditions. If too little oxygen is present, the ethanol concentration rises so much that the yeast cells are killed. The secret to making beer and wine is not to let the conditions become too anaerobic. It is commercially beneficial to develop new strains of yeast that are tolerant to high concentrations of ethanol. This is a major occupation of microbiologists working for brewing companies.

Lactic acid fermentation

Lactic acid is the end product of anaerobic respiration in animals. Even though our body prefers to generate most of its energy using aerobic methods, some circumstances, such as strenuous exercise, require energy production faster than our bodies can adequately deliver oxygen to, in this case, our working muscles. In these cases, the working muscles generate energy anaerobically with a build-up of lactic acid. Once the strenuous exercise slows down, oxygen is now available once again and lactic acid reverts back to pyruvate, allowing continued aerobic metabolism and energy for the body's recovery from the strenuous event.

Aerobic respiration produces a lot of energy. In anaerobic respiration, glucose is not broken down as completely as it is in aerobic respiration and so less energy is released as a consequence. A lot of energy still remains locked up in the ethanol or lactic acid molecules.

WOW

Evolution of ethanol producing yeast

When flowering plants evolved 130 million years ago, a type of yeast, *Saccharomyces*, evolved to feed off them. The yeast evolved the ability to partially break down the sugar to ethanol. The yeast was competing with bacteria that also fed off the fruit. As ethanol kills bacteria, the yeast killed off its competition.

Lactic acid fermentation is an important pathway for energy production. This anaerobic respiration in animal cells is represented by the equation below.

Anaerobic respiration:

$$\text{glucose} \rightarrow \text{lactic acid} + \text{adenosine triphosphate}$$
$$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH + 2\ ATP$$

WOW

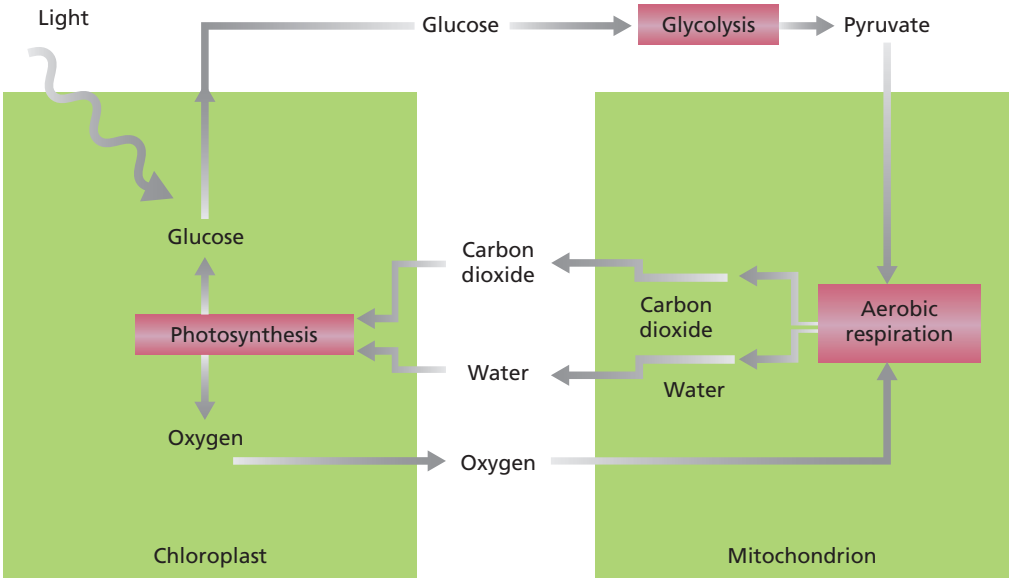
Rats on treadmills

Some people are born with less ability to take up oxygen and transfer energy than others. Scientists exploring this phenomenon bred rats over 11 generations to be good or poor runners. The best runners could last up to 42 minutes on a treadmill before becoming exhausted, whereas the less fit rats averaged just 14 minutes. This research suggests that the ability to use oxygen efficiently is genetically determined as well as being elevated by training.

Some of us don't have the genes to become a super athlete no matter how hard we train. Furthermore, the rats with high aerobic capacity were less likely to have risk factors linked to cardiovascular disease and stroke, such as high blood pressure and high cholesterol levels. The main player here seems to be the mitochondria. Researchers found that low-aerobic-capacity rats had comparatively reduced levels of enzymes and proteins used by the mitochondria.

Photosynthesis and aerobic cellular respiration are closely related and interdependent

The outputs of photosynthesis are the inputs of aerobic cellular respiration. Similarly, the outputs of aerobic cellular respiration are the inputs of photosynthesis. In plants and other autotrophs, the two processes can occur in the same individual cells when both chloroplasts and mitochondria are present. But many cells in green plants, such as root cells, do not have chloroplasts. These cells, and those of heterotrophs, depend on the products of photosynthesis to carry out cellular respiration. Thus, there is a dependency between autotrophs and heterotrophs.



◀ **Figure 9.23**
Photosynthesis uses the products of cellular respiration, and cellular respiration uses the products of photosynthesis.

EXPERIMENT 9.2

THE EFFECT OF LIGHT ON PHOTOSYNTHESIS

Living plant cells carry out cellular respiration all the time. When light is present, green plant cells also undergo photosynthesis.

When studying water plants, the rate of photosynthesis and cellular respiration can be measured by the amount of carbon dioxide used or produced. This can be observed indirectly by recording the pH of the surrounding water. When carbon dioxide dissolves in water, it forms carbonic acid (H_2CO_3) according to the following equation:

$$CO_2 + H_2O \rightarrow H_2CO_3$$

If the amount of carbon dioxide increases, more carbonic acid forms and the acidity increases (pH decreases). If the amount of carbon dioxide decreases, carbonic acid levels also decrease, reducing the amount of acid so that the pH increases and the surrounding watery solution becomes more basic.

Aim

To test the effect of light on the rate of photosynthesis

Materials

- two *Elodea* healthy leaf shoots (or an alternative freshwater oxygenator available from the local aquarium or biological supplier)
- five test tubes (four of them with stoppers)
- aluminium foil
- phenol red indicator in a dropper bottle
- 0.1 M ammonia solution in a dropper bottle
- 0.1 M hydrochloric acid in a dropper bottle
- beaker
- grow-lux lamp or bright light

What are the risks in doing this experiment?	How can you manage these risks to stay safe?
Hydrochloric acid and ammonia can be corrosive.	Avoid contact with skin and eyes. If spilt or splashed, rinse affected area immediately with plenty of water and report any accidents to your teacher. Eye wash facilities and equipment should be readily available.
Phenol red indicator can irritate skin and eyes.	Take care to avoid contact with skin and eyes.
Elodea is a noxious weed.	Dispose of *Elodea* safely, away from waterways.

Procedure

1. Half-fill a test tube with tap water and add two to three drops of phenol red indicator. Note the colour.
2. Add five drops of 0.1 M ammonia solution. Note any colour change.
3. Add five drops of 0.1 M hydrochloric acid. Note any colour change, and then add a further five drops of hydrochloric acid. Record your results. (Note that this part of the experiment is simply to produce some reference indicator colours.)
4. Collect enough aged tap water in a beaker (preferably the water the *Elodea* was in) to fill four test tubes in a beaker. Add five to six drops of phenol red to the water (in the beaker) to give it a good colour.
5. Place an *Elodea* shoot into each of two test tubes and fill with the water–phenol red mixture. Label the tubes A and B. Fill the other two test tubes with more of the same water and label these C and D.
6. Stopper the four tubes and record their colours.
7. Wrap tube A and tube C with aluminium foil.
8. Place all tubes under a grow-lux lamp or in bright light (but not direct).
9. Observe all four tubes the next day. Record any colour changes.
10. Remove the foil from the tubes and leave them in the light for another day. Observe and record their colours.

Results

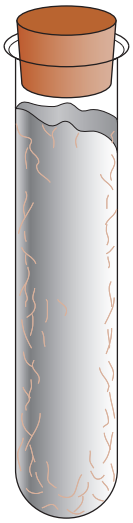
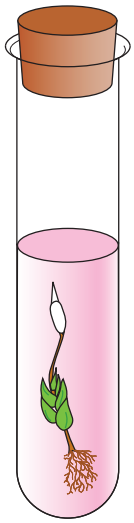
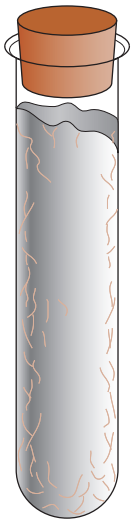
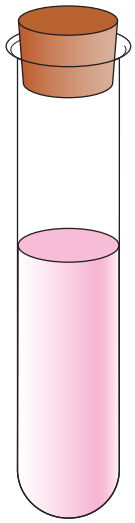
1 Copy Table 9.4 and record the indicator colours for:
   - **a** tap water and phenol red.
   - **b** tap water, phenol red and 0.1 M ammonia.
   - **c** tap water, phenol red, 0.1 M ammonia and 0.1 M hydrochloric acid.

2 Copy Table 9.5 and record the colour of tubes A to D.

Table 9.4 Reference indicator colours

Tap water and phenol red	Tap water, phenol red and 0.1 M ammonia	Tap water, phenol red, 0.1 M ammonia and 0.1 M hydrochloric acid

Table 9.5 Measuring effect of light on photosynthesis

	Tube A	Tube B	Tube C	Tube D
	Plant with foil wrap 	Plant without foil wrap 	Foil wrap without plant 	No plant no foil wrap 
Initial observations				
Colour **pH**				
Day 1 observations				
Colour **pH**				
Day 2 observations				
Colour **pH**				

Pool your data with other groups in the class to establish repetition of samples.

3 Explain the results seen in tube A after one day.

4 Explain the results seen in tube B after one day.

5 Describe the colour of the indicator in tubes A and C the day after the foil was removed. Explain why the indicator was this colour for each tube.

Discussion

1 Explain why aluminium foil was used to wrap around the test tubes instead of putting them in a dark place.
2 Name the tubes that were the control. List the variables being tested in this experiment.
3 Write balanced equations for:
   a photosynthesis.
   b cellular respiration.
4 Describe the conditions under which plants carry out photosynthesis. Describe the conditions under which plants carry out cellular respiration.
5 Explain why it was necessary to stopper the test tubes.
6 Explain why tubes C and D were used.
7 Predict what gas, other than carbon dioxide, could be used as a measure of the rate of photosynthesis and respiration. Explain why the concentration of carbon dioxide is easier to measure in this experiment.
8 Discuss the advantages of using water plants for this experiment.
9 Identify some possible sources of error in your experiment.

Taking it further

Devise an experiment to investigate the effect of different light intensities on the rate of photosynthesis.

Conclusion

1 Propose conclusions about the rate of photosynthesis compared to the rate of respiration if:
   a there is a net production of carbon dioxide.
   b there is a net use of carbon dioxide.
   c the amount of carbon dioxide remains the same.
2 Discuss what effect light has on the rate of photosynthesis compared to the rate of cellular respiration.

QUESTION SET 9.4

Remembering

1 Write a balanced equation for aerobic cellular respiration.
2 Identify the initial substrate in the glycolysis pathway and the final product.
3 Describe where glycolysis takes place in all cells.
4 List two differences between aerobic respiration and fermentation.

Understanding

5 Compare the products of anaerobic respiration with those of aerobic respiration in animals and plants.
6 Name the source of the by-product, carbon dioxide, in aerobic cellular respiration.

Applying

7 Describe the conditions under which aerobic cells carry out fermentation.

Evaluating

8 Discuss why alcohol fermentation is used in cake and bread making rather than lactic acid fermentation.
9 'Muscle cells cannot contract when deprived of oxygen.' Evaluate this statement by using your knowledge of pathways for cellular respiration.

CHAPTER SUMMARY

- Cells continually assemble, rearrange and break down organic compounds by carrying out many different chemical reactions.
- Internal cellular membranes allow many chemical reactions to occur at the same time. Membrane-bound cellular structures can store products and concentrate reactants. Increasing the surface area of membrane-bound cellular structures allows for more enzymes to be available for chemical reactions.
- Metabolic pathways are ordered, enzyme-regulated reaction sequences.
- The main metabolic pathways that transfer energy through living systems are photosynthesis and respiration.
- Anabolic reactions build large molecules from smaller molecules; they require an input of energy.
- Catabolic reactions break down large molecules to smaller ones; energy is released.
- Endergonic reactions use energy; exergonic reactions release energy. Energy from exergonic reactions fuel endergonic reactions.
- Enzymes enhance reaction rates by lowering the amount of activation energy required to start a reaction.
- Chemical reactions in cells occur in a series of regulated steps called biochemical pathways.
- The molecular structure of proteins, including enzymes, determines how they perform their functions. The active site of an enzyme has a highly specific shape for a particular substrate to which it attaches.
- There are two models of enzyme action: the lock-and-key model and the induced-fit model.
- Enzymes work very rapidly. They are not destroyed or altered by reactions – they can be reused. Enzymes can work in either direction of a metabolic reaction.
- Enzymes work best when the cellular environment stays within a limited range of temperature and pH.
- When temperatures are higher than the enzyme's range, the enzyme is denatured and cannot work again. When temperatures are lower than the enzyme's range, the rate of reaction is slowed down but the enzyme is not destroyed.
- Enzymes can be inhibited by a large amount of substrate, or by competitive inhibitors binding to the active site.
- Some enzymes need other molecules such as cofactors and coenzymes to activate them.
- ATP is the main energy-carrier in a cell. When one of the phosphates of the ATP is removed, ADP is produced. Energy is released in this process.
- Photosynthesis is a series of steps where cells utilise light energy to break down water and carbon dioxide molecules and build up glucose, oxygen and water molecules.
- The light-dependent stage of photosynthesis occurs when light is absorbed by chlorophyll in thylakoid membranes of chloroplasts. In this stage, water molecules are split into hydrogen ions and oxygen gas.
- The light-independent stage of photosynthesis occurs in the stroma of a chloroplast; hydrogen ions and carbon dioxide are combined to produce glucose.
- Aerobic cellular respiration is a series of steps where cells break down glucose and oxygen molecules and build up carbon dioxide and water molecules.
- Glycolysis is the first stage of cellular respiration occurring in the cytoplasm; glucose is broken down to pyruvate with a net yield of 2 ATP molecules for each glucose molecule.
- If oxygen is available, pyruvate produced from glycolysis enters mitochondria where aerobic cellular respiration is completed; there is a net yield of 36 ATP molecules for each glucose molecule.
- If oxygen is not available, pyruvate produced from glycolysis undergoes a series of steps in anaerobic cellular respiration to either produce ethanol and carbon dioxide (alcohol fermentation) or lactic acid (lactic acid fermentation) in the cytoplasm; there is a net yield of 2 ATP molecules.
- The outputs of photosynthesis are the inputs of aerobic cellular respiration. The outputs of aerobic cellular respiration are the inputs of photosynthesis.

CHAPTER GLOSSARY

activation energy the energy required to initiate a reaction

active site the place on the surface of an enzyme molecule where substrate molecules attach

adenosine diphosphate (ADP) a low-energy compound composed of adenine and ribose and two phosphate groups attached; it is converted to ATP for energy storage when it gains a phosphate group

adenosine triphosphate (ATP) a high-energy compound composed of adenine and ribose and three phosphate groups attached; it releases energy for cellular reactions when its last phosphate group is removed and it is converted to ADP

alcohol fermentation a form of anaerobic respiration (no oxygen present); glucose is converted to ethanol, a type of alcohol

anabolic reaction a reaction that builds up complex molecules from more simple ones

catabolic reaction a reaction, such as cellular respiration, that involves the breakdown of complex molecules to simpler products

catalyst a substance that speeds up chemical reactions without being used up in the reaction

cellular metabolism all of the chemical processes occurring in a living cell

coenzyme a small non-protein organic substance that needs to be present in addition to an enzyme to catalyse a certain reaction

cofactor a small inorganic substance that needs to be present in addition to an enzyme to catalyse a certain reaction

competitive inhibitor a substance that competes with a substrate for an enzyme's active site

denature the process by which the structure of a protein is changed by factors such as pH and temperature; the change in structure often destroys the shape of the active site of the molecule and results in a loss of function

endergonic reaction an energy-requiring chemical reaction

enzyme–substrate complex a substance formed when an enzyme and a substrate molecule join

exergonic reaction a reaction that releases energy

glycolysis an energy-yielding process occurring in the cell cytosol in which glucose is partially broken down to pyruvate in enzyme reactions that do not require oxygen; this first stage of cellular respiration produces two ATP molecules

grana the stack of thylakoid membranes in a chloroplast that contain chlorophyll

induced-fit model a model to explain that the shape of an enzyme's active site undergoes specific changes, induced by the substrate, to achieve a high degree of specificity with the substrate

lactic acid fermentation a form of anaerobic respiration (no oxygen present) that occurs in animal cells and some anaerobic bacteria; glucose is converted to lactic acid

light-dependent stage the first stage of photosynthesis; it requires light energy that is absorbed by chlorophyll; water molecules split to produce oxygen and hydrogen ions and ATP

light-independent stage the second stage of photosynthesis; through a series of reactions carbon dioxide, hydrogen ions and ATP produce carbohydrate

lock-and-key model a model suggesting that the shape of a substrate molecule is an exact fit to the shape of an enzyme's active site

non-competitive inhibitor a molecule that binds to an enzyme at a site other than the active site; this changes the shape of the enzyme so that the substrate can no longer bind to the active site

product the substance at the end of a metabolic reaction

psychrophile an organism that lives in extremely cold conditions

pyruvate the end product of glycolysis

stroma the jelly-like, semifluid interior of a chloroplast

substrate a substance that enters a reaction; also called reactants or precursors

thermophile an organism that lives in high-temperature environments

thylakoid membrane the interconnected, folded membranes within chloroplasts

CHAPTER REVIEW QUESTIONS

Remembering

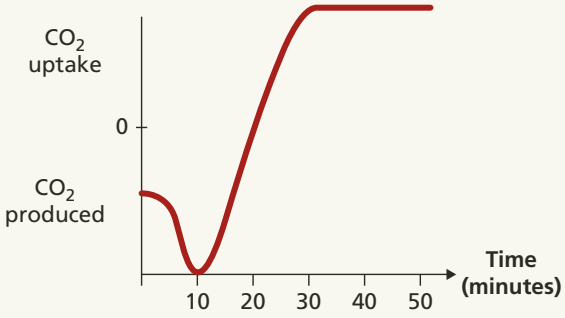
1 Describe the structure and role of the active site of an enzyme.
2 Describe factors that alter the speed of enzyme-controlled reactions.
3 List the conditions necessary for photosynthesis.

Understanding

4 Identify each of the following as either an anabolic or catabolic process, and justify your choice in each case.
   a Protein synthesis
   b Digestion
   c DNA synthesis
   d Photosynthesis
   e Cellular respiration
5 Describe two structural differences and two structural similarities between chloroplasts and mitochondria.
6 Compare alcoholic fermentation, lactic acid fermentation and aerobic respiration. In your comparison, you should consider starting materials, end products and energy produced.
7 Are photosynthesis and cellular respiration exact opposites? Explain.
8 Explain why enzyme action is said to be highly specific.
9 Describe how the lock-and-key model explains the effect of denaturing enzymes.
10 Describe the inputs of photosynthesis that the oxygen gas produced comes from.
11 ATP is an energy-carrier molecule. Explain how its structure is related to its function.

Applying

12 The pH of human blood and body fluids (excluding gastric juices) is approximately 6.8–7.0. Explain why maintaining this level of pH is important.
13 Figure 9.24 shows the relationship between net carbon dioxide production and uptake by a green plant.
   a Determine at what time the rate of photosynthesis would be equal to the rate of respiration.
   b Decide if the plant was put in light or dark conditions for the first 10 minutes of the experiment. Explain why you think so.
   c Predict what happened at 10 minutes to cause the change.
   d Suggest a reason why the carbon dioxide uptake levelled off at 30 minutes. Predict some limiting factors.
   e Predict what would happen to cellular metabolism if the temperature surrounding the plant increased beyond the plant's limit.
   f Explain why the carbon dioxide concentration in the air surrounding the plant can be used as a measure of the rate of photosynthesis.



▲ **Figure 9.24**
Inputs and outputs of carbon dioxide over time

14 Organisms such as the bacterium *Thermophilus* can thrive in hot springs at about 80°C. Use resource materials to find out why some enzymes are more heat-stable than others.
15 Investigate the use of a commercial enzyme and record your findings under the following subheadings: 'Source of the enzyme', 'Properties or action of the enzyme' and 'Industrial or commercial applications'. Some trade names of commercial enzymes are Neutrase, Lipolase, Lactozyme and Termamyl.
16 ATP is like currency in an economy. Use the ATP–ADP cycle to explain how it can be likened to spending and earning money.
17 The compensation point is when the rate of photosynthesis exactly equals the rate of cellular respiration. Predict how plant growth is likely to be affected at compensation point.

Analysing

18 If plant cells make their own food, explain why they need mitochondria.

19 Predict what would happen to a cell if its mitochondria failed to work.

20 During a heart attack, blood flowing to the heart muscle is interrupted by a blockage of a coronary artery. Predict how you would expect the metabolism in the heart to change.

21 Find out how the antibiotic penicillin affects enzyme action in bacteria.

22 A jar of preserved fruit looks frothy and smells of alcohol. Explain what has happened. Suggest what could be done to prevent this happening again.

23 A human protease works best at 37°C.

- **a** Predict what would happen to the enzyme's activity at very low temperatures.
- **b** Propose how this may differ from the activity of the enzyme at very high temperatures.
- **c** Describe what has happened to the active site in both cases.

24 Explain why arsenic is called a 'chronic poison'.

Evaluating

25 Enzymes are responsible for both sperm and male sex hormone production in the testicles of human males. Some of these enzymes have an optimal temperature of 33°C, which is about 4°C lower than body temperature. Discuss whether an increase in temperature would affect sperm production.

26 Many cut fruits will brown quickly when exposed to air. This is caused by the naturally occurring enzyme polyphenol oxidase. If the freshly cut fruit is rubbed with lemon juice, the brown discoloration almost disappears. Explain why this happens.

27 Chloroplasts were extracted from plants from two different habitats and observed. Plant A grows in open grassland and Plant B grows in a dense tropical rainforest. Predict which plant would have the most thylakoid membranes in their chloroplasts. Explain the reason for your choice.

28 You are given two test tubes containing two types of yeast cells that are the same in every way except that one can carry out only aerobic respiration and the other can carry out only anaerobic respiration. The tubes are labelled A and B. Yeast in tube A grows rapidly, whereas the yeast in tube B grows slowly. Predict which tube contains the cells capable of performing only aerobic respiration. Justify your choice. Devise an experiment to explain the test result.

29 Explain why cooling organs and tissues used in medical transplants prolongs their life. Refer to the effect of temperature on enzymes.

Creating

30 Your friend says she doesn't believe the air she breathes out contains carbon from the food she's eaten. Outline what you would say to convince her. Explain how what you have learned in this chapter has given you the confidence to answer her correctly.

Reflecting

31 Reflect on what you understand is the importance of enzymes for living beings.