SCHOLAR Study Guide

Higher Biology
Unit 2: Metabolism and Survival

Authored by:

Bryony Clutton (North Berwick High School)

Reviewed by:

Fiona Stewart (Perth Grammar School)

Previously authored by:

Jaquie Burt

Eileen Humphrey

Lorraine Knight

Nadine Randle

Fergus Forsyth

Patrick Hartie

Heriot-Watt University

Edinburgh EH14 4AS, United Kingdom.

First published 2018 by Heriot-Watt University.

This edition published in 2018 by Heriot-Watt University SCHOLAR.

Distributed by the SCHOLAR Forum.

SCHOLAR Study Guide Higher Biology: Unit 2

Higher Biology Course Code: C807 76

ISBN 978-1-911057-36-9

Print Production and Fulfilment in UK by Print Trail www.printtrail.com

Acknowledgements

Thanks are due to the members of Heriot-Watt University's SCHOLAR team who planned and created these materials, and to the many colleagues who reviewed the content.

We would like to acknowledge the assistance of the education authorities, colleges, teachers and students who contributed to the SCHOLAR programme and who evaluated these materials.

Grateful acknowledgement is made for permission to use the following material in the SCHOLAR programme:

The Scottish Qualifications Authority for permission to use Past Papers assessments.

The Scottish Government for financial support.

The content of this Study Guide is aligned to the Scottish Qualifications Authority (SQA) curriculum.

Contents

1 Metabolic pathways 1
- 1.1 Introduction to metabolic pathways . . . 3
- 1.2 Membranes and metabolic pathways . . . 3
- 1.3 Metabolic pathways . . . 4
- 1.4 Enzyme action . . . 5
- 1.5 Control of enzyme activity . . . 10
- 1.6 Learning points . . . 14
- 1.7 Extended response question . . . 16
- 1.8 End of topic test . . . 16

2 Cellular respiration 21
- 2.1 The role of ATP . . . 23
- 2.2 The chemistry of respiration . . . 25
- 2.3 Learning points . . . 31
- 2.4 Extended response question . . . 32
- 2.5 End of topic test . . . 32

3 Metabolic rate 35
- 3.1 Measuring metabolic rate . . . 36
- 3.2 Oxygen delivery . . . 37
- 3.3 Learning points . . . 39
- 3.4 End of topic test . . . 40

4 Metabolism in conformers and regulators 41
- 4.1 Introduction . . . 42
- 4.2 Conformers . . . 42
- 4.3 Regulators . . . 45
- 4.4 Negative feedback control of body temperature . . . 47
- 4.5 Learning points . . . 53
- 4.6 Extended response question . . . 54
- 4.7 Extension materials . . . 54
- 4.8 End of topic test . . . 56

5 Maintaining metabolism 57
- 5.1 Introduction . . . 58
- 5.2 Dormancy . . . 58
- 5.3 Migration . . . 61
- 5.4 Learning points . . . 64
- 5.5 End of topic test . . . 64

6 Environmental control of metabolism 67

6.1 Microorganisms 68
6.2 Growth of microorganisms 72
6.3 Patterns of growth 75
6.4 Penicillin production 79
6.5 Learning points 80
6.6 Extended response question 81
6.7 Extension materials 81
6.8 End of topic test 83

7 Genetic control of metabolism 87
7.1 Improving wild strains of microorganisms 89
7.2 Mutagenesis 89
7.3 Selective breeding 90
7.4 Recombinant DNA 90
7.5 Recombinant DNA technology 91
7.6 Bovine somatotrophin (BST) 94
7.7 Learning points 96
7.8 Extension materials 97
7.9 End of topic test 98

8 End of unit test 99

Glossary 108

Answers to questions and activities 111

Topic 1

Metabolic pathways

Contents

1.1 Introduction to metabolic pathways . . . 3
1.2 Membranes and metabolic pathways . . . 3
1.3 Metabolic pathways . . . 4
1.4 Enzyme action . . . 5
1.4.1 Enzyme properties . . . 5
1.4.2 Enzymes and activation energy . . . 8
1.5 Control of enzyme activity . . . 10
1.5.1 Controlling enzyme activity . . . 10
1.5.2 Competitive inhibition . . . 10
1.5.3 Non-competitive inhibition . . . 11
1.5.4 Feedback inhibition . . . 12
1.5.5 Experimental evidence of altering enzyme activity . . . 14
1.6 Learning points . . . 14
1.7 Extended response question . . . 16
1.8 End of topic test . . . 16

Prerequisites

You should already know that:

- the cell membrane consists of phospholipids and proteins and is selectively permeable;
- enzymes speed up cellular reactions and are unchanged in the process;
- enzymes function as biological catalysts and are made by all living cells;
- they speed up cellular reactions and are unchanged in the process;
- the shape of the active site of enzyme molecules is complementary to a specific substrate.

Learning objective

By the end of this topic, you should be able to:

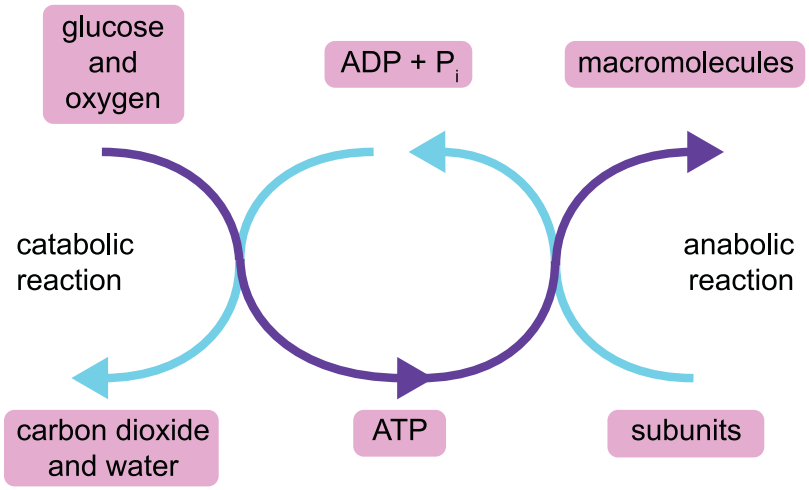
- understand that metabolism is the sum total of all chemical reactions taking place within an organism;
- describe how reactions occur in sequences, each one mediated by a specific enzyme;
- describe these sequences as pathways and explain how they are linked and may be reversed;
- explain the differences between anabolic and catabolic pathways;
- describe the functions of protein embedded in the plasma membrane;
- understand that metabolic pathways are controlled by enzymes within the pathway;
- state that the rate of a pathway's reaction is controlled by the rates of the enzymes' reaction;
- describe the relationship between the shape (configuration) and activity of an enzyme;
- describe the 'induced fit' model of enzyme action;
- explain the role and function of the active site;
- explain what is meant by the term 'activation energy' and state that enzymes lower the activation energy of a reaction;
- explain how the concentrations of both substrate and end product affect the rate of enzyme action;
- explain why some genes are constantly expressed and how they are regulated;
- describe inhibition of enzymes in terms of a change in configuration;
- explain how metabolic pathways can be controlled through competitive and non-competitive inhibition of enzymes;
- explain how metabolic pathways can be controlled by feedback inhibition of enzymes.

1.1 Introduction to metabolic pathways

Metabolism is the term used to describe the enormous number of integrated and complex biochemical reactions that occur in an organism. These reactions are ordered into pathways and controlled at each stage by an enzyme. By means of these metabolic pathways, the cell is able to transform energy, degrade macromolecules and synthesise new organic molecules that are needed for life.

A **catabolic** reaction releases energy through the breakdown of a large molecule into smaller units (cellular respiration is a good example of this). An **anabolic** reaction uses energy to build small molecules into large ones, such as the synthesis of a protein from amino acids.

Many of the pathways are reversible, but some are not. For those that have stages that cannot be reversed, there are often alternative pathways that can overcome the blockage.



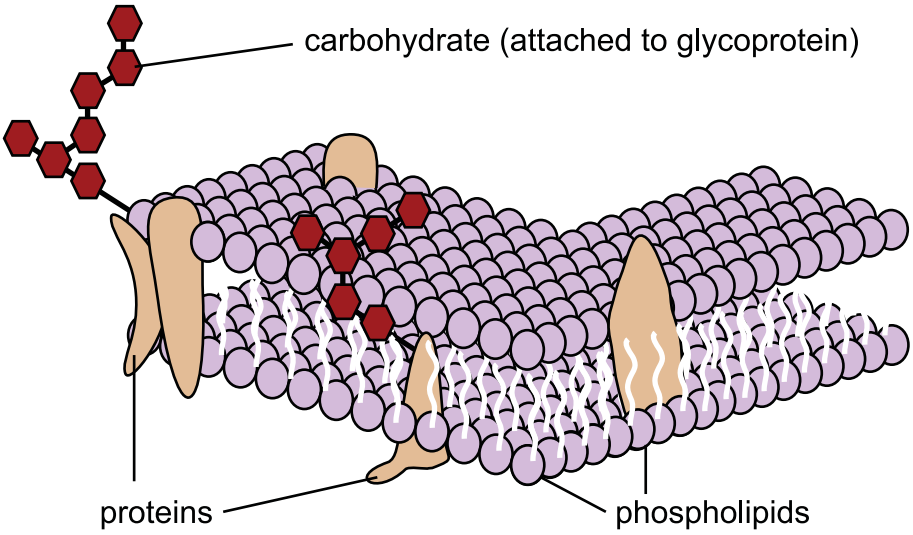
Energy coupling

1.2 Membranes and metabolic pathways

Membranes are vital to the activity of all cells. The plasma membrane separates the cell from its immediate environment whilst other membranes, similar in structure to the plasma membrane, divide the contents of the eukaryotic cell into specialised compartments. Molecules on the surface of the plasma membrane are involved in cell communication, and the membrane system as a whole is essential for transport both within cells and between cells.

The plasma membrane surrounding the cell is approximately 8nm wide, it is **selectively permeable**, and its unique structure determines both its function and physical characteristics.

Currently, the accepted model of membrane structure is the fluid mosaic model. This states that the membrane is made up of a bilayer of phospholipids with proteins embedded in it. The fluid mosaic model, illustrated below, highlights the complexity of the membrane, which is dynamic in nature.



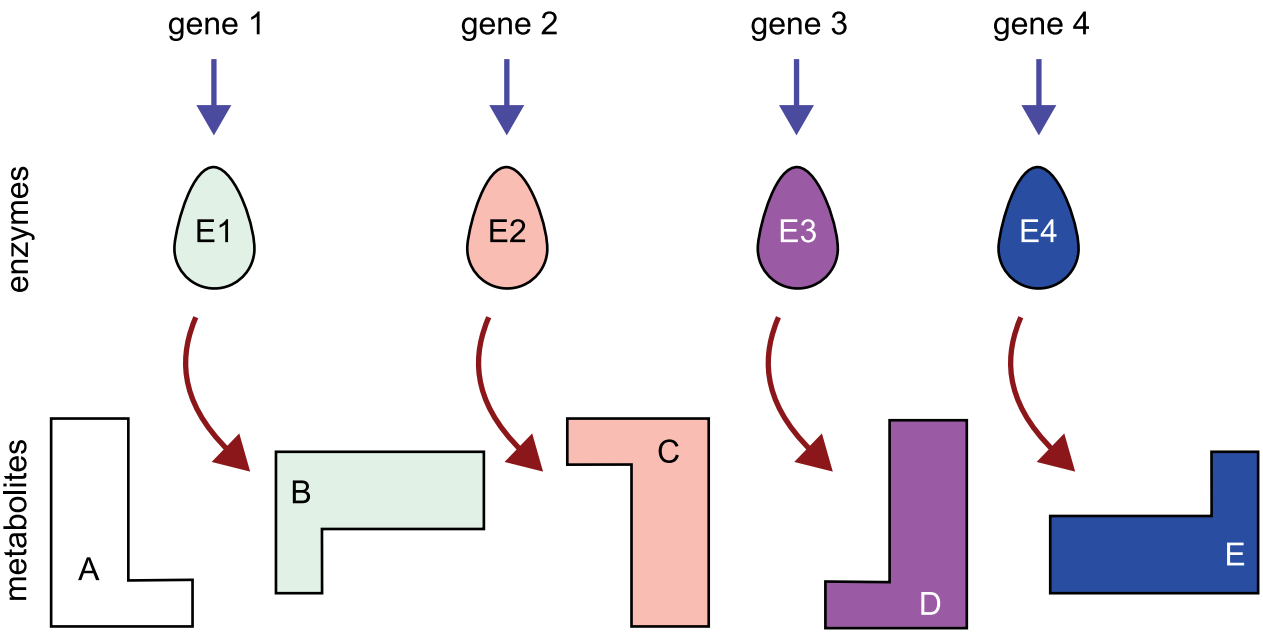
Fluid mosaic model of membrane structure

The membrane has proteins dispersed and embedded in the phospholipid bilayer that vary in both structure and function. The variety of functions carried out by membrane proteins are described below.

- *Channel (pore) proteins* - these proteins allow specific molecules and ions to pass through the membrane, for example, a protein channel found in the plasma membrane allows chloride ions (Cl^-) to cross the membrane.
- *Carrier (pump) proteins* - as the name suggests, carrier proteins bind to specific molecules or ions temporarily, enabling them to cross the membrane. This involves a change to the conformation of the carrier protein, which may require energy provided by ATP. The sodium-potassium pump is an example of a carrier protein involved in the transport of ions.
- *Enzymes* - some proteins in the membrane catalyse a specific reaction. Some receptor proteins have enzymatic activity, in which the cytoplasmic portion of the protein catalyses a reaction in response to binding by a ligand.
- *Structural support* - some membrane proteins are linked to the cytoskeleton and help to maintain the shape of the cell.

1.3 Metabolic pathways

Metabolism is the term used to describe all of the chemical reactions that occur within an organism. A metabolic pathway is a sequence of reactions that is controlled by enzymes that change one **metabolite** to another.



A metabolic pathway

Problems occur in metabolic pathways if the enzymes are not synthesised correctly, due to mutations in the genes that code for them. The next reaction in the pathway is then unable to occur and the intermediate metabolite builds up in the system.

Metabolic pathways are controlled by altering the presence and/or activity of key enzymes within the pathway. The regulation is brought about by signalling molecules from within the cell or from other cells.

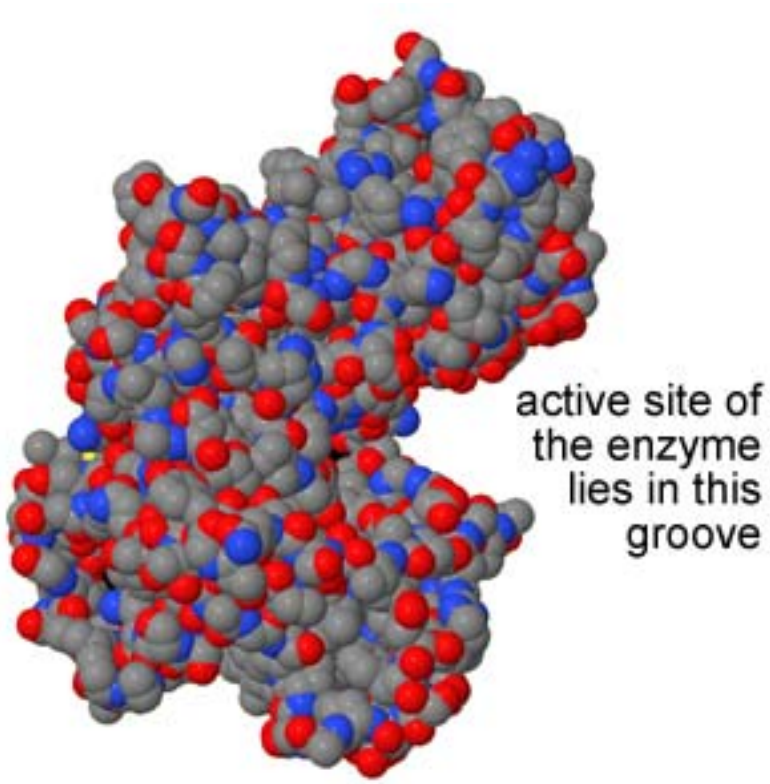
1.4 Enzyme action

This section covers enzyme properties, enzymes and activation energy, and enzymes groups and multi-enzyme complexes.

1.4.1 Enzyme properties

Enzymes are three-dimensional **biological catalysts** comprising of globular protein molecules that are only produced in living organisms. They possess a small region called the **active site** where the substrate binds and the reaction occurs, and are specific in the reactions that they catalyse (one enzyme, one substrate).

Enzyme activity conforms to the **induced fit** model. The substrate molecule induces a slight change in the shape of the active site to allow the substrate molecule to fit perfectly. The change in shape of the active site facilitates the reaction by correctly orienting the reactants. After the reaction is complete, the products have a low affinity for the active site and are released; the active site resumes its normal shape and the enzyme is free to attach to more substrate molecules. This can be summarised as the catalytic cycle. A space-filling model of the enzyme hexokinase is shown below. This enzyme uses ATP to phosphorylate glucose to glucose-6-phosphate. The active site lies in a groove (as labelled), which closes when glucose is bound to the enzyme. This facilitates the reaction and increases the efficiency with which the enzyme binds ATP.



Space-filling model of the enzyme hexokinase showing the location of the active site

Enzymes are proteins, which means that their activity and/or structure will be affected by changes in conditions such as temperature and pH. The rate of an enzyme-catalysed reaction is also affected by:

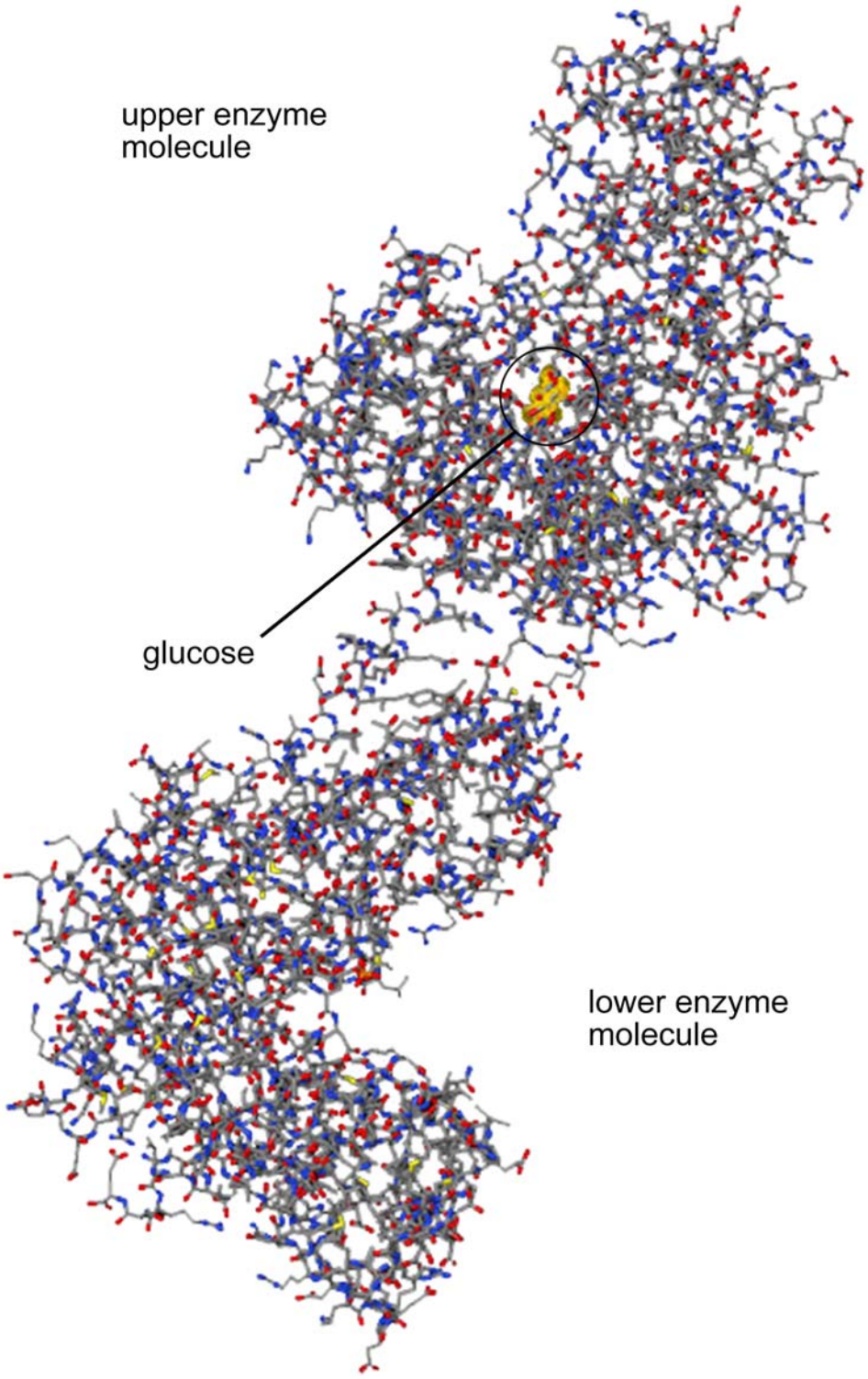
- the concentration of the enzyme;
- the concentration of the substrate.

Since enzymes are catalysts, they:

- are required only in relatively small amounts;
- remain unchanged at the end of a reaction.

Hexokinase and the induced fit model of enzyme activity: Interactive 3D model Go online

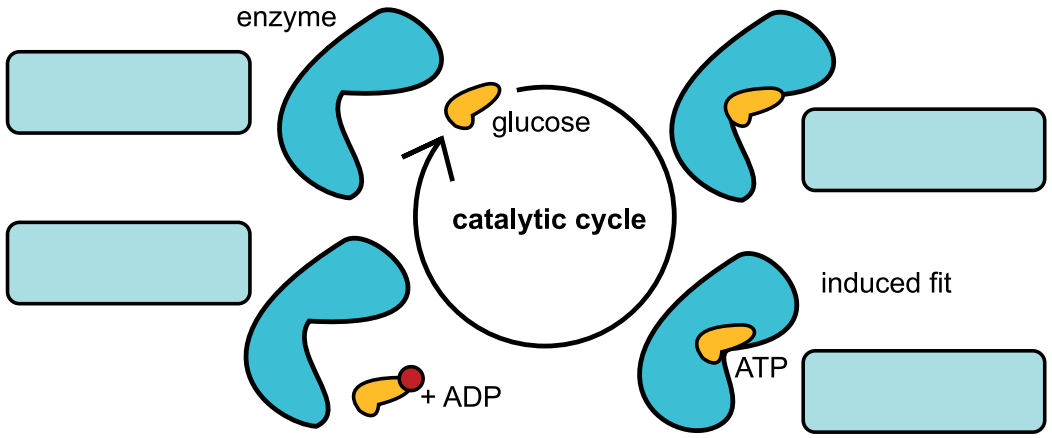
An activity that shows a molecular model of hexokinase (dimer) as an interactive 3D model is available in the online materials at this point. The following illustration gives an idea of what to expect. The position of a glucose molecule in the upper enzyme molecule is shown.



Enzyme properties: Question Go online

The following illustrates the induced fit model of enzyme activity as applied to hexokinase.

Q1: Complete the diagram of the catalytic cycle for hexokinase by inserting the labels in the appropriate places.

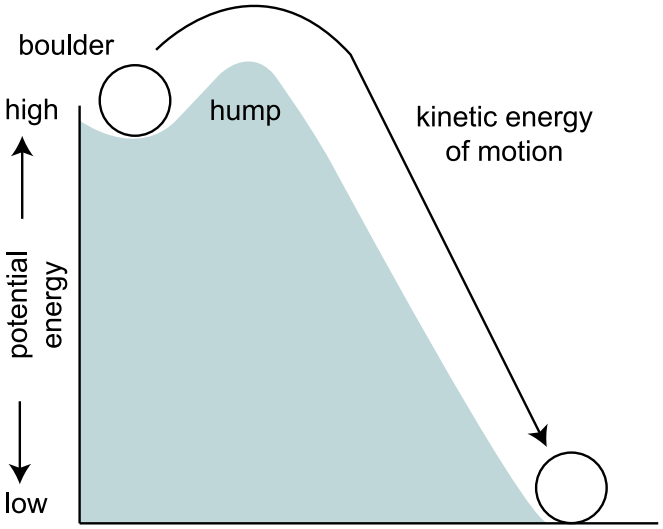


enzyme available again | products released (G-6-P and ADP) | glucose binds | ATP binding enhanced

1.4.2 Enzymes and activation energy

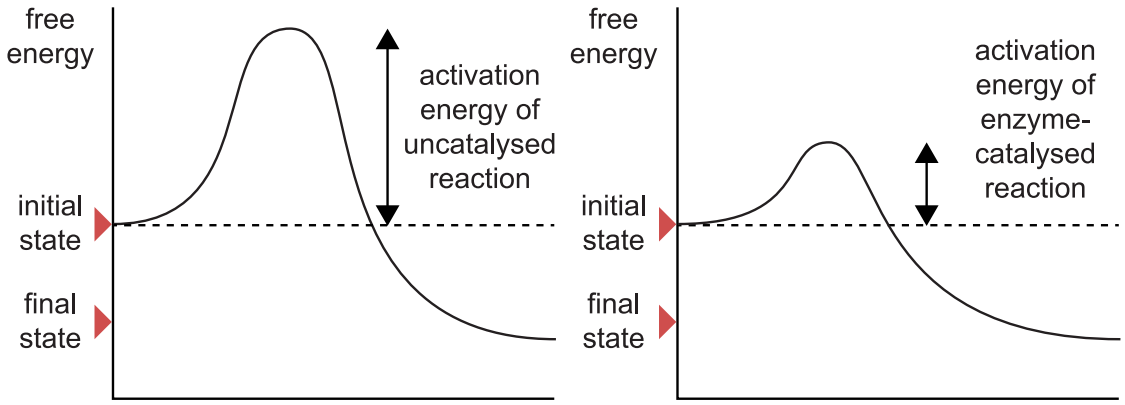
Chemical reactions can involve the build up or a break down of a substance. In either case, the energy required to initiate the reaction is known as the **activation energy**.

If a catalyst is absent, the energy required to cause a chemical reaction is quite large and the speed of the reaction extremely slow. The presence of a catalyst ensures that the energy requirement is lowered and that the reaction takes place faster. In living systems, an enzyme lowers the activation energy by forming an enzyme-substrate complex that accelerates the rate of reaction. It is like rolling a boulder down a hill, but having to push it up a small hump first - this initial push takes energy, but after that the boulder rolls on. Enzymes make the small hump even smaller!



Activation energy

The effect of an enzyme on the activation energy of a reaction is shown below.



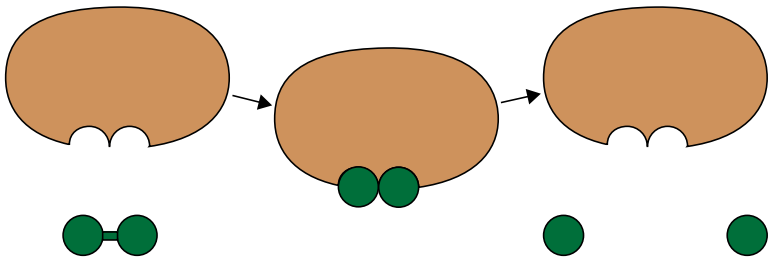
Effect of an enzyme on activation energy

Enzymes work by:

- bringing the substrates of a reaction close together (at the active site) so that they can react;
- lowering the activation energy of the reaction, so reactions can occur.

Enzyme action: Visualisation Go online

A simple enzyme-substrate reaction is modelled below.



1.5 Control of enzyme activity

This section covers controlling enzyme activity, competitive inhibition, non-competitive inhibition, feedback inhibition and experimental evidence of altering enzyme activity.

1.5.1 Controlling enzyme activity

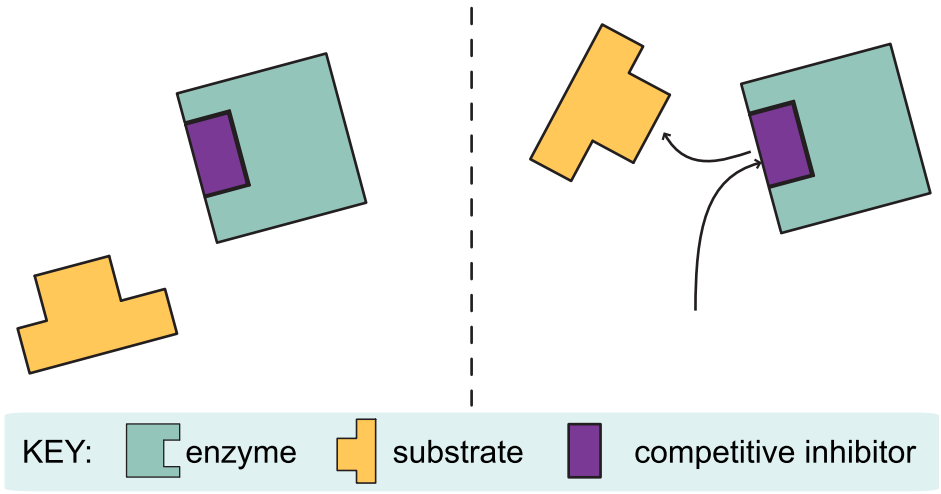
The number of enzyme-catalysed reactions in a cell is enormous, so the presence and number of enzyme molecules must be tightly controlled to ensure metabolic efficiency. Regulation of enzyme activity can be achieved in several ways:

- control of the number of enzyme molecules actually present in the cell - this is generally achieved at the level of gene expression;
- compartmentalisation, for example the enzymes required for the citric acid cycle and the electron transfer chain (two stages of respiration you will learn about in the next section) are contained in the mitochondria;
- change of enzyme shape - by far the most effective way of regulating an enzyme is to change its shape and therefore enzyme efficiency: a change in shape may either reduce or enhance enzyme activity, depending on the precise events taking place.

1.5.2 Competitive inhibition

Competitive inhibitors bind at the active site preventing the substrate from binding. This type of inhibition can be reversed by increasing the concentration of the correct substrate in the reaction.

Competitive inhibition: Visualisation Go online



A good example of a reaction where the enzyme is subject to competitive inhibition is the conversion of succinate to fumarate in the citric acid cycle. This reaction is catalysed by the enzyme succinate dehydrogenase. However, if both succinate and malonate (which are very similar in structure) are present in the reaction vessel, they will compete for the active site of the enzyme. This reduces the rate of reaction because some of the active sites of the enzyme molecules are being occupied by the malonate. To increase the rate of reaction again, more succinate is added to the reaction vessel, ensuring that the enzyme is more likely to collide with the correct substrate molecule.

Competitive inhibition: Questions Go online

Q2: Explain the importance of the active site.

. . . . . .

Q3: How is a competitive inhibitor related to the substrate of an enzyme-catalysed reaction?

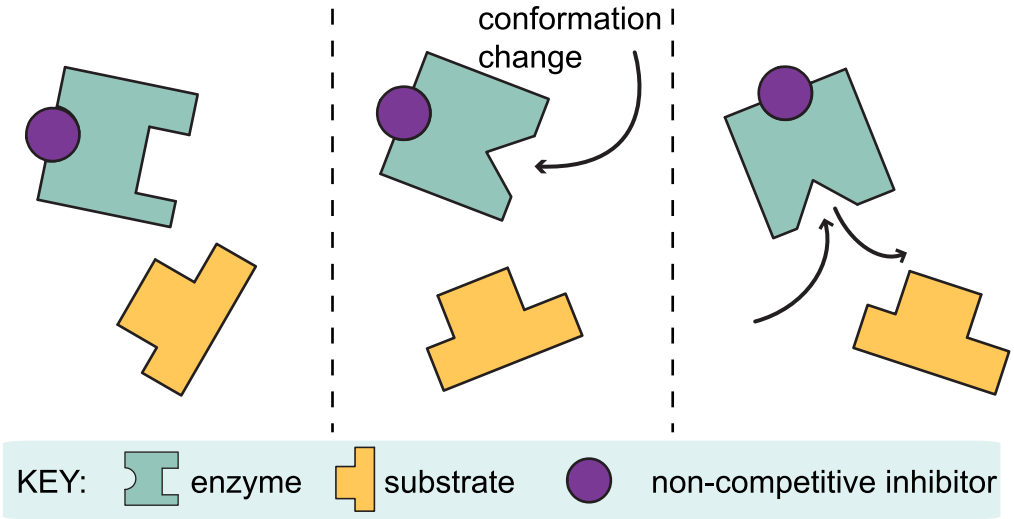
. . . . . .

Q4: How can competitive inhibition be overcome in experimental situations?

1.5.3 Non-competitive inhibition

Non-competitive inhibitors bind away from the active site but change the shape of the active site preventing the substrate from binding. Non-competitive inhibition cannot be reversed by increasing substrate concentration.

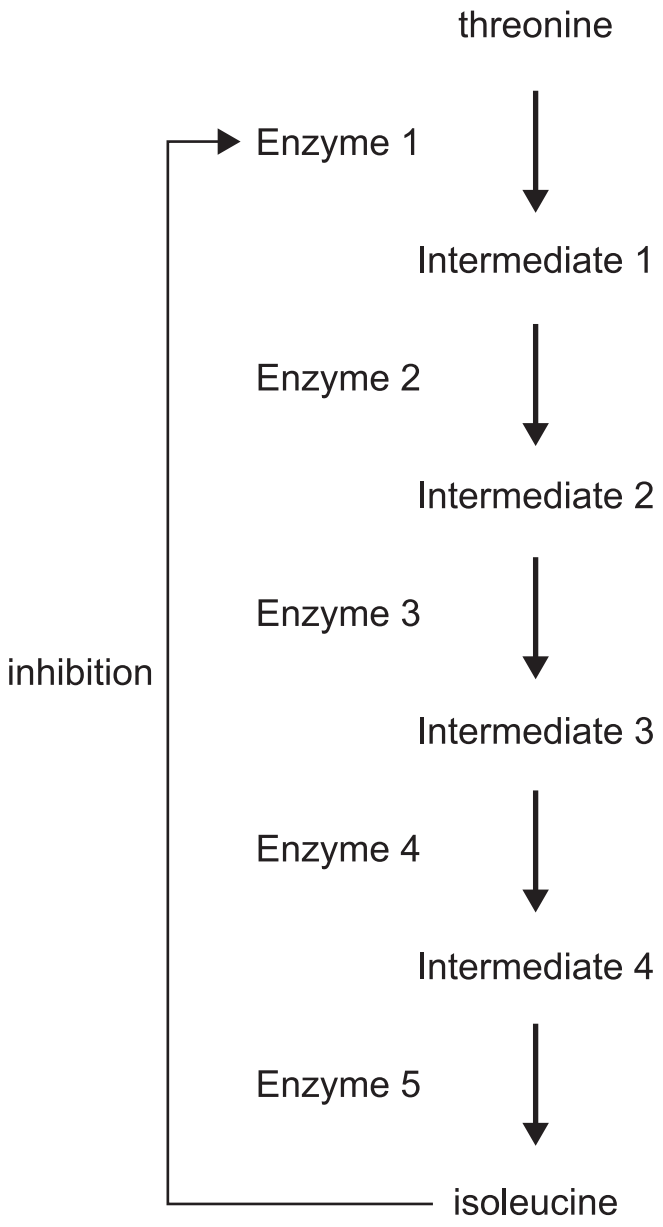
Non-competitive inhibition: Visualisation Go online



1.5.4 Feedback inhibition

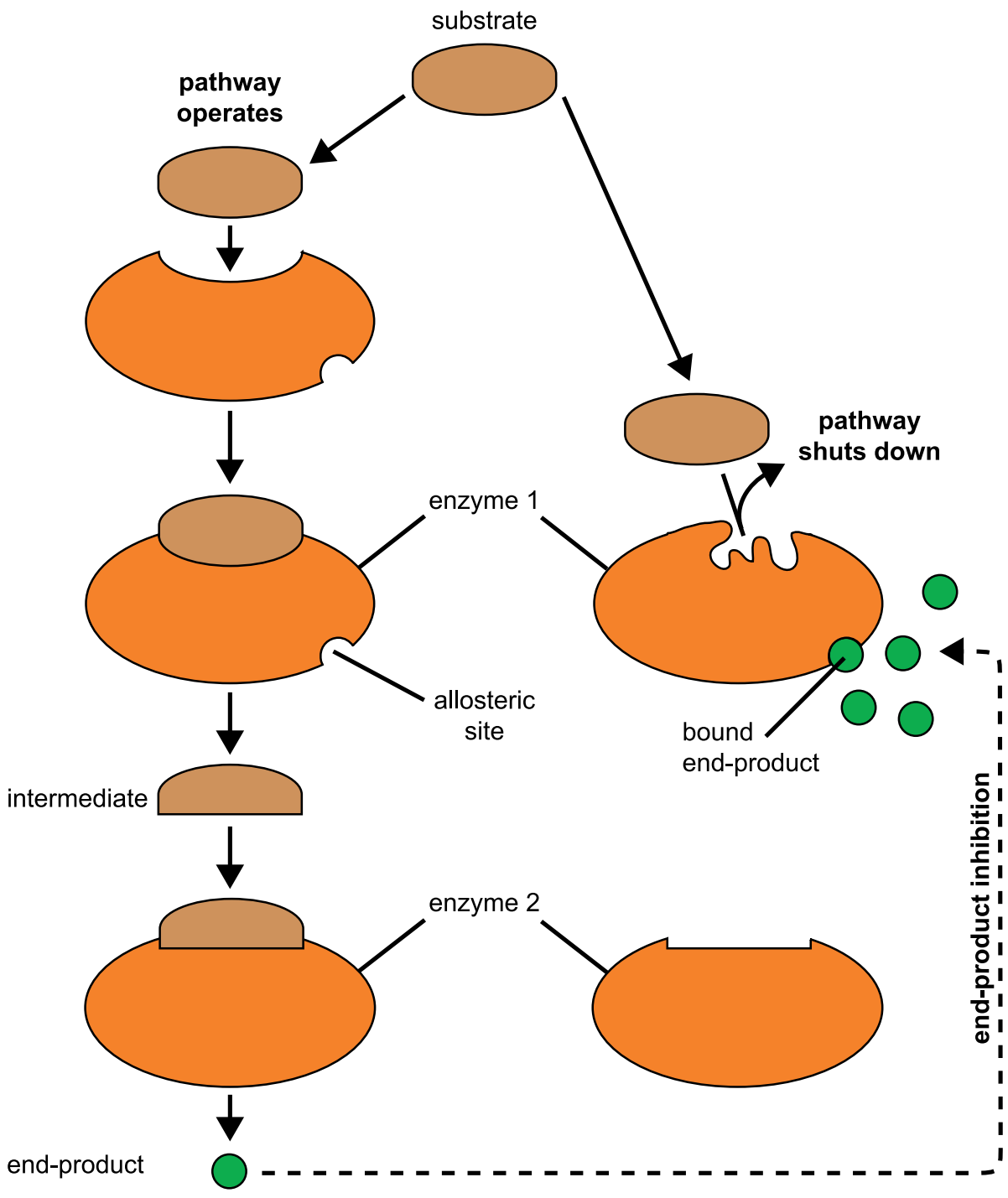
Feedback inhibition occurs when the end-product in the metabolic pathway reaches a critical concentration. The end-product then inhibits an earlier enzyme, blocking the pathway, and so prevents further synthesis of the end-product.

An example takes place during the production of the amino acid isoleucine in bacteria, where the initial substrate is threonine which is converted by five intermediate steps to isoleucine. As isoleucine begins to accumulate, it binds to and inhibits the first enzyme in the pathway, thereby slowing down its own production. By this mechanism of end-product, or negative feedback, inhibition, the cell does not produce any more isoleucine than is necessary.



Feedback inhibition pathway

Feedback inhibition: Visualisation Go online



Feedback inhibition: Question Go online

Q5: What is the advantage of end-product inhibition to the cell?

1.5.5 Experimental evidence of altering enzyme activity

The table below shows the results obtained in an experiment to investigate the effect of enzyme concentration on the rate of a reaction. The reaction that was investigated is the breakdown of starch into the disaccharide maltose by α-amylase. Starch hydrolysis was measured by the change in iodine staining (as determined using a spectrophotometer). The solutions of starch, phosphate buffer, and amylase were added to seven labelled test tubes.

Tube	Starch (0.02%, w/v) (ml)	Phosphate buffer (ml)	Amount of amylase added (ml)	μg amylase in reaction	Reaction rate (μg starch min^{-1})
1	5.0	4.5	0.5	50	34.7
2	5.0	4.0	1.0	100	50.0
3	5.0	3.5	1.5	150	56.5
4	5.0	3.0	2.0	200	73.9
5	5.0	2.5	2.5	250	82.1
6	5.0	5.0	0.0	0	0
7	0.0	5.0	5.0	500	0

The effect of amylase concentration on the rate of hydrolysis of starch to maltose

Q6: Draw a graph of the reaction rate against μg amylase in the reaction.

. . . . . .

Q7: What is the effect of an increase in the amount of enzyme on the rate of the reaction.

. . . . . .

Q8: What does the graph that you have drawn indicate about the reaction?

. . . . . .

Q9: Explain the purpose of tubes 6 and 7.

. . . . . .

Q10: What will happen to the reaction rate as the amount of amylase is increased above 250μg?

1.6 Learning points

Summary

- Metabolism is the term given to all the reactions that take place in the cell.
- Reactions in cells are controlled and co-ordinated by enzymes.

Summary continued

- Enzyme reactions do not take place in isolation but in pathways.
- Many of these pathways are reversible, but some are not.
- Where pathways are irreversible, or energetically unfavourable, alternative pathways are usually available.
- Reactions within metabolic pathways can be anabolic or catabolic. Anabolic reactions build up large molecules from small molecules and require energy. Catabolic reactions break down large molecules into smaller molecules and release energy.
- Most of these reactions are linked; the energy given off by one pathway is used to power another.
- Proteins within the membranes perform several functions, such as pores, embedded enzymes and channels.
- Metabolic pathways are controlled by a series of enzyme reactions.
- The rate of metabolism is dictated by the rate at which the enzymes work.
- Enzyme activity is closely related to its shape.
- Because enzymes are made from protein, their structure is flexible.
- When an enzyme and its substrate come together, the shape of the active site of the enzyme changes to allow a tighter fit with the substrate. This is called the 'induced fit' model.
- The active site of an enzyme creates an energetically favourable environment for the reaction to take place and lowers the activation energy.
- When the products are produced, they leave with a low affinity for the active site.
- The rate of enzyme reaction is affected by the concentration of the substrate and the end product.
- Most metabolic pathways are reversible. The direction will often depend on the quantity of substrate or the end product.
- Some genes, such as those for metabolism, are expressed continuously.
- These enzymes are always present, and are controlled through their rates of reaction.
- Enzymes can be inhibited by the binding of other particles.
- Non-competitive inhibitors bind away from the active site but change the shape of the active site, preventing the substrate from binding. Non-competitive inhibition cannot be reversed by increasing substrate concentration.
- Competitive inhibitors bind at the active site, preventing the substrate from binding. Competitive inhibition can be reversed by increasing substrate concentration.
- Feedback inhibition occurs when the end-product in the metabolic pathway reaches a critical concentration. The end-product then inhibits an earlier enzyme, blocking the pathway, and so prevents further synthesis of the end-product.

1.7 Extended response question

The activity which follows presents an extended response question similar to the style that you will encounter in the examination.

You should have a good understanding of the control of the enzyme activity by inhibition before attempting the question.

You should give your completed answer to your teacher or tutor for marking, or try to mark it yourself using the suggested marking scheme.

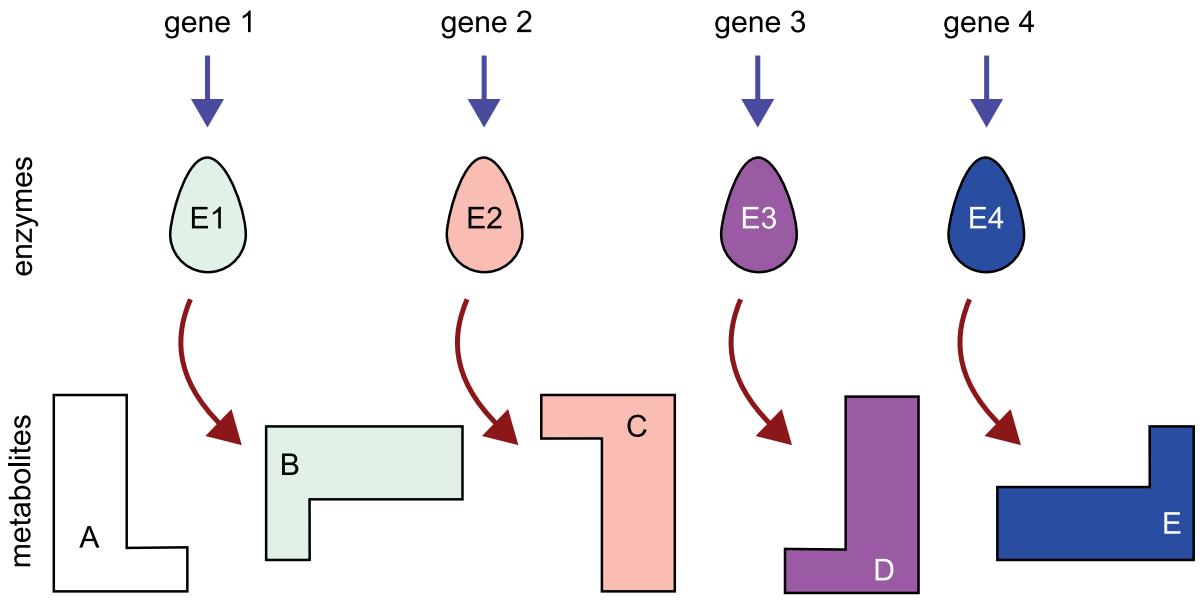
Extended response question: The control of the enzyme activity by inhibition

Give an account of the control of the enzyme activity by inhibition. *(8 marks)*

1.8 End of topic test

End of Topic 1 test Go online

Q11: The following diagram shows a metabolic pathway controlled by enzymes. The genes which code for each enzyme in the pathway are also shown.



A mutation in a gene can result in the disruption of a metabolic pathway. Explain how such a mutation could result in a buildup of metabolite D.

. . . . . .

Q12: What is metabolism?

. . . . . .

Q13: Reactions which release energy are said to be _____ and reactions which require energy are described as _____. *(Choose between 'anabolic' and 'catabolic' for each gap.)*

. . . . . .

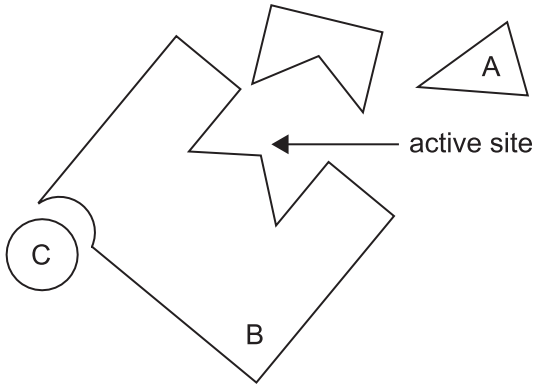
Q14: Name the substances which control metabolic pathways.

. . . . . .

Q15: Describe the role of pore proteins in the plasma membrane.

. . . . . .

Q16: The following diagram shows the molecules involved in an enzyme controlled reaction.



Which list of molecule names identifies labels A, B and C from the diagram in order?

a) Non-competitive inhibitor, enzyme, competitive inhibitor.
b) Non-competitive inhibitor, substrate, competitive inhibitor.
c) Competitive inhibitor, substrate, non-competitive inhibitor.
d) Competitive inhibitor, enzyme, non-competitive inhibitor.

. . . . . .

Q17: With respect to enzyme activity, what is meant by the term 'induced fit'?

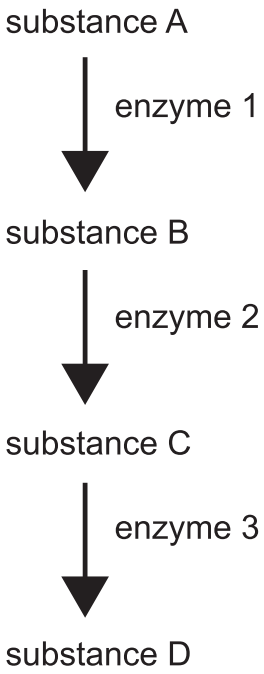
a) An inhibitor binds to the active site preventing the substrate from binding.
b) Hydrogen and ionic bonds hold the substrate in the active site.
c) The correct conditions are created for substrate and enzyme to interact.
d) When the substrate binds to the active site, the shape of the active site is changed.

. . . . . .

Q18: Enzymes _____ *(choose between 'increase' and 'lower')* the activation energy and release products with a _____ *(choose between 'high' and 'low')* affinity for the active site.

. . . . . .

Q19: The following diagram shows the process of feedback inhibition.

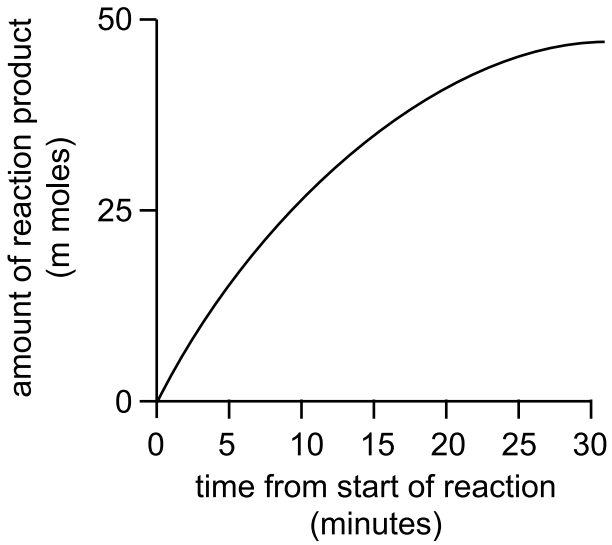


If substance D were to bring about feedback inhibition, which of the following will it interact with?

a) Enzyme 1
b) Enzyme 3
c) Substance A
d) Substance C

.....

Q20: The figure below shows the progress of an enzyme-catalysed reaction.



When is the rate of the reaction at its highest?

a) 0 to 5 minutes from the start of the reaction.
b) 5 to 15 minutes from the start of the reaction.
c) 15 to 25 minutes from the start of the reaction.
d) 25 to 30 minutes from the start of the reaction.

. . . . . .

Q21: In an enzyme-catalysed reaction, the amount of product decreased when substance X was added. The addition of more substrate did not increase the amount of product. Substance X could be:

a) product
b) a competitive inhibitor
c) a non-competitive inhibitor
d) a cofactor

. . . . . .

Q22: _____ inhibitors decrease the activity of an enzyme by binding to the active site.

. . . . . .

Q23: The following illustrates a metabolic pathway.

$$A \rightarrow B \rightarrow C \rightarrow D \rightarrow E \rightarrow F$$

Assuming that molecule F regulates the first reaction in the pathway (the formation of B from A), which of the following would describe its mode of action?

a) Coenzyme activation
b) Feedback inhibition
c) Competitive inhibition
d) Non-competitive inhibition

Topic 2

Cellular respiration

Contents

2.1 The role of ATP 23
2.2 The chemistry of respiration 25
2.2.1 Glycolysis 25
2.2.2 Citric acid cycle 26
2.2.3 The electron transport chain 28
2.2.4 Fermentation 29
2.2.5 Measuring the rate of respiration 30
2.3 Learning points 31
2.4 Extended response question 32
2.5 End of topic test 32

Prerequisites

You should already know that:

- the chemical energy stored in glucose must be released by all cells through a series of enzyme-controlled reactions called respiration;
- the energy released from the breakdown of glucose is used to generate ATP from ADP and phosphate;
- the chemical energy stored in ATP can be released by breaking it down to ADP and phosphate;
- ATP can be regenerated during respiration;
- each glucose molecule is broken down via pyruvate to carbon dioxide and water in the presence of oxygen;
- the breakdown of each glucose molecule via the fermentation pathway yields two molecules of ATP when oxygen is not present;
- in the absence of oxygen in animal cells, glucose is broken down into lactate via pyruvate;
- in the absence of oxygen in plant and yeast cells, glucose is broken down into alcohol/ethanol and carbon dioxide via pyruvate;

Prerequisites continued

- fermentation occurs in the cytoplasm;
- aerobic respiration starts in the cytoplasm and is completed in the mitochondria.

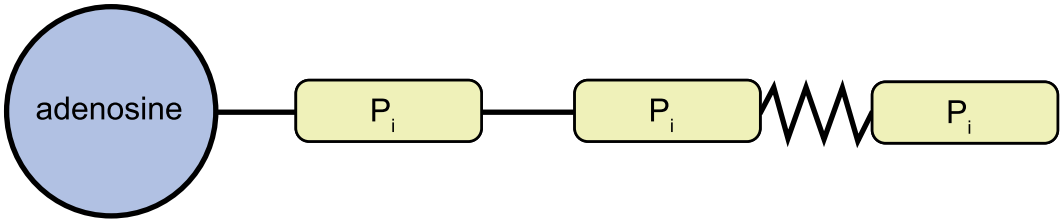
Learning objective

By the end of this topic, you should be able to:

- understand that cellular respiration is the core pathway in cells which delivers energy for cell metabolism;
- describe how glucose is broken down to ultimately deliver ATP;
- explain that ATP is used to transfer energy to carry out cell processes;
- explain the reversible nature of ATP production;
- describe how ATP is synthesised;
- describe glycolysis;
- describe the progression of respiration pathways, both in the presence and absence of oxygen;
- describe the citric acid cycle;
- understand that respiration is a series of enzyme mediated reactions;
- explain the importance of the products of the citric acid cycle;
- describe the electron transport chain as a membrane bound system;
- explain the role of dehydrogenase enzymes;
- understand the key role of NAD and their reduced forms;
- explain the role of oxygen in the electron transport chain and the consequences of its absence.

2.1 The role of ATP

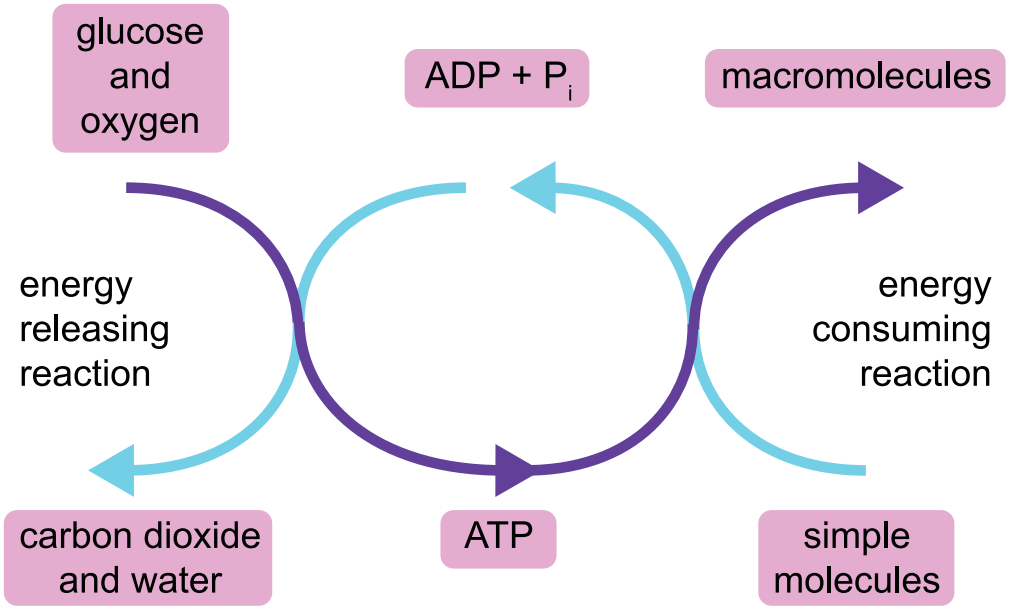
ATP is essential to biological systems as it is the link between reactions that release energy (catabolic) and those that use energy (anabolic).



The structure of ATP

ATP is sometimes known as the 'energy currency' of the cell as it is spent during cellular work such as muscular contraction or the formation of proteins, and is 'banked' or stored when glucose is broken down during cellular respiration.

The illustration below shows the coupling of catabolic and anabolic reactions through ATP. You can see that energy to form ATP comes from the breakdown of food, and that the energy then contained in the ATP molecule is used to build up complex molecules from simple ones (such as proteins from amino acids).



ATP cycling

When an energy rich substance, such as glucose, is broken down in a living cell, it releases energy which is used to produce ATP. Many molecules of ATP are present in every living cell. Since ATP can rapidly be broken down to ADP + P_i (phosphate), it is able to make energy available for processes which need energy (e.g. muscular contraction, synthesis of proteins etc).

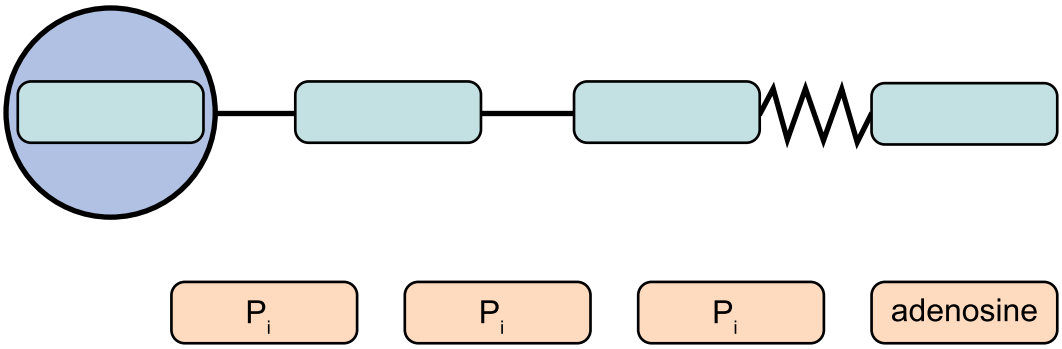
ATP is important, therefore, in that it provides the link between energy releasing reactions and energy consuming reactions. It provides the means by which chemical energy is transferred from one type of reaction to the other in a living cell. ATP is constantly manufactured in all living cells from ADP and P_i. The rate of production of ATP varies to meet the demands of the cell.

ATP also has a role in carrying out phosphorylation reactions within living cells. A phosphorylation reaction involves a phosphate group being added to a substrate. This is an enzyme controlled process. The formation of ATP from ADP and P_i is a phosphorylation reaction since a phosphate group is added to ADP to form ATP.

ATP can be used to phosphorylate other molecules within the cell. For example, during the first stage of respiration (glycolysis), ATP is broken down to ADP and P_i and the phosphate group is used to phosphorylate the substrate of glycolysis. This process initiates the reaction.

The role of ATP: Questions Go online

Q1: Complete the diagram using the labels provided.



Q2: Define the term anabolic reaction.

. . . . . .

Q3: Give an example of an anabolic reaction.

. . . . . .

Q4: Define the term catabolic reaction.

. . . . . .

Q5: Give an example of a catabolic reaction.

2.2 The chemistry of respiration

Cellular respiration is a metabolic pathway. It consists of a series of enzyme-controlled reactions that release the energy contained in food, by oxidation.

There are three sets of reactions in cellular respiration:

1. **glycolysis**;
2. the **citric acid cycle**;
3. the **electron transport chain**.

2.2.1 Glycolysis

Glycolysis takes place in the cytoplasm of the cell and does not require oxygen. It is the breakdown, in a series of enzyme-catalysed reactions, of the sugar glucose into two molecules called pyruvate.

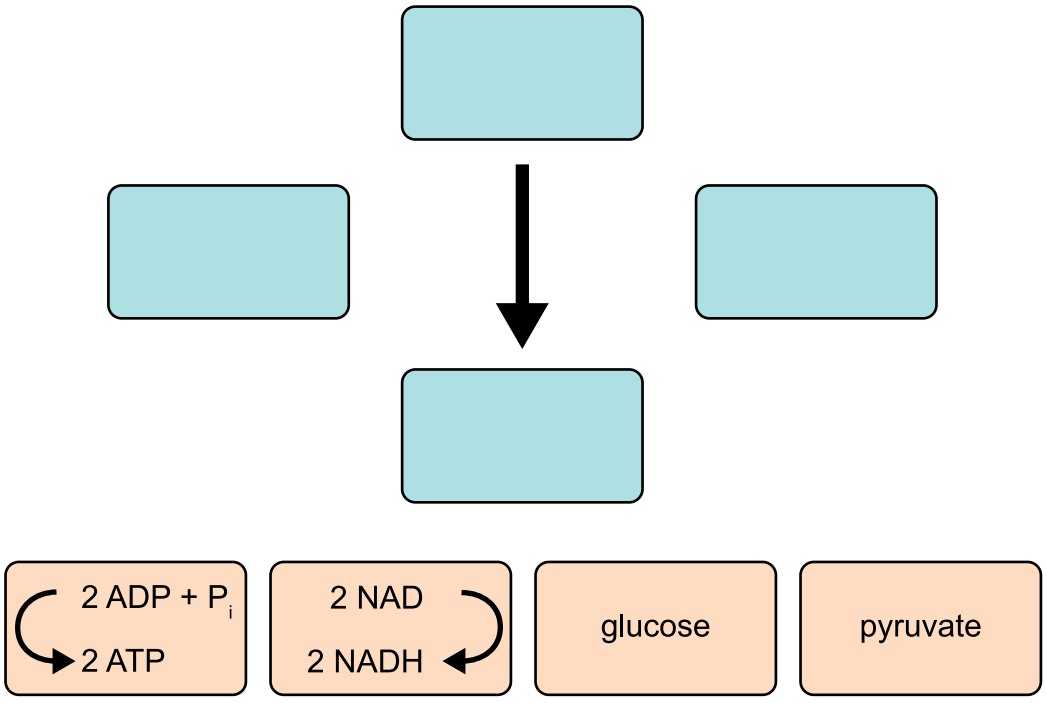
To start the process off, energy from two ATP molecules is needed. This can be thought of as an energy investment phase where ATP is used to phosphorylate intermediates in glycolysis. The series of reactions eventually produces four ATP molecules, so there is a net gain of two ATP from glycolysis (energy pay-off stage).

During the transformation of glucose into pyruvate, **dehydrogenase** enzymes remove hydrogen ions and electrons that are passed to a coenzyme called **NAD** which is reduced to form NADH. In a later process of cell respiration, the NADH will be used to produce ATP.

If oxygen is present pyruvate progresses to the **citric acid cycle**.

Glycolysis: Questions Go online

Q6: Complete the diagram using the labels provided.



Q7: Where does glycolysis take place in the cell?

. . . . . .

Q8: What is the net gain in ATP molecules from one glucose molecule during glycolysis?

a) 6
b) 2
c) 8
d) 4

. . . . . .

Q9: Is oxygen required for glycolysis?

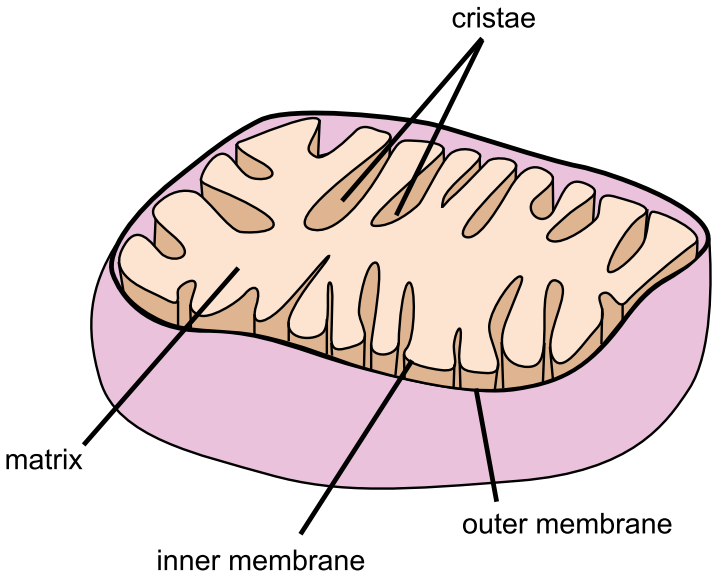
. . . . . .

Q10: Name the final product of glycolysis.

2.2.2 Citric acid cycle

In the presence of oxygen, cell respiration continues on from **glycolysis** in the **mitochondria** of the cell.

Mitochondria (mitochondrion - singular) possess a double membrane. The inner membrane of each mitochondrion is folded into many cristae, which provide a large surface area. It is here that the reactions of the **electron transport chain** occur. The cristae project into a fluid-filled interior matrix which contains the enzymes involved in the **citric acid cycle** reactions.



A mitochondrion

During the citric acid cycle, pyruvate diffuses into the matrix of the mitochondrion, where it is broken down into an acetyl group. The acetyl group combines with coenzyme A forming acetyl coenzyme A (acetyl CoA). During the conversion of pyruvate to acetyl coenzyme A, dehydrogenase enzymes remove hydrogen ions and electrons from pyruvate which are passed to the coenzyme **NAD**, forming NADH.

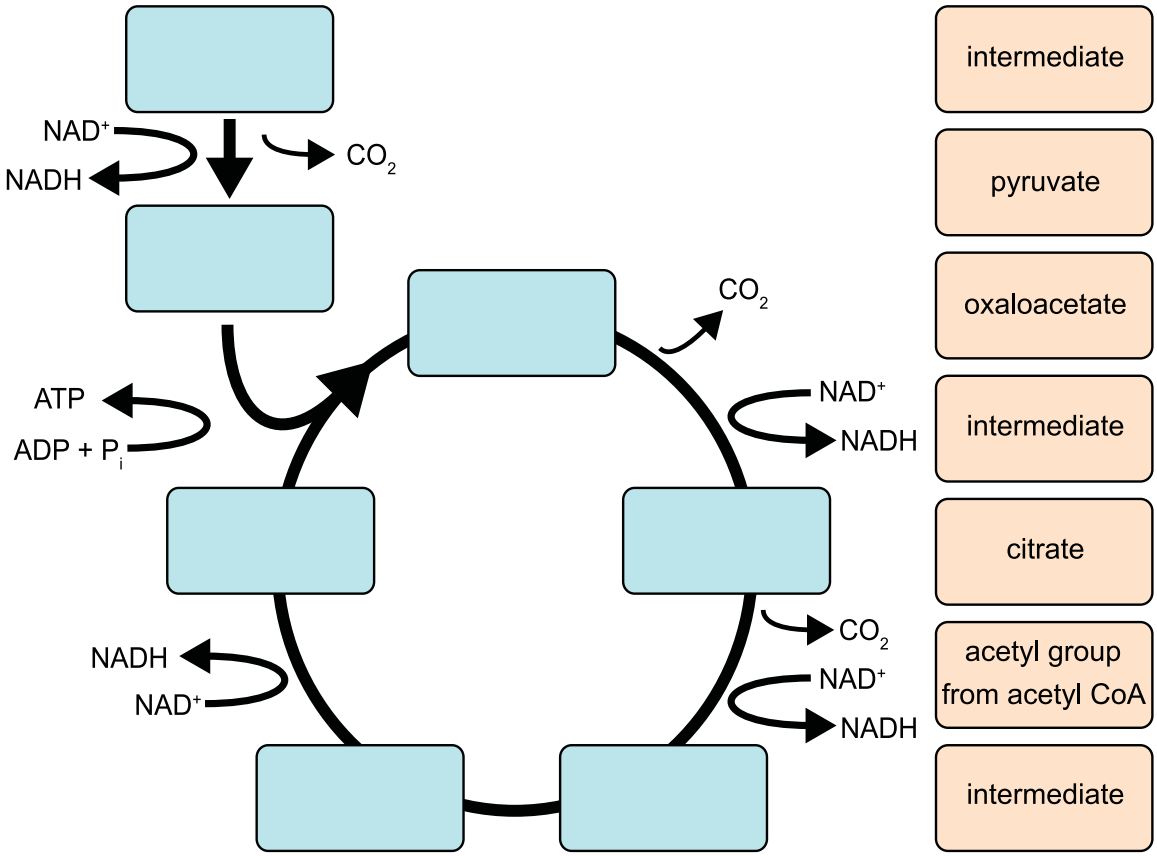
The acetyl group from acetyl coenzyme A combines with oxaloacetate to form citrate. During the citric acid cycle, citrate is converted through a series of enzyme-catalysed reactions back into oxaloacetate. In the process, both carbon (in the form of carbon dioxide) and hydrogen ions (along with electrons) are released.

Hydrogen ions and electrons become bound to NAD to form NADH. NADH will be used in the next stage of respiration to release energy for ATP production. Carbon dioxide diffuses out of the cell as a waste product and is expired from the organism by breathing out or by diffusion over the body surface.

Citric acid cycle: Questions Go online

The following diagram illustrates the citric acid cycle.

Q11: Complete the diagram using the labels provided.



Q12: Name the molecule produced when oxaloacetate combines with an acetyl group.

.....

Q13: In which organelle does the citric acid cycle occur?

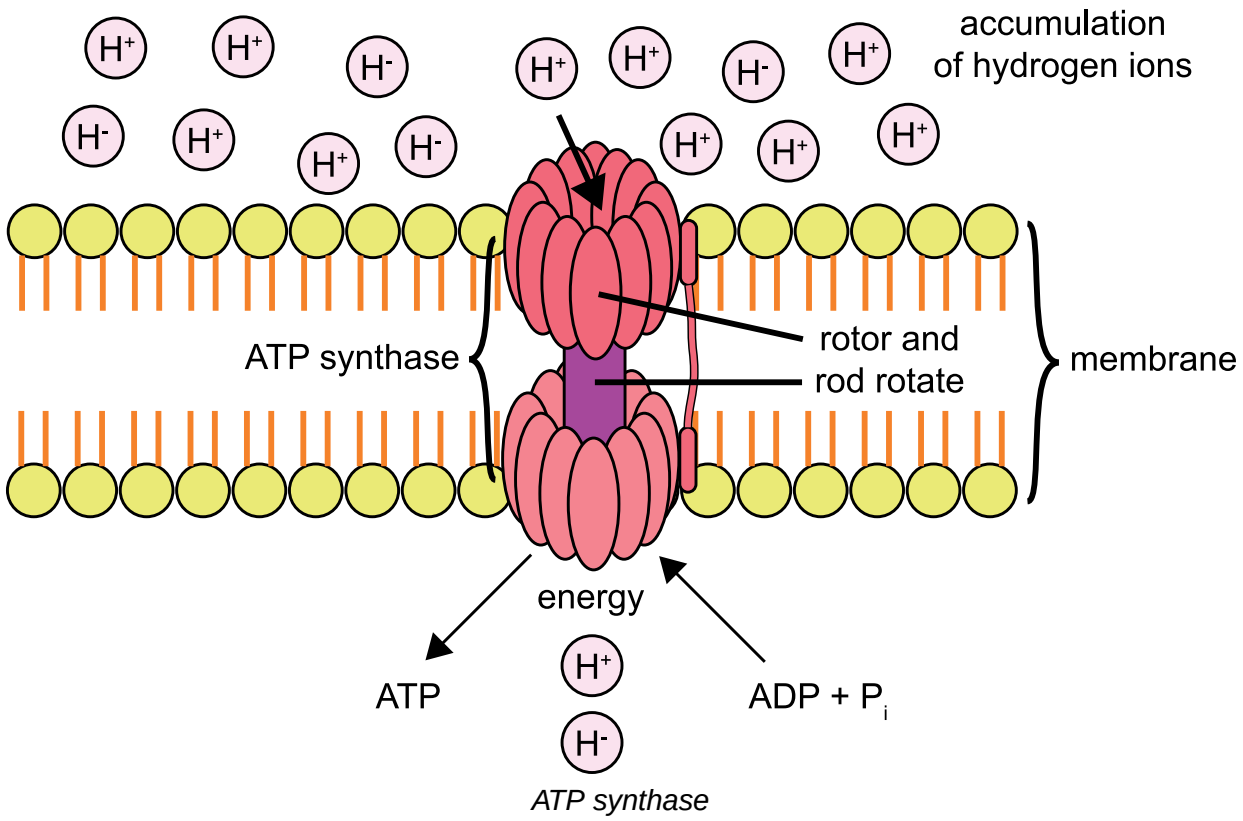
a) Chloroplast
b) Lysosome
c) Mitochondrion

. . . . . .

Q14: Is citric acid cycle an aerobic or anaerobic process?

2.2.3 The electron transport chain

So far, many hydrogen ions and electrons have been transferred to **NAD** from **glycolysis** and the **citric acid cycle**. The electrons are passed to the **electron transport chain** on the inner **mitochondrial** membrane. The electrons are used to pump hydrogen ions across the mitochondrial membrane. The return flow of these hydrogen ions rotates part of the membrane protein **ATP synthase**. ATP synthase is an enzyme which catalyses the synthesis of ATP.



Oxygen is the final electron acceptor, which combines with H ions and electrons, forming water.

The presence of oxygen is necessary for both the citric acid cycle and the electron transport chain to function. Cell respiration will not occur after glycolysis if oxygen is not present. The complete oxidation of one molecule of glucose results in a total of 38 ATP molecules. In glycolysis there is a net gain of two ATP molecules. In the citric acid cycle and during hydrogen ion transfer through the electron transport chain, 36 ATP molecules are produced, making a total of 38.

2.2.4 Fermentation

In the absence of oxygen, only **glycolysis** takes place and pyruvate follows a **fermentation** pathway in the cytoplasm. Fermentation results in much less ATP being produced than in aerobic respiration.

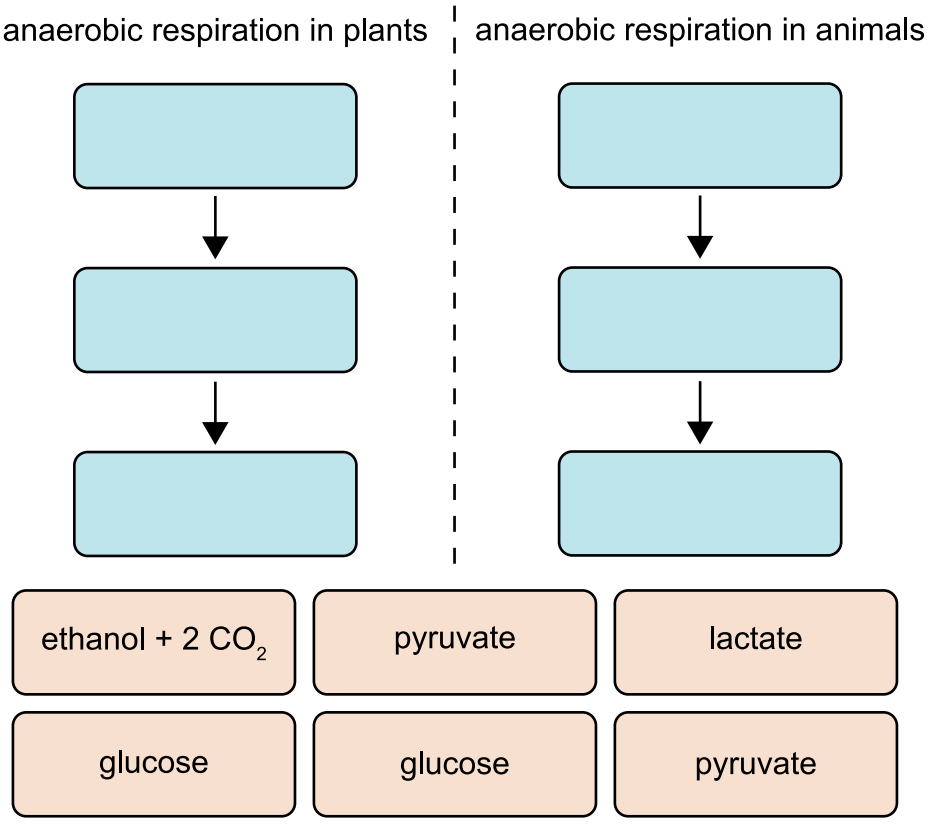
In animal cells, pyruvate is broken down into lactate. This can happen in the muscle cells of humans during vigorous exercise when all the oxygen available is used up. This process is reversible, as lactate can be converted back into pyruvate when oxygen becomes available again.

In plant and yeast cells, the fermentation pathway converts pyruvate into ethanol and carbon dioxide. This is an irreversible process because carbon dioxide is lost from the cell.

Anaerobic respiration: Questions Go online

The following provides a summary of anaerobic respiration in plants and animals.

Q15: Complete the diagram using the labels provided.



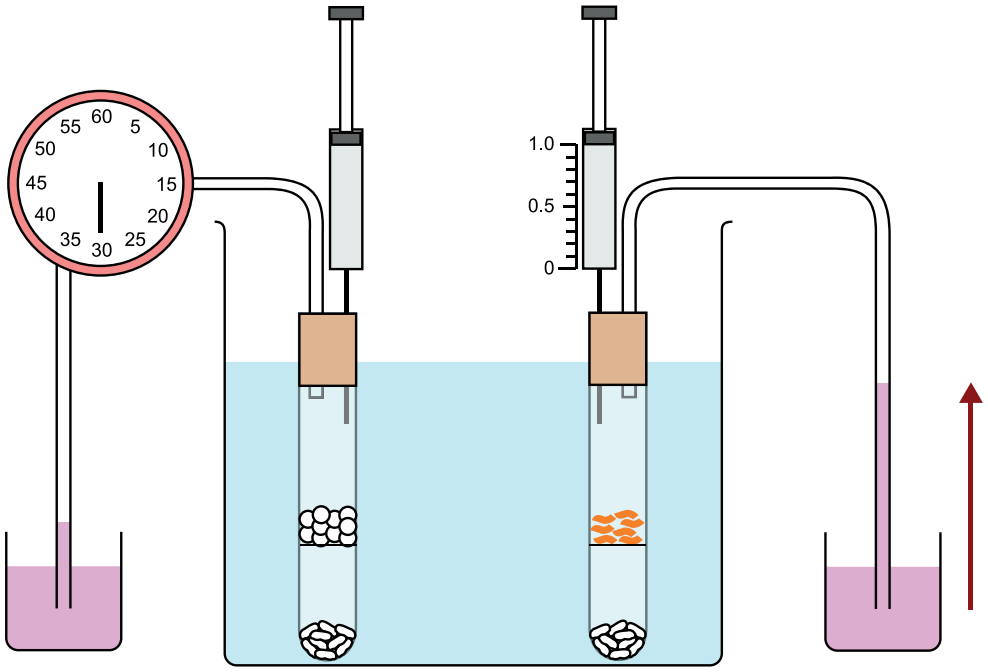
2.2.5 Measuring the rate of respiration

Measuring the rate of respiration Go online

The **respirometer** on the right has been designed to measure the rate of respiration of some maggots. The respirometer on the left contains glass beads instead of maggots - this acts as a control.

As the maggots use oxygen for respiration, the level of the liquid in the glass tube will rise. Carbon dioxide produced by the maggots is absorbed by sodium hydroxide beads that have been placed underneath them.

The timer has been set for 30 minutes. After that time, the syringe above the maggots is pressed down to return the liquid in the tube to its initial level. The volume of oxygen used by the maggots can then be determined (the syringe is calibrated in units of 0.1 ml).



Q16: How much oxygen is consumed by the maggots in 30 minutes?

a) 0.1 ml
b) 0.2 ml
c) 0.3 ml
d) 0.4 ml

. . . . . .

Q17: What is the rate of respiration (expressed as ml of oxygen consumed per hour)?

a) 0.3
b) 0.4
c) 0.5
d) 0.6

2.3 Learning points

Summary

- Glycolysis is the breakdown of glucose to pyruvate in the cytoplasm.
- ATP is required for the phosphorylation of glucose and intermediates during the energy investment phase of glycolysis. This leads to the generation of more ATP during the energy pay-off stage and results in a net gain of ATP.
- In aerobic conditions, pyruvate is broken down to an acetyl group that combines with coenzyme A forming acetyl coenzyme A.
- In the citric acid cycle, the acetyl group from acetyl coenzyme A combines with oxaloacetate to form citrate.
- During a series of enzyme controlled steps, citrate is gradually converted back into oxaloacetate which results in the generation of ATP and release of carbon dioxide.
- The citric acid cycle occurs in the matrix of the mitochondria.
- Dehydrogenase enzymes remove hydrogen ions and electrons and pass them to the coenzyme NAD, forming NADH. This occurs in both glycolysis and the citric acid cycle.
- The hydrogen ions and electrons from NADH are passed to the electron transport chain on the inner mitochondrial membrane.
- The electron transport chain is a series of carrier proteins attached to the inner mitochondrial membrane.
- During ATP synthesis electrons are passed along the electron transport chain releasing energy.
- This energy allows hydrogen ions to be pumped across the inner mitochondrial membrane. The flow of these ions back through the membrane protein ATP synthase results in the production of ATP.
- Finally, hydrogen ions and electrons combine with oxygen to form water.
- In the absence of oxygen, fermentation takes place in the cytoplasm.
- In animal cells, pyruvate is converted to lactate in a reversible reaction.
- In plants and yeast, ethanol and CO2 are produced in an irreversible reaction.
- Fermentation results in much less ATP being produced than in aerobic respiration.
- ATP is used to transfer energy to cellular processes which require energy.

2.4 Extended response question

The activity which follows presents an extended response question similar to the style that you will encounter in the examination.

You should have a good understanding of the stages of cellular respiration before attempting the question.

You should give your completed answer to your teacher or tutor for marking, or try to mark it yourself using the suggested marking scheme.

Extended response question: The stages of cellular respiration

Give an account of the first two stages of cellular respiration under the following headings.

A) Glycolysis.

B) Citric acid cycle.

(8 marks)

2.5 End of topic test

End of Topic 2 test Go online

Q18: The production of ATP from ADP and P_i is called _____.

. . . . . .

Q19: The break down of ATP releases _____, some of which is used in the synthesis of complex molecules.

. . . . . .

Q20: Which statement referring to glycolysis is correct?

a) It is an anabolic reaction that requires oxygen.
b) It is a catabolic reaction that does not require oxygen.
c) It is an anabolic reaction that does not require oxygen.
d) It is a catabolic reaction that requires oxygen.

. . . . . .

Q21: Which of the following does glycolysis produce?

a) Carbon dioxide
b) Citrate
c) Acetyl CoA
d) Pyruvate

. . . . . .

Q22: Where in the cell does glycolysis take place?

a) In both the cytoplasm and the matrix of the mitochondrion.
b) On the surface of the inner mitochondrial membrane.
c) In the matrix of the mitochondrion.
d) In the cytoplasm.

. . . . . .

Q23: The citric acid cycle occurs in the _____ of the mitochondrion.

. . . . . .

Q24: During the citric acid cycle _____ combines with an acetyl group to form _____, this is gradually turned back into _____ by a series of _____ controlled reactions.

. . . . . .

Q25: In the electron transport chain, what is the final acceptor of hydrogen?

a) Water
b) Carbon dioxide
c) Oxygen
d) NAD

. . . . . .

Q26: Name the enzyme required to produce ATP

. . . . . .

Q27: Name the coenzyme which transports hydrogen ions and electrons to the electron transport chain.

Topic 3

Metabolic rate

Contents

3.1 Measuring metabolic rate 36
3.2 Oxygen delivery 37
3.3 Learning points 39
3.4 End of topic test 40

Prerequisites

You should already know that:

- the pathway of blood through human heart, lungs and body;
- the structure of the human heart including the right and left atria and ventricles;
- red blood cells contain haemoglobin and are specialised to carry oxygen.

Learning objective

By the end of this topic, you should be able to:

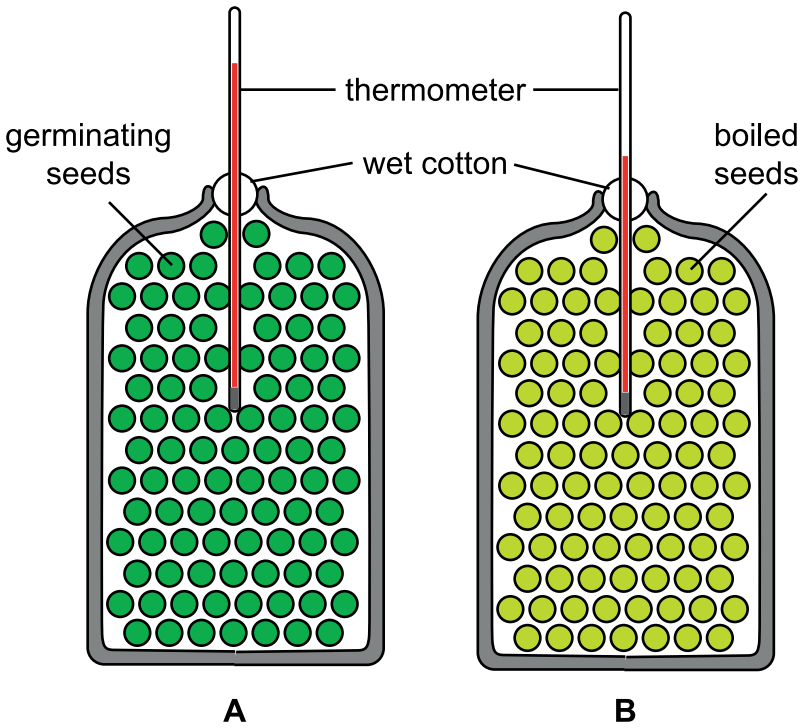
- understand how the rate of metabolism can be measured;
- describe the mechanisms of delivery of oxygen in terms of the cardiovascular system of different animals.

3.1 Measuring metabolic rate

Metabolic rate is the quantity of energy used by the body over a given time. It is measured in kilojoules (or kilocalories). Metabolic rate can be measured in a number of ways, through the rate of oxygen consumption, carbon dioxide evolution or heat generation. The methods for detecting and measuring these factors range from very simple, low technology to more modern methods giving real time readings.

A common method used to calculate metabolic rate is to measure the quantity of oxygen used because in many cases respiration is aerobic. A figure frequently quoted is 350 litres per day for an average man. This equates to approximately 7000 kJ (or 1700 kcal) per day. Remember, the rate of oxygen uptake by an organism can be measured using a **respirometer**; for more information on respirometers look back to the interactivity in section 3.2.5 "Measuring the rate of respiration".

An organism's metabolic rate can also be calculated using a **calorimeter**. This piece of equipment monitors the heat generated by an organism and calculates the metabolic rate from the results collected. A simple set-up to measure heat generation by germinating peas is shown below. The boiled peas act as a control; there should be no temperature rise in this flask.



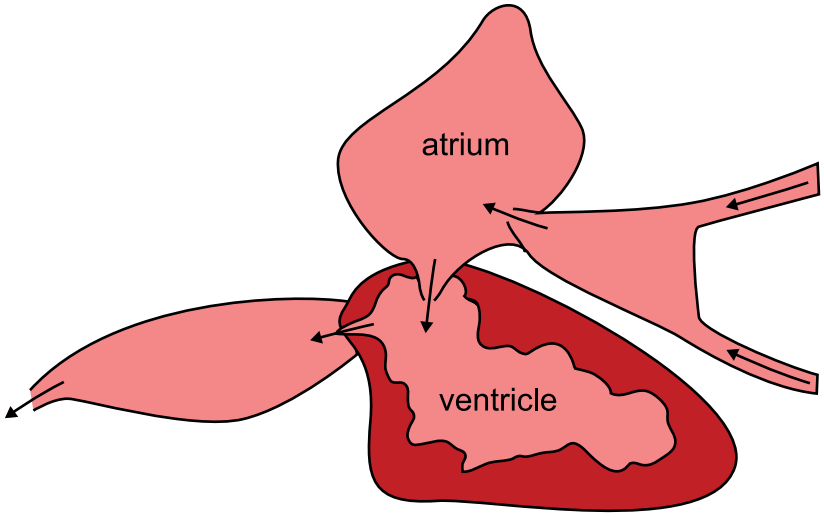
Using a simple calorimeter to measure generated heat

3.2 Oxygen delivery

Oxygen is consumed during aerobic respiration. If the rate at which energy is demanded rises, then so too must the rate of oxygen delivery. In simple organisms, oxygen can dissolve and diffuse through cell membranes. However, with increasing complexity, multi-cellular organisms must devise oxygen delivery systems. This gives rise to the cardiovascular systems seen in higher animals.

Organisms with high metabolic rates require more efficient delivery of oxygen to cells. Birds and mammals have higher metabolic rates than reptiles and amphibians, which in turn have higher metabolic rates than fish.

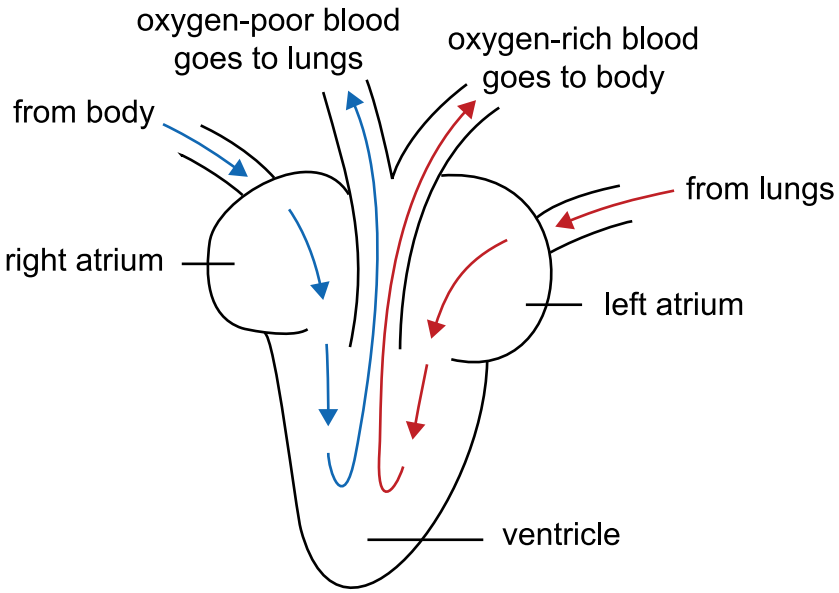
Fish have a single circulatory system consisting of one atrium and one ventricle. This model of a heart, which has an atrium to collect blood and a muscular ventricle to pump the blood around the body, is the basis for all vertebrates. In fish, the two-chambered heart results in a single circulatory system where blood passes through the heart once in each complete circuit of the body.



The heart of a fish

The hearts of amphibians and reptiles have evolved to a three-chambered organ, which has become necessary as there is a need to separate the deoxygenated blood from the oxygenated blood returning from the lungs. The circulatory system is described as an incomplete double circulatory system.

There are two atria in the heart which collect the blood. One collects blood from the body on the right (which is shown on the left in the diagram), and the other collects blood from the lungs on the left (which is shown on the right). Both chambers deliver blood to a single ventricle. There is relatively little mixing of the oxygenated and deoxygenated blood by means of a combination of timing of arterial contractions and the beginnings of a septum in the ventricle.

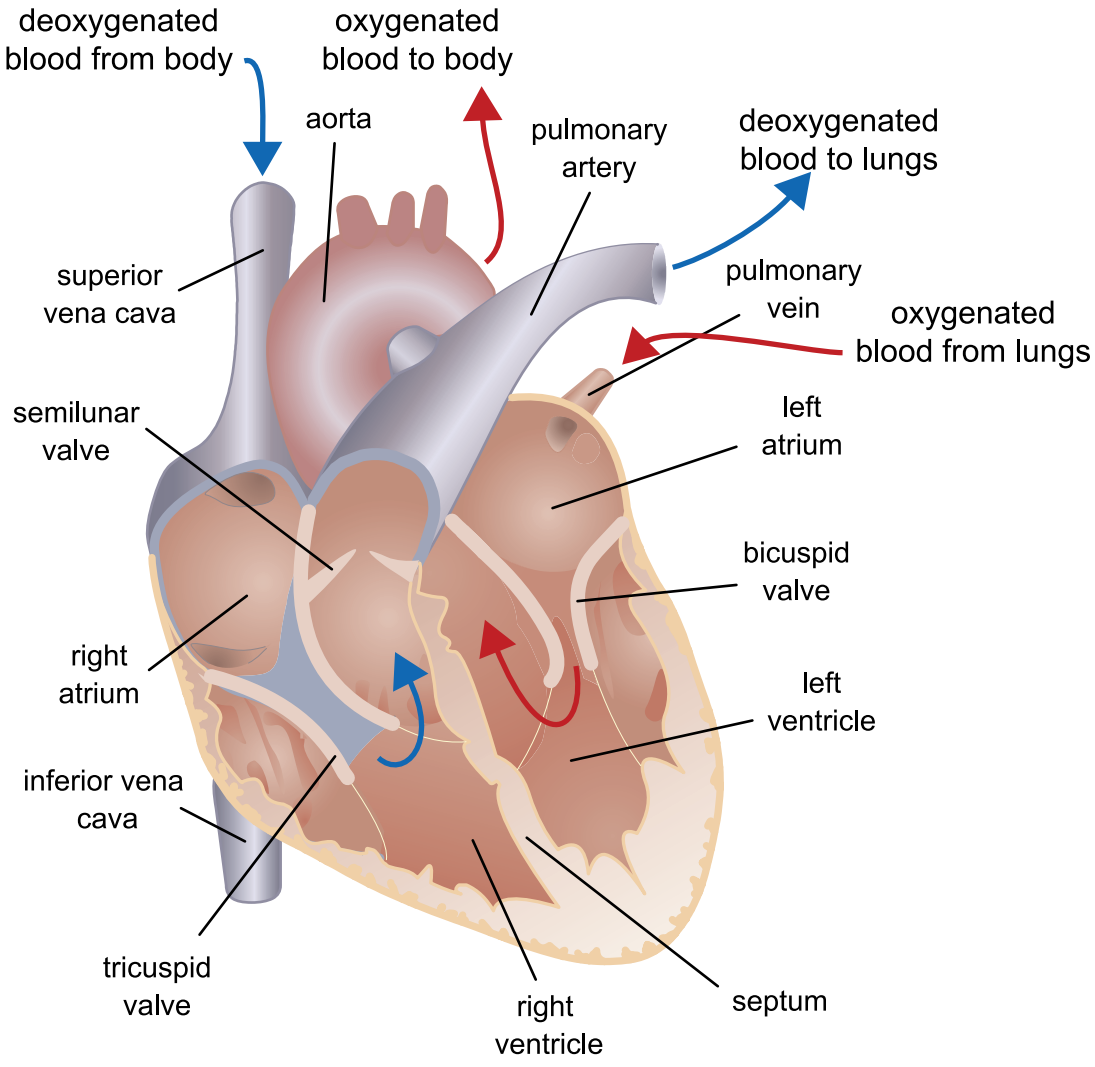


The heart of an amphibian

Birds and mammals have a high energy demand which requires efficient delivery of oxygen to the tissues.

The bird heart is essentially similar to that of the mammal. Blood returning from the body enters an atrium and is then pushed into the ventricle below. When the ventricle contracts, the blood leaves the heart and travels to the lungs where it is oxygenated. Blood leaves the lungs and travels back to the heart, entering the other atrium. Blood passes down into the ventricle below. When the ventricle contracts, the blood leaves the heart and travels to the body tissues.

Birds and mammals therefore have a complete double circulatory system consisting of two atria and two ventricles. Complete double circulatory systems enable higher metabolic rates to be maintained. There is no mixing of oxygenated and deoxygenated blood and the oxygenated blood can be pumped out at a higher pressure. This enables more efficient oxygen delivery to cells.



The heart of a mammal

3.3 Learning points

Summary

- The comparison of metabolic rates of organisms at rest is achieved by measuring either the oxygen uptake, carbon dioxide output or the heat produced.
- Metabolic rate can be measured using respirometers, oxygen probes, carbon dioxide probes and calorimeters.
- During aerobic respiration, oxygen needs to be delivered to respiring cells.
- Fish have a single circulatory system consisting of one atrium and one ventricle.
- Amphibians and most reptiles have an incomplete double circulatory system consisting of two atria and one ventricle.

Summary continued

- Birds and mammals have a complete double circulatory system consisting of two atria and two ventricles.
- Complete double circulatory systems enable higher metabolic rates to be maintained. There is no mixing of oxygenated and deoxygenated blood and the oxygenated blood can be pumped out at a higher pressure. This enables more efficient oxygen delivery to cells.

3.4 End of topic test

End of Topic 3 test Go online

Q1: What instrument can be used to measure respiration?

. . . . . .

Q2: Which of the following measurements do not allow metabolic rate to be calculated?

a) Heart rate
b) Heat generation
c) Oxygen consumption
d) Carbon dioxide production

. . . . . .

Q3: The Mufflin formula for Resting Metabolic Rate (RMR) for a man is:

$RMR = 10w + 6.25h - 5a + 5$

Where: w = weight in kg; h = height in cm; a = age in years; and RMR is in kcal.

Calculate the RMR for a 45 year old man weighing 80 kg who is 170 cm tall.

. . . . . .

Q4: A fish heart has ____ chambers.

An amphibian heart has ____ chambers.

. . . . . .

Q5: What is the significance of the development of four-chambered heart?

. . . . . .

Q6: A mammal has a _____ circulatory system whereas fish have a _____ circulatory system. *(choose from 'single' and 'double' for each gap)*

Topic 4

Metabolism in conformers and regulators

Contents

4.1 Introduction . . . 42
4.2 Conformers . . . 42
4.3 Regulators . . . 45
4.4 Negative feedback control of body temperature . . . 47
4.5 Learning points . . . 53
4.6 Extended response question . . . 54
4.7 Extension materials . . . 54
4.8 End of topic test . . . 56

Prerequisites

You should already know that:

- abiotic factors are non living factors such as temperature;
- the nervous system allows messages / signals to pass from one part of the body to another.

Learning objective

By the end of this topic, you should be able to:

- explain that an organism's ability to maintain its metabolic rate is affected by external abiotic factors;
- explain the differences between conformers and regulators, and the mechanisms they use in response to changes in the environment;
- explain the importance of regulating temperature during metabolism;
- describe the negative feedback control of temperature in mammals, including the role of the hypothalamus, nerves, effectors and skin.

4.1 Introduction

The metabolic rate of an organism will be affected by external conditions. How the organism responds to these changes in the external environment will alter according to the type of organism.

At the heart of metabolism are enzymes. It is the enzymes that regulate each and every reaction. Enzyme function is reliant on its three-dimensional configuration or shape. Anything that alters this will have an effect on the enzyme's ability to catalyse its specific reaction.

Of the many external environmental factors which may affect enzymes, pH, salinity and temperature are possibly the most obvious. For metabolism to remain efficient, enzymes should be in an environment that is maintained within fairly narrow parameters; extremes should be avoided as this would lead to denaturing of the enzymes.

Some organisms can do little to regulate their internal environment (conformers) while others can take extensive measures to regulate their internal environment (regulators).

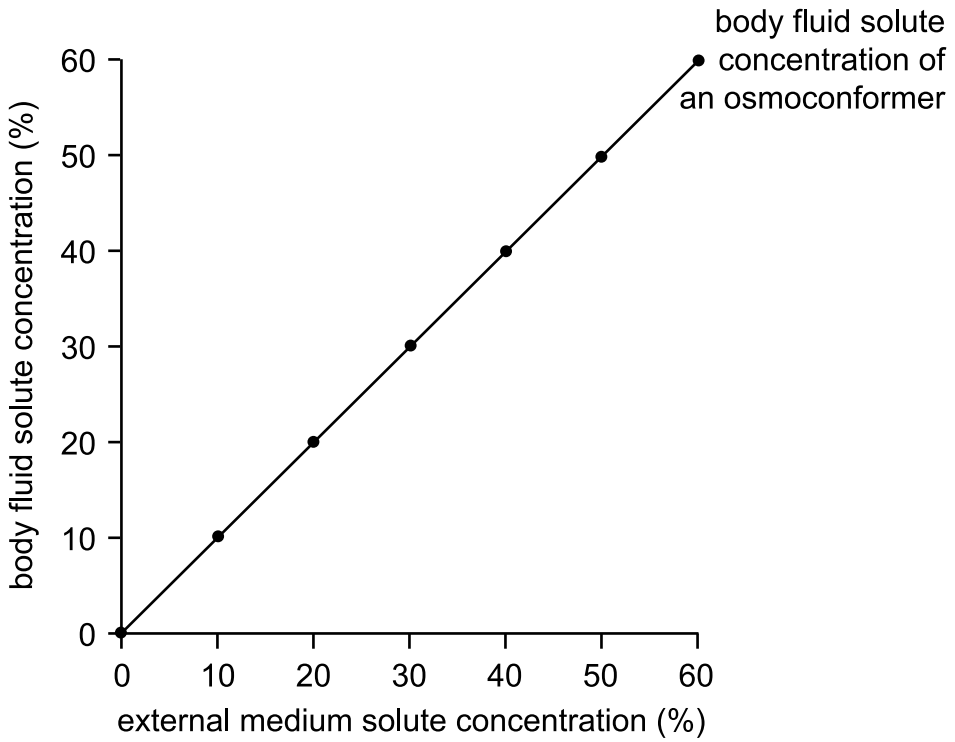
4.2 Conformers

A conformer's internal environment is dependent upon its external environment. In other words, their internal conditions are controlled by environmental conditions. Conformers use behavioural responses to maintain optimum metabolic rate. Behavioural responses by conformers allow them to tolerate variation in their external environment to maintain optimum metabolic rate.

There are some advantages to being a conformer in that they have low metabolic costs, which means that little energy has to be used to drive mechanisms such as contractile vacuoles or other forms of active transport. There is, however, some disadvantage in that it will frequently restrict these organisms to narrow ecological niches and lower activity rates.

Two areas where this can be seen are responses to changes in osmolarity and temperature.

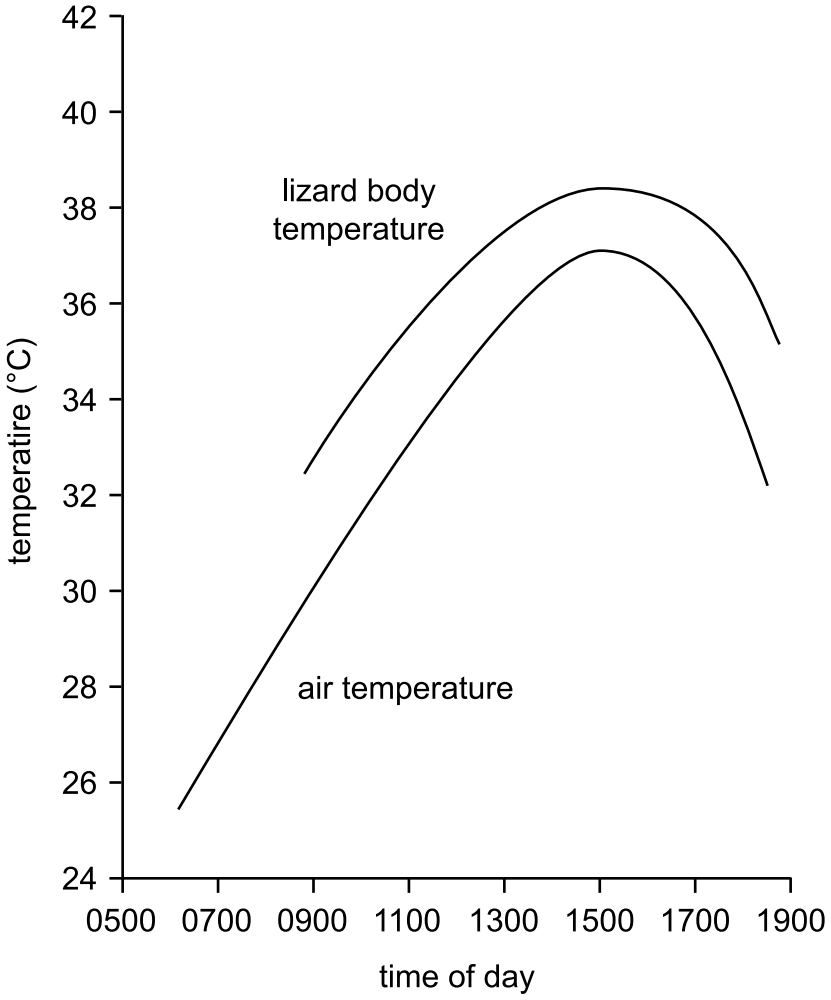
Osmolarity is a measure of the quantity of dissolved ions in water. Osmoconformers (frequently marine animals, such as squid) have body fluids that are at the same osmolarity as their surroundings. This, in turn, means that they have no need for structures such as kidneys, but it does leave them vulnerable to changes in their habitat.



Osmolarity of body fluids and the environment in osmoconformers

If the conditions change rapidly, then cell or organism death will result either by water leaving or entering in an overwhelming manner.

Thermoconformers are described as animals that cannot regulate their body temperature internally. This includes most insects and reptiles. The most frequent method of control is that of adapted behaviour.



Body and external temperature in a thermoconformer, e.g. a lizard

The behaviours that are exhibited include:

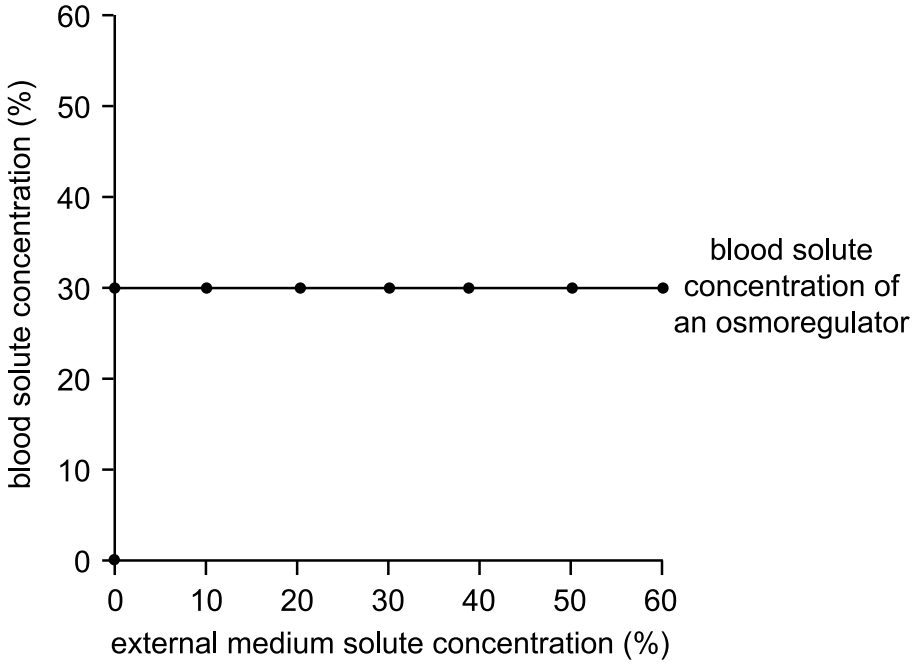
- vaporisation (getting wet);
- convection (losing or gaining heat to an airflow);
- conduction (lying next to some colder or warmer surface or material);
- radiation (finding shade or lying on hot rocks).

The major advantage of this behaviour is that little energy is required to maintain a steady body temperature. As the food/energy requirement is much lower for thermoconformers, they can survive erratic food supplies and so increase their chances of survival. However, should the temperature rise, their need for food will also rise as the metabolic rate increases with the surrounding temperature.

4.3 Regulators

Regulators are organisms which are able to use metabolic means to regulate their internal environments in response to external environmental changes. In order to achieve this, large quantities of energy are required in order to power specialist organs and body systems. The advantage of this, however, is that it allows these organisms to live more independently of their external environment and occupy a wider range of ecological niches. The maintenance of an internal environment in a 'steady state' is called homeostasis. It requires energy to achieve homeostasis, therefore, regulators have higher metabolic costs than conformers.

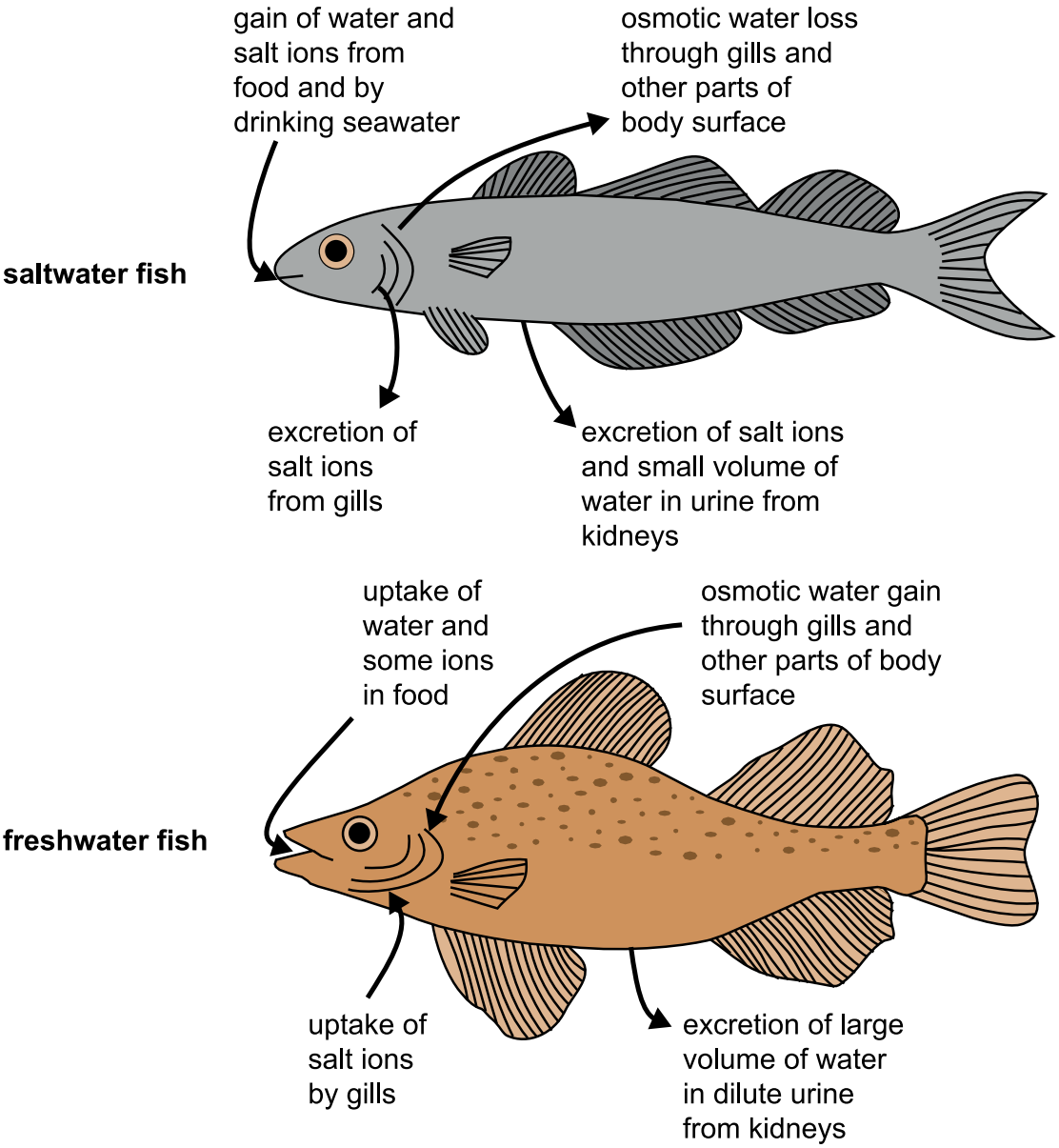
An osmoregulator can maintain its osmolarity despite wide variations in its habitat. It can move between a wide variety of habitats and niches and not be adversely affected. This group includes arthropods and vertebrates.



Osmolarity of body fluids and the environment in osmoregulators

Fish have adapted to usually either marine or fresh water environments (although some can range between both) by using salt organs in the gills and kidneys. In marine habitats, the overwhelming problem is that there is a the tendency is for water to be drawn out of the body. In addition, there is a diffusion gradient by which salts will move into the fish. To overcome these problems, the fish drinks water constantly and excretes salt from its gills. It also produces a small volume of concentrated urine.

Freshwater fish have the opposite problem, which is that water is moving into the fish while it is losing salts to the external environment. In this instance, the mechanisms are reversed so that salt cells in the gills actively absorb salt from the environment and the kidneys produce large volumes of very dilute urine.



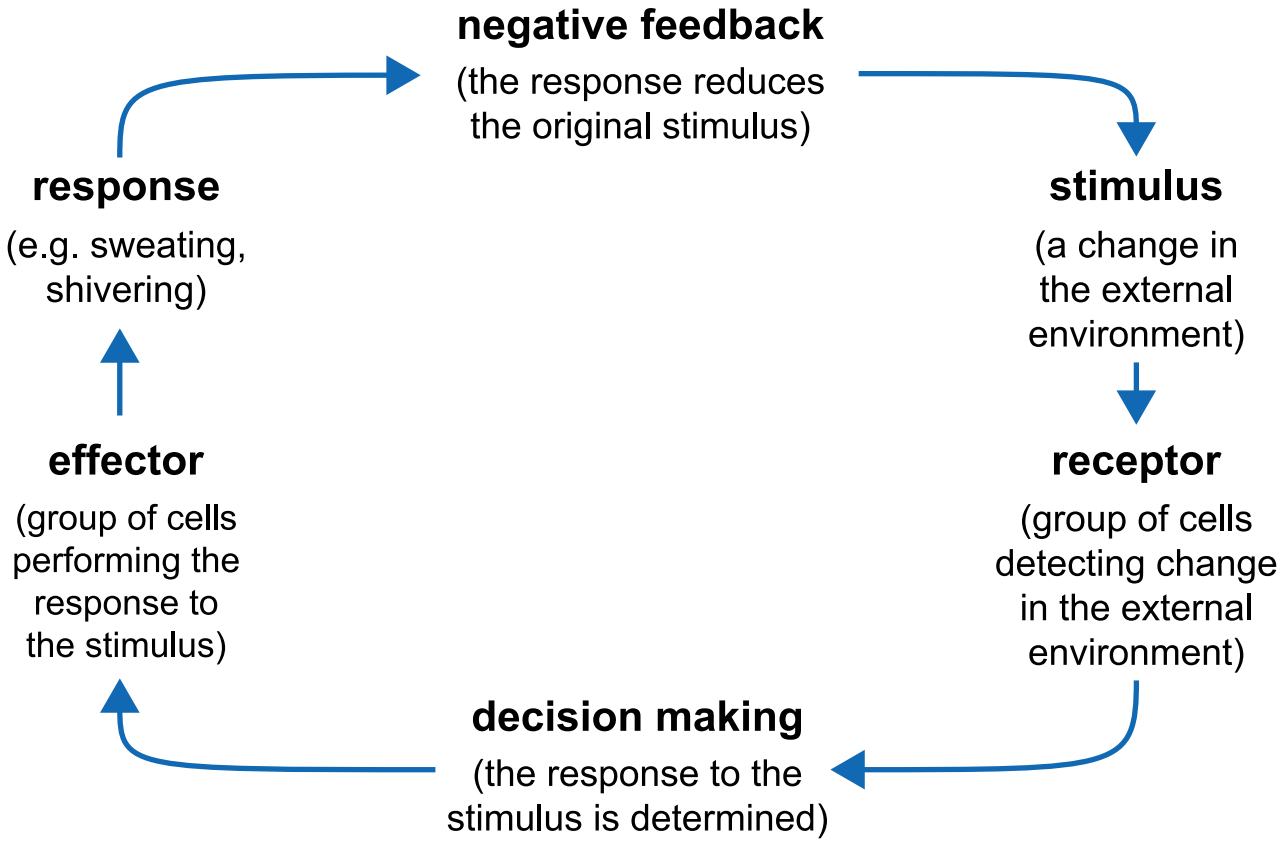
Control of blood salt concentration in saltwater and freshwater fish

Thermoregulators are organisms which can regulate their internal temperature, for example birds and mammals. For more information on thermoregulation read through the section.

4.4 Negative feedback control of body temperature

Feedback is used to regulate the response to a change in the environment. In **negative feedback**, when a condition changes, the opposite effect is produced by the body to return itself to normal. For example, if the internal temperature increases, the body responds by stimulating the processes to cool itself down.

The body is covered in **receptor** cells (both inside and outside) that detect changes in the environment. The change acts as a stimulus for the receptor cells, which then send a message to a control unit (usually the brain). A decision is made as to how the body is going to respond to the environmental change and a message is sent to the appropriate **effector** cells. These cells perform the response and return the body to its normal state. All of this happens very, very quickly so that almost as soon as a change occurs, a response is initiated.



Negative feedback

The messages sent between the receptor and effector cells are hormonal messages or nerve impulses.

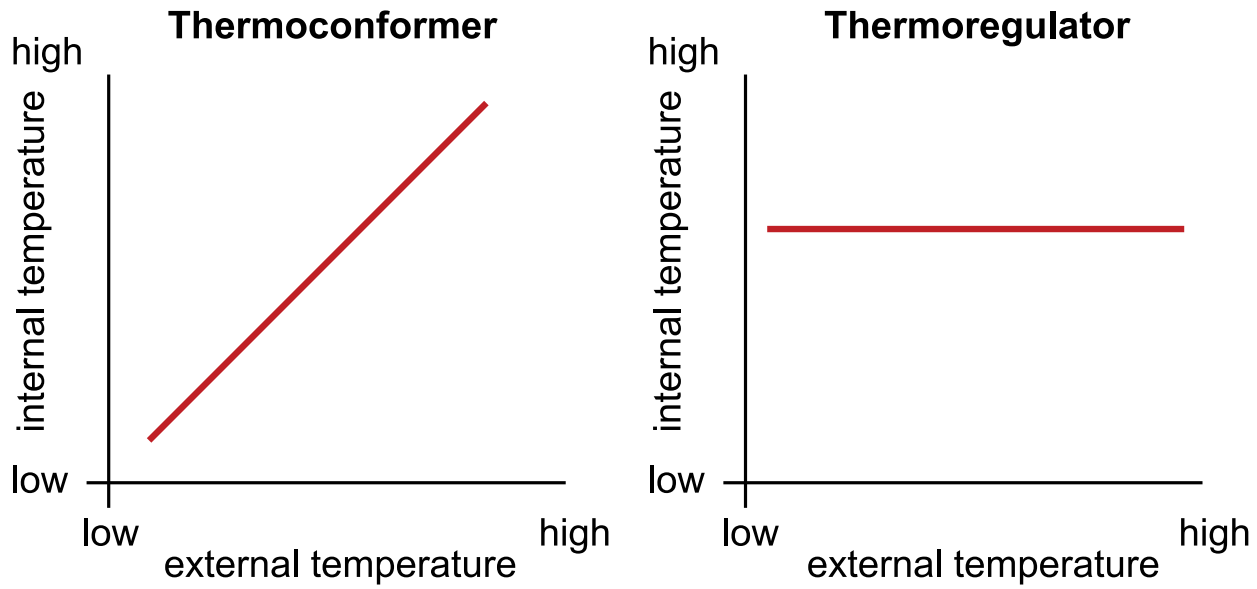
Regulating body temperature

The internal body temperature of a mammal needs to remain constant in order for the body to function properly. Although humans can withstand a wide range of environmental temperatures, we need to keep our internal body temperature as close to 37 °C as possible. Slight changes can cause major problems for the body and can even result in death. For example:

- a temperature increase denatures enzymes and blocks metabolic pathways;
- a temperature decrease slows metabolism and affects the functioning of the brain.

Different types of animals control their internal body temperature in different ways. Lizards, along with other reptiles, invertebrates, amphibians and fish, are thermoconformers. This means that they regulate their body temperature by absorbing heat from the surrounding environment. Therefore, their body temperature changes with that of the environment.

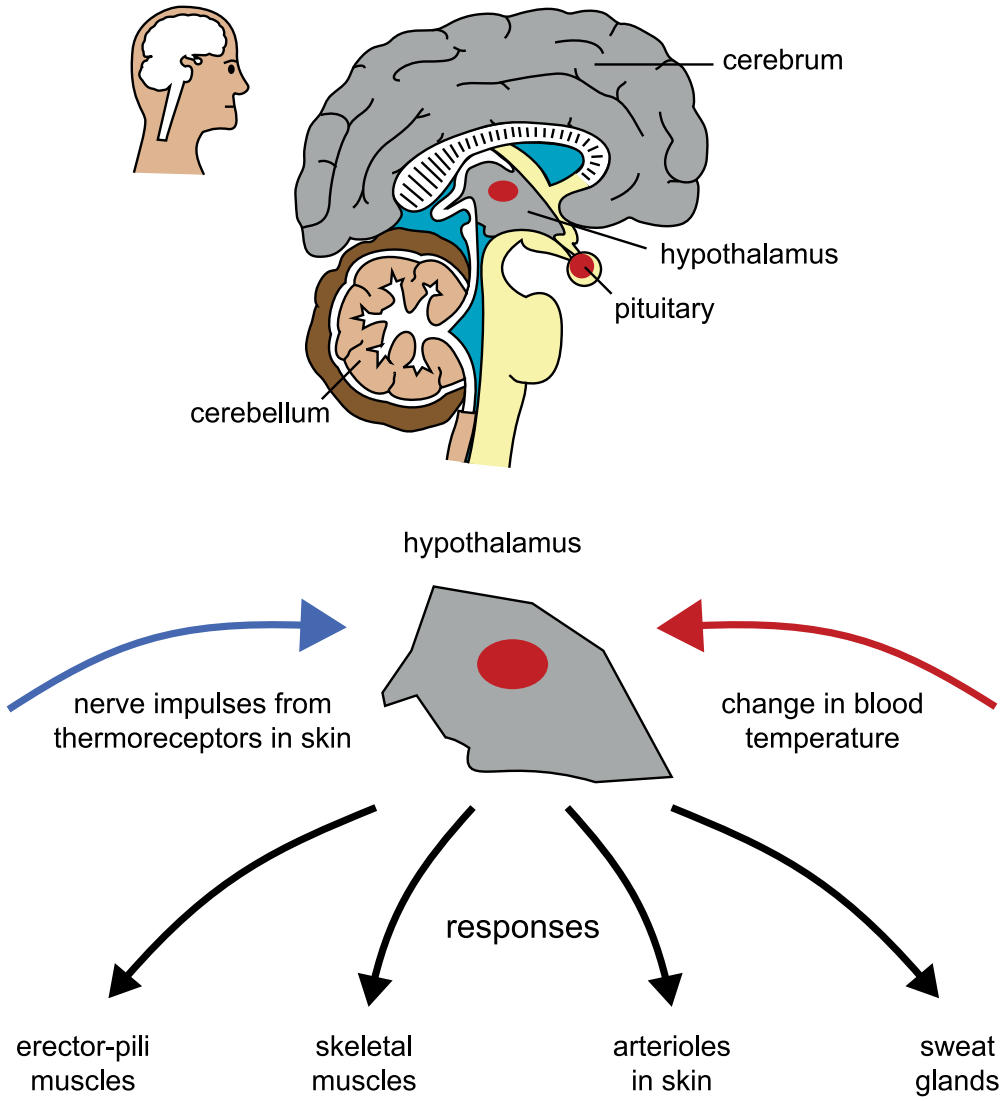
On the other hand, thermoregulators, such as mammals and birds, are able to maintain a constant body temperature regardless of the environmental temperature. They do not need to absorb heat from the surroundings because they gain heat energy from their own metabolism. Thermoregulators regulate their body temperature using homeostatic mechanisms and are able to live in a wider range of temperatures than thermoconformers (think about how widespread humans are in comparison with crocodiles).



Body temperature in thermoconformers and thermoregulators

One of the roles of the **hypothalamus** is monitoring and responding to changes in body temperature - it is the body's thermostat, found in the brain. Thermoreceptors in the hypothalamus detect changes in the temperature of the blood, which correspond to temperature changes of the core of the body. The hypothalamus also responds to changes in the surface temperature of the body. These temperature changes are detected by millions of thermoreceptors in the skin which send messages, via nerve impulses, to the hypothalamus.

The hypothalamus processes the information it receives from the thermoreceptors and decides what response is needed (i.e. does it need to heat the body up or cool it down?). Electrical impulses are sent through nerves to the **effectors** which perform the chosen response to the stimulus. Feedback tells the body when its temperature has returned to normal.



The hypothalamus

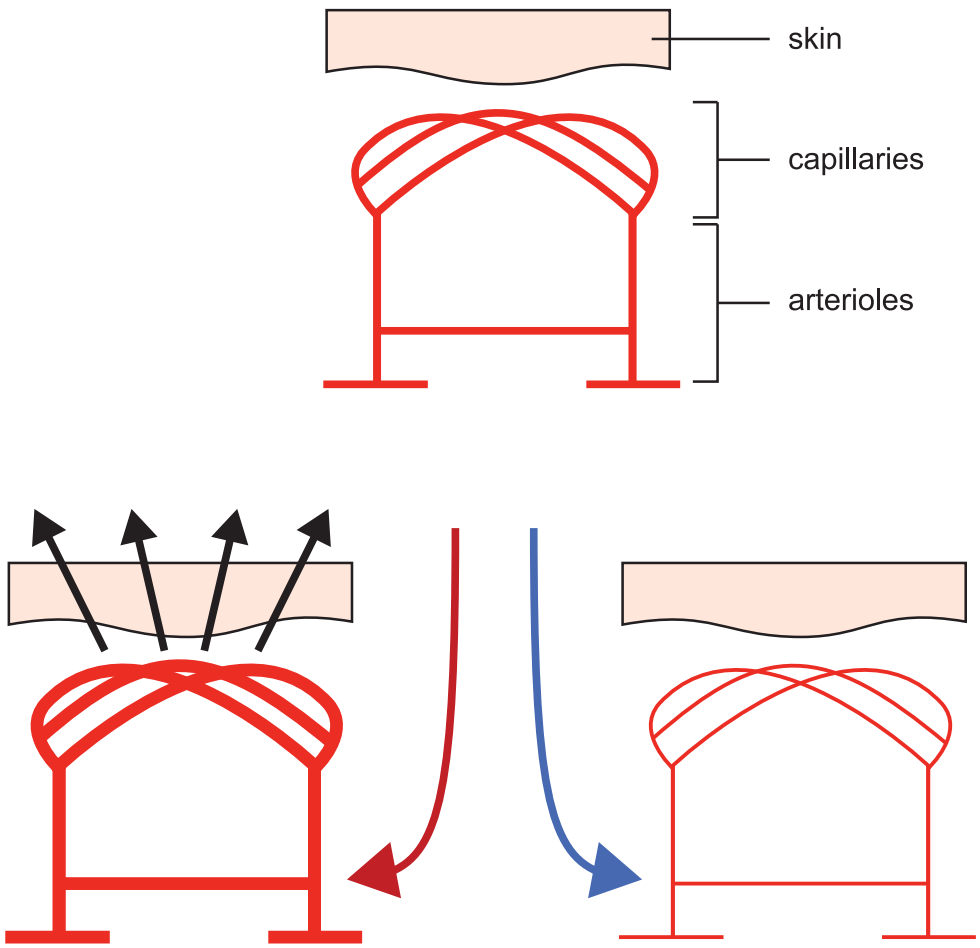
The skin is a highly complex organ that plays a very important role in regulating the temperature of a mammal. Not only does the skin contain **receptor** cells that detect temperature changes, but it also acts as an effector in response to nerve impulses from the hypothalamus.

When the hypothalamus detects an increase in body temperature it responds by taking measures to cool the body down (and vice versa). The main mechanisms employed by the skin to regulate the body temperature of a mammal are:

- **vasodilation** - the blood vessels (arterioles) that supply blood to the skin dilate (widen), increasing the amount of blood flowing to the skin. This increases the surface area from which heat can be lost to the environment by radiation.
- **vasoconstriction** - the arterioles that supply blood to the skin constrict (narrow), reducing the amount of blood flowing to the skin. As a result less heat is lost by radiation from the surface of the body.

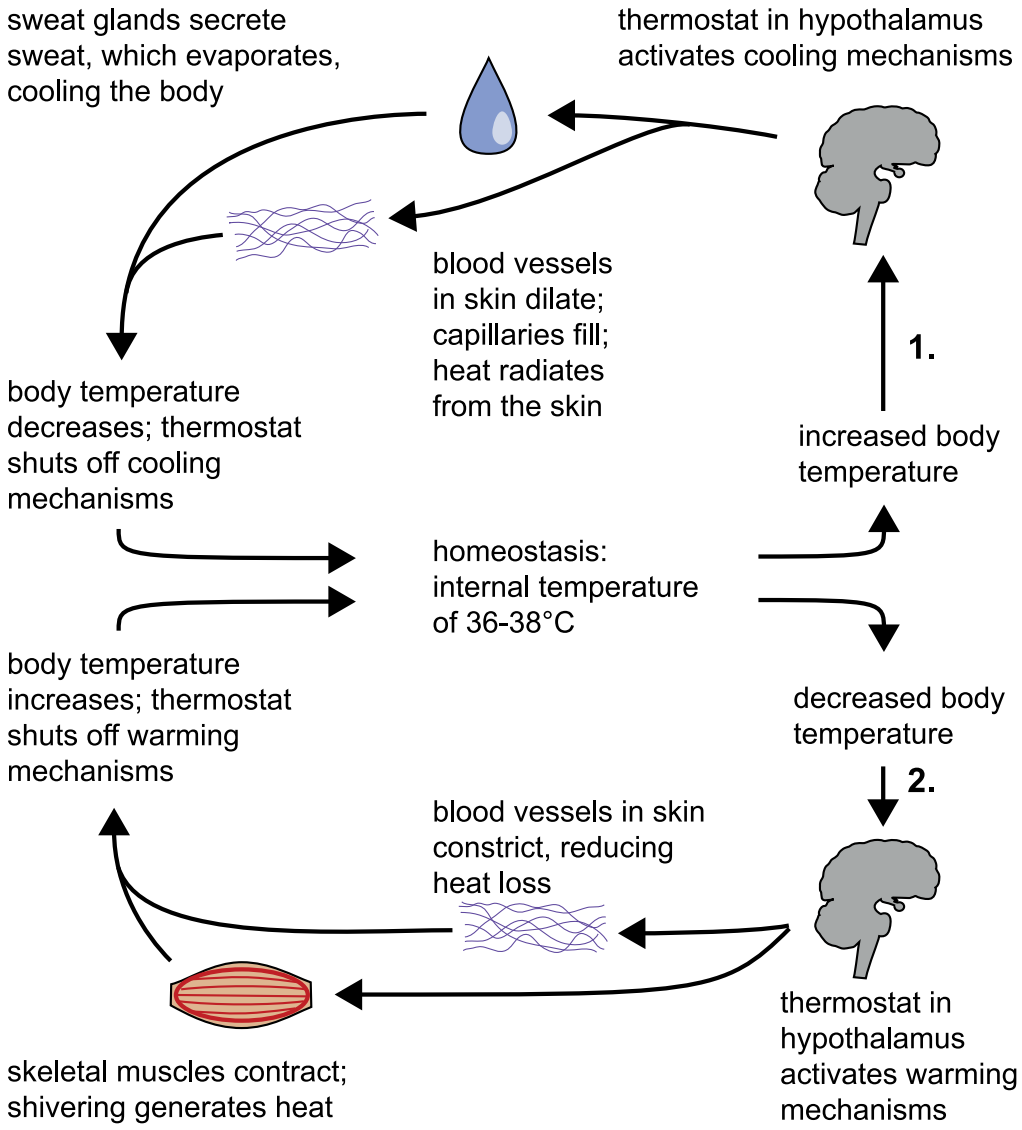
Vasodilation and vasoconstriction Go online

The following illustrates vasodilation and vasoconstriction.



If a nerve impuse from the hypothalamus signals that the body temperature is hot, the arterioles dilate, sending more blood to the capillaries so that heat is lost from the surface of the skin.

If a nerve impuse from the hypothalamus signals that the body temperature is cold, the arterioles constrict, sending less blood to the capillaries so that the heat loss from the surface of the skin is minimised.

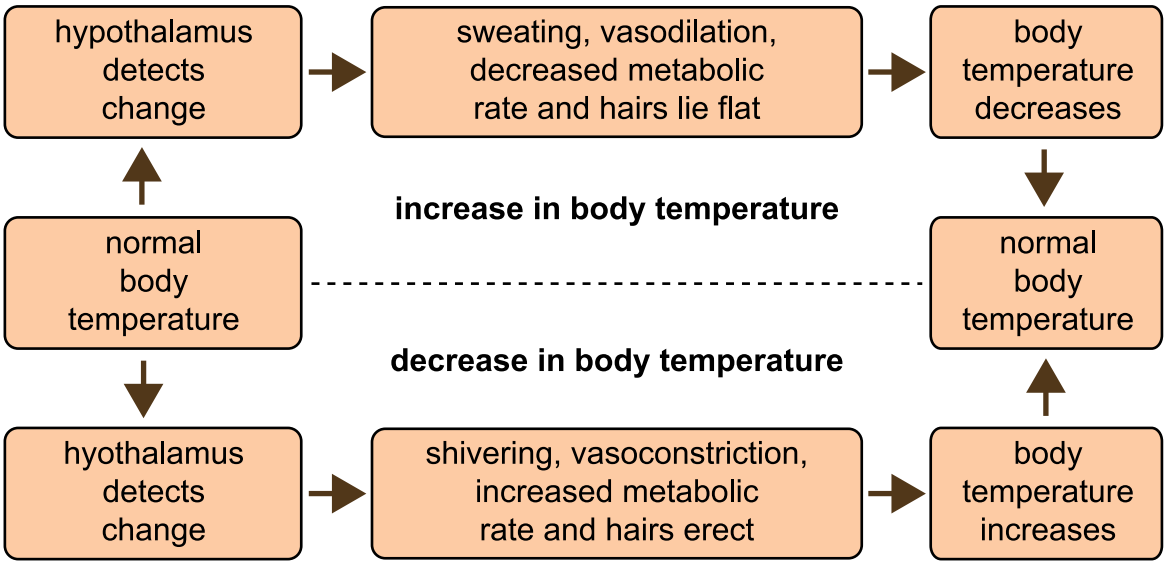


When the body is hot, sweat glands in the skin secrete sweat which cools the body down when it evaporates from the surface of the skin. When the body is cool, less sweat is produced to conserve heat.

Hairs are embedded in the sublayers of the skin and are attached to the skin epidermis by erector pili muscles. When the body is cold, nerve impulses from the hypothalamus contract the erector pili muscles, causing the hairs to stand up. This traps a layer of air close to the body, which acts as insulation. This mechanism is less effective in humans than it is in birds and furry mammals.

The body also employs other mechanisms to regulate its body temperature. For example, shivering warms the body up, as does increasing the rate of metabolism (this generates more heat energy). On the other hand, decreasing the rate of metabolism reduces the amount of heat energy produced by the body.

Not all temperature regulation mechanisms are physiological. When a person feels hot they will usually drink more water, take some of their clothes off or stay in the shade. However, when they feel cold they may have a hot drink, put more clothes on or hug a hot water bottle!



Negative feedback control of body temperature

Regulating body temperature: Question Go online

Q1: Complete the table with the temperature responses listed.

Decrease in body temperature	***Increase in body temperature***

Temperature responses: active sweat glands, decrease in metabolic rate, hair erector muscles contracted, hair erector muscles relaxed, inactive sweat glands, increase in metabolic rate, vasoconstriction, vasodilation.

Q2: Which part of the brain is responsible for regulating body temperature in mammals?

. . . . . .

Q3: In which of the following ways does the body respond when its temperature falls?

a) Vasoconstriction and sweating
b) Contraction of hair erector muscles and vasodilation
c) Vasodilation and decreased rate of metabolism
d) Shivering and vasoconstriction

. . . . . .

Q4: Why do you sweat and your skin become flushed during exercise?

4.5 Learning points

Summary

- The ability of an organism to maintain its metabolic rate is affected by the external environment.
- Abiotic factors in the external environment include temperature, pH and salinity.
- Conformers are organisms which do not regulate their internal environment by metabolic means.
- The internal environment of a conformer is dependent upon its external environment.
- There is an energetic advantage to this as they do not have to expend much metabolic energy.
- The result however is that they are confined to a narrow range of ecological niches.
- They can respond to a limited extent by behaviour responses to help maintain optimum internal conditions.
- Regulators maintain their internal environment regardless of external environment.
- This allows them to occupy a greater range of ecological niches.
- This level of control demands a high energy expenditure, with a considerable proportion of metabolic activity devoted to maintaining a steady state.
- The process is called homeostasis.
- To maintain a steady state negative feedback must be employed.
- Negative feedback occurs when a trend is reversed back to a 'normal level'. For example if body temperature rises above the set 'norm' then negative feedback returns it toward that 'norm'.
- For such a mechanism to work there have to be in place receptors to detect change in a given parameter (e.g. temperature), effectors to bring about any change, and a communication system between the two (usually nerves).
- It is important to regulate temperature (thermoregulation) for optimal enzyme activity and high diffusion rates to maintain metabolism.
- The hypothalamus is the temperature monitoring centre. Information is communicated by electrical impulses through nerves to the effectors, which bring about corrective responses to return temperature to normal.
- If body temperature increases above normal, processes such as sweating (body heat used to evaporate water in the sweat, cooling the skin), vasodilation (increased blood flow to the skin increases heat loss) and decreased metabolic rate (less heat produced) help to bring body temperature back down to normal.
- If body temperature decreases below normal, processes such as shivering (muscle contraction generates heat), vasoconstriction (decreased blood flow to skin decreases heat loss), hair erector muscles contract (traps layer of insulating air) and increased metabolic rate (more heat produced) help to bring body temperature back up to normal.

4.6 Extended response question

The activity which follows presents an extended response question similar to the style that you will encounter in the examination.

You should have a good understanding of internal body temperature regulation in mammals before attempting the question.

You should give your completed answer to your teacher or tutor for marking, or try to mark it yourself using the suggested marking scheme.

Extended response question: Internal body temperature regulation in mammals

Give an account of how internal body temperature is regulated in mammals. *(7 marks)*

4.7 Extension materials

The material in this section is not examinable. It includes information which will widen your appreciation of this section of work.

Extension materials: ADH

The term osmoregulation describes the mechanisms by which the body maintains a constant level of water, ions and salts in its cells. Controlling the amount of water in the body is very important as it can be just as dangerous to be over-hydrated as dehydrated.

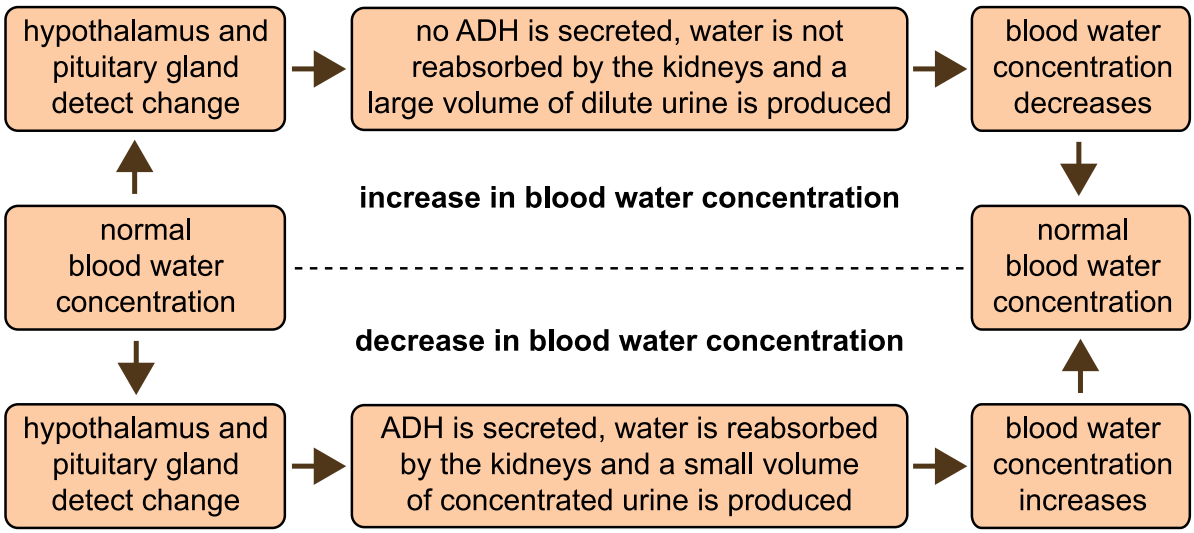
The kidneys act as osmoregulators in the human body. They respond to a hormone produced by the pituitary gland and reabsorb water back into the blood.

The concentration of water in the blood decreases if a person loses a lot of water, fails to take in enough water or consumes lots of salty food.

So, how does the body know that its cells need more water? There is a group of cells found in the hypothalamus, next to the pituitary gland in the brain. These act as the receptor cells that monitor the water concentration in the blood. If the concentration of water falls these receptor cells are stimulated and cause the pituitary gland to increase the secretion of a hormone into the bloodstream. When the kidneys detect this hormone they increase the rate at which they reabsorb water back into the bloodstream. As a result a smaller volume of more concentrated urine is produced by the body (to minimise water loss).

The hormone secreted by the pituitary gland is called antidiuretic hormone (ADH). (Its name describes its function: diuresis is the production of large volumes of dilute urine; ADH produces the opposite.) ADH works by making the ducts and tubules in the kidneys more permeable to water. Therefore, water is able to move (by osmosis) into the tissues and bloodstream from the kidneys more easily.

To prevent you from becoming too dehydrated the receptors in the brain that increase the secretion of ADH also make you feel thirsty. Drinking, along with the re-absorption of water in the kidneys, helps the body to restore the normal blood water concentrations. However, if you drink too much liquid the body reduces the production of ADH and the kidneys re-absorb less water. This allows the body to remove the excess water - in other words, you have to go to the toilet a lot!



Effect of ADH

4.8 End of topic test

End of Topic 4 test Go online

Q5: Which of the following factors do not affect the ability of an animal to maintain its metabolic rate?

a) Light intensity
b) pH
c) Salinity
d) Temperature

. . . . . .

Q6: Explain why it is important to maintain an organism's body temperature within a relatively narrow range.

. . . . . .

Q7: The internal environment of _____ is dependent upon the external environment.

_____ control their internal environment.

(Choose between 'conformers' and 'regulators' for each gap.)

. . . . . .

Q8: Give an advantage and a disadvantage of conformers over regulators.

. . . . . .

Q9: Which part of the brain detects body temperature?

. . . . . .

Q10: What term describes the process whereby the blood vessels immediately under the skin become narrower to restrict blood flow and reduce heat loss?

. . . . . .

Q11: Which of the following occur when the body is too hot?

a) Decreased metabolic rate
b) Increased metabolic rate
c) Vasoconstriction
d) Vasodilation

. . . . . .

Q12: How are signals sent from the temperature-monitoring centre of the brain to effectors?

Topic 5

Maintaining metabolism

Contents

5.1 Introduction 58
5.2 Dormancy 58
5.3 Migration 61
5.4 Learning points 64
5.5 End of topic test 64

Prerequisites

You should already know that:

- an adaptation is an inherited characteristic that makes an organism well suited to survival in its environment/niche.

Learning objective

By the end of this topic, you should be able to:

- describe the mechanisms and adaptations of organisms to survive in adverse conditions;
- describe how reducing metabolic rate (dormancy) can be used as a strategy for surviving adverse conditions;
- describe how relocation (migration) can be used as a method of avoiding adverse conditions.

5.1 Introduction

All organisms need to grow, develop and reproduce. Varying quantities of energy are devoted to each of these processes by organisms, and different strategies are employed to enhance the organism's chances of survival. Organisms also respond to changes in their external environment in different ways. In order to survive, organisms need to overcome the pressures of their environment and generate enough energy to complete their life-cycle.

When environmental conditions vary beyond the tolerable limits for normal metabolic activity, for example extremes of temperature or lack of water, organisms frequently resort to two types of survival mechanism: the first is to devise mechanisms to survive the condition (dormancy) and the second is to avoid them (migration).

5.2 Dormancy

One commonly employed method of ensuring survival involves **dormancy**. Dormancy is part of the lifecycle of some organisms to allow survival during a period when the costs of continued normal metabolic activity would be too high. The metabolic rate can be reduced during dormancy to save energy. During dormancy, there is also a decrease in heart rate, breathing rate and body temperature.

Dormancy can be predictive or consequential.

A predictive strategy allows dormancy to occur before the onset of unfavourable conditions. For example, decreasing temperature and day lengths are cues in seasonal environments that predict the onset of winter.

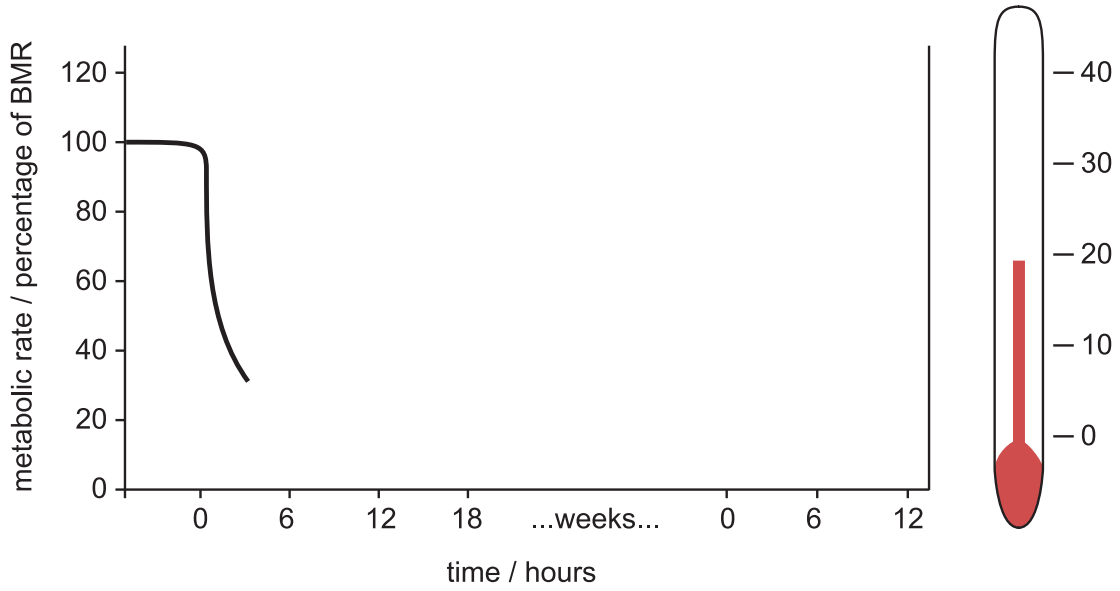
A consequential strategy enables the organism to react immediately to environmental cues. Organisms only enter a state of dormancy after they have been exposed to the adverse conditions. This is typically found in unpredictable environments where conditions may change very quickly. There is an enormous disadvantage to this, as a sudden change in conditions may result in high mortality rates. However, the organisms can delay dormancy until adverse conditions arise, meaning that they can make full use of the resources available in the habitat for as long as possible.

An organism may become dormant in response to changes in the environment or dormancy may be part of its life cycle. There are several different ways in which organisms save energy:

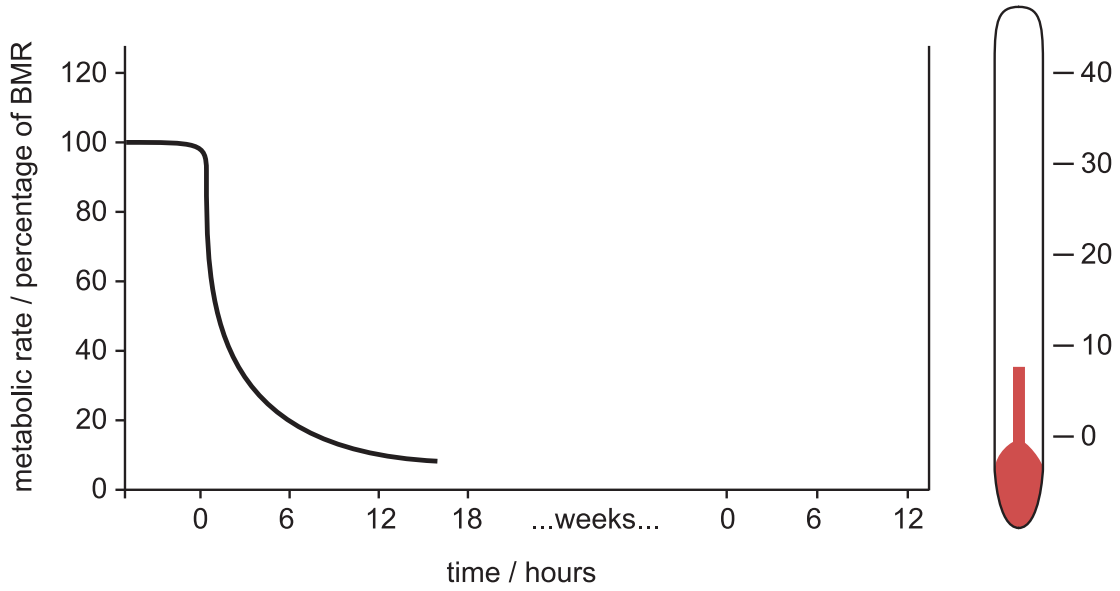
- **Daily torpor** involves the reduction of an organism's activity and metabolic rate for part of the day. Daily torpor often involves a reduction in heart rate and breathing rate. This allows organisms with high metabolic rates to save energy when they would not be able to find food. For example, house mice are active during the night and experience torpor through the day when it would be dangerous for them to be out in the open foraging for food.
- **Hibernation** is used by many organisms to escape cold weather conditions and scarce food supplies. The normal body functions of an organism change dramatically during hibernation. For example, the heart rate of the jumping mouse falls from 600 beats per minute to just 30 beats per minute. Animals prepare for hibernation by eating lots of food in the late summer and autumn. This builds up a layer of fat which keeps them warm and acts as a food source during the hibernation period. Hibernation can be either a predictive or consequential strategy. This form of dormancy is commonly seen in mammals such as hedgehogs, bears and dormice.

- **Aestivation** is a form of dormancy entered into by organisms in response to very hot and dry conditions. For example, the garden snail and some worms become dormant until moisture levels rise again. The snail retreats into its shell and seals the end and the worm coils up in a pocket of air surrounded by mucus. A more amazing example of aestivation is the lungfish, found in South America and Africa. This fish survives drought by burying itself in the mud on the river bed; the mud dries with the fish inside where it is able to survive until the next rainy season. Aestivation is a consequential strategy.

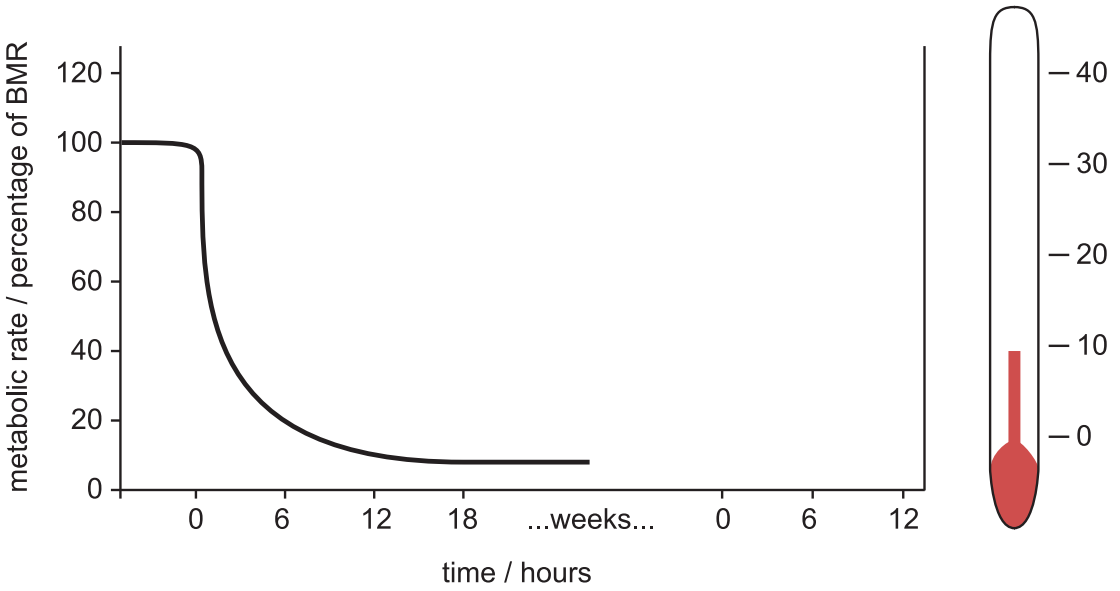
Hibernation Go online



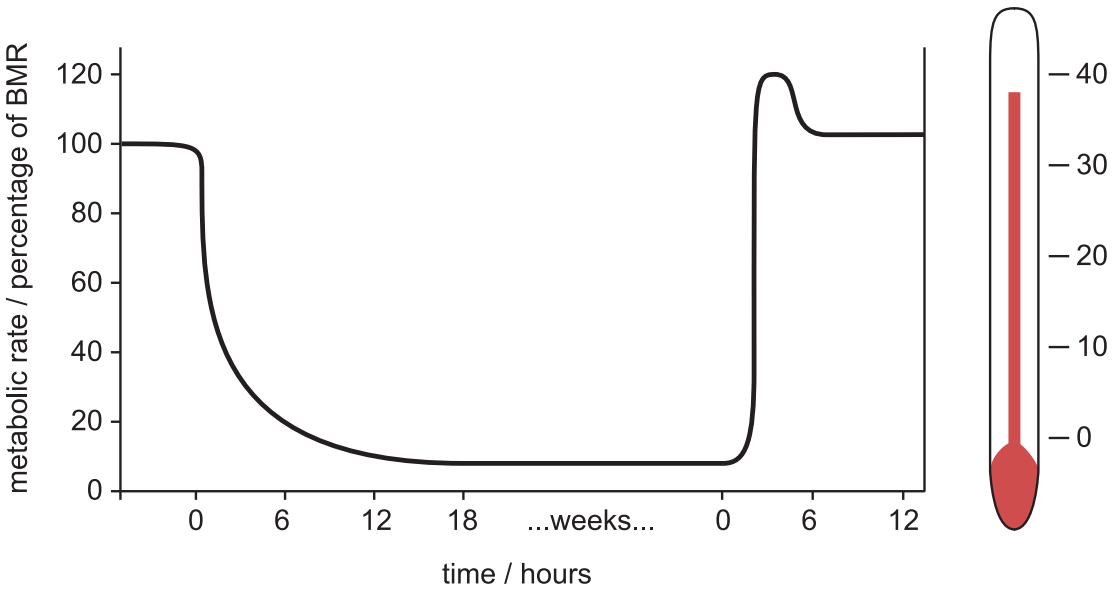
A change in the environment causes an organism to enter hibernation.



The organism's metabolic activity and body temperature fall.



An organism may hibernate for several weeks or even months. During this period, its metabolic activity and body temperature remain constant, but at a lower level than in the active organism.



As it emerges from hibernation, the organism's metabolic activity and body temperature increase rapidly. Initially, it metabolises at a higher rate than normal to generate energy for its normal active functions.

Dormancy: Questions Go online

Q1: Complete the table using the descriptions listed.

Term	*Definition*
Hibernation	
Aestivation	
Daily torpor	

Description list:

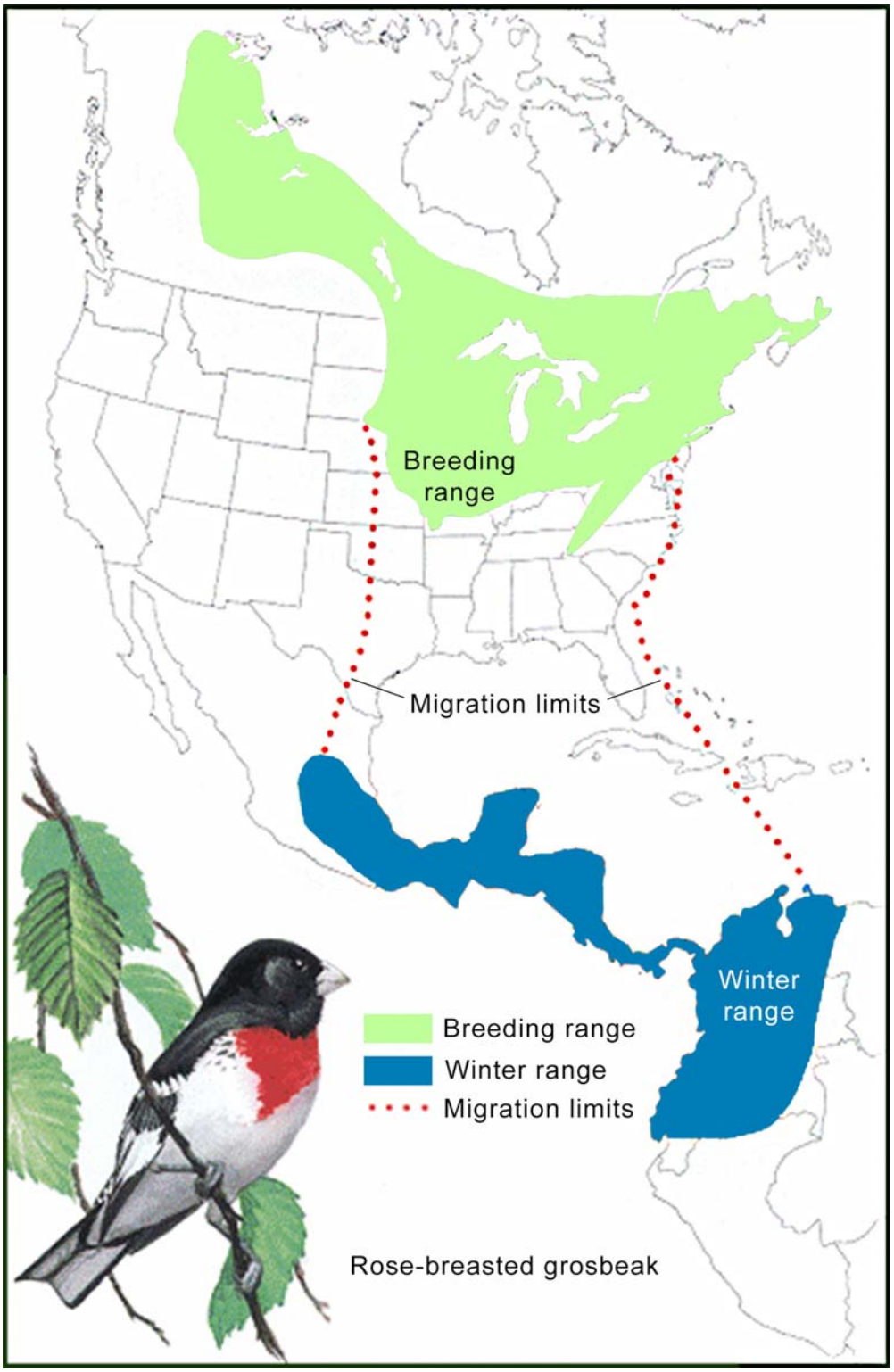
- Dormancy in response to hot, dry conditions
- Dormancy in response to low temperatures
- A period of reduced activity in some animals with high metabolic rates

5.3 Migration

In addition to physiological responses, organisms use behavioural strategies to avoid adverse environmental conditions. Many organisms, such as swallows, whales and moose, **migrate** to areas with more favourable conditions. All migration involves a considerable investment of energy from the individuals concerned, but is beneficial in the long-term, as it allows them to relocate to a more suitable environment.

Observation of migration goes back at least to the times of the ancient Greeks. Although some of the explanations may seem a little strange today. Aristotle thought that robins turned into redstarts at the onset of summer and the barnacle goose grew as a fruit from trees and had an intermediate state as goose barnacles on drift wood.

As time progressed, more critical observation was able to demonstrate that a large variety of animals, mammals, birds, fish and insects made long journeys from typically feeding and breeding grounds to over-wintering areas and back again the following year. To improve accuracy, early tagging methods were employed in conjunction with capture and release techniques.



Rose-breasted grosbeak's summer and winter areas and migration routes

Capture and release technique

Advances in technology allow for radio tags which can be followed by VHF receivers or satellite. This allows real-time tracking of an individual. Such methods are now used with many species of animal.

Work carried out by Dr. Peter Berthold and others has now established that the ability to successfully migrate is most likely to be hereditary. By observing the behaviour of a species of bird which has populations that migrate and others that do not, it was found that after breeding between the two types a significant proportion of the offspring had the ability to successfully migrate. The fact that not all of the offspring received the ability suggests that there is more than one gene involved.

It was further found that this inbuilt ability was controlled by a circannual rhythm. That is these birds have an inbuilt 'clock' that measures time. It was thought to have a natural cycle of approximately ten months. It will most likely be coordinated by day length.

Migratory behaviour is thought to be influenced by both innate and learned behaviour. Innate behaviour is inherited from parents to offspring and is likely to be the biggest influence on successful migration. Learned behaviour is gained by experience. Learned behaviours may come from parents or other members of a social group.

5.4 Learning points

Summary

- Many environments experience a range of conditions which will not support life.
- To be able to survive such conditions, organisms have evolved strategies to offset the conditions.
- One strategy is to lower metabolic rate, which is known as dormancy.
- Dormancy is described as a period when growth, development and activity are temporarily suspended.
- Dormancy is either predictive (occurs before the onset of adverse conditions) or consequential (occurs after the onset of adverse conditions).
- Hibernation in mammals is mostly in response to the onset of winter, with reduced temperature and shorter day length.
- Aestivation, usually in summer, is induced by high temperature and lack of water.
- Some organisms which have small body size but high metabolic rates, e.g. hummingbirds, conserve energy by decreasing physiological activity over nights, a process known as daily torpor.
- Some organisms avoid adverse conditions by relocating to a more suitable (survivable) environment. This is migration.
- During migration, the energy that would have been used to survive adverse conditions is used to travel to another environment.
- Techniques have been developed to study long distance migration. These include tagging, radio tracking, capture and release, and direct observation.
- It is believed that the ability of animals to follow migratory pathways is a combination of inherited (innate) and learned behaviour.

5.5 End of topic test

End of Topic 5 test Go online

Q2: Which strategy is used by an organism that enters a state of dormancy **after** it has been exposed to adverse conditions?

a) Complete dormany
b) Consequential dormancy
c) Predetermined dormancy
d) Predictive dormancy

. . . . . .

Q3: Dormancy entered into by organisms in response to very hot and dry conditions is called:

a) aestivation
b) daily torpor
c) hibernation

. . . . . .

Q4: Dormancy during the winter, in which the body temperature and metabolic rate of the animal drops significantly, is called:

a) aestivation
b) daily torpor
c) hibernation

. . . . . .

Q5: Aestivation is a form of:

a) consequential dormancy.
b) predictive dormancy.
c) both predictive and consequential dormancy.

. . . . . .

Q6: Hibernation is a form of:

a) consequential dormancy.
b) predictive dormancy.
c) both predictive and consequential dormancy.

. . . . . .

Q7: Dormice hibernate for five to seven months of the year. During this time, their heart rate can drop from 600 beats per minute to 30 beats per minute.

What is the percentage decrease in the heart rate of the dormouse during hibernation?

. . . . . .

Q8: Successful migration depends on a combination of _____ and _____ behaviours.

. . . . . .

Q9: Name one method of studying migration.

Topic 6

Environmental control of metabolism

Contents

6.1 Microorganisms . . . 68
6.1.1 Archaea . . . 69
6.1.2 Bacteria . . . 69
6.1.3 Fungi . . . 70
6.1.4 Protozoa . . . 71
6.1.5 Algae . . . 72
6.2 Growth of microorganisms . . . 72
6.3 Patterns of growth . . . 75
6.4 Penicillin production . . . 79
6.5 Learning points . . . 80
6.6 Extended response question . . . 81
6.7 Extension materials . . . 81
6.8 End of topic test . . . 83

Learning objective

By the end of this topic, you should be able to:

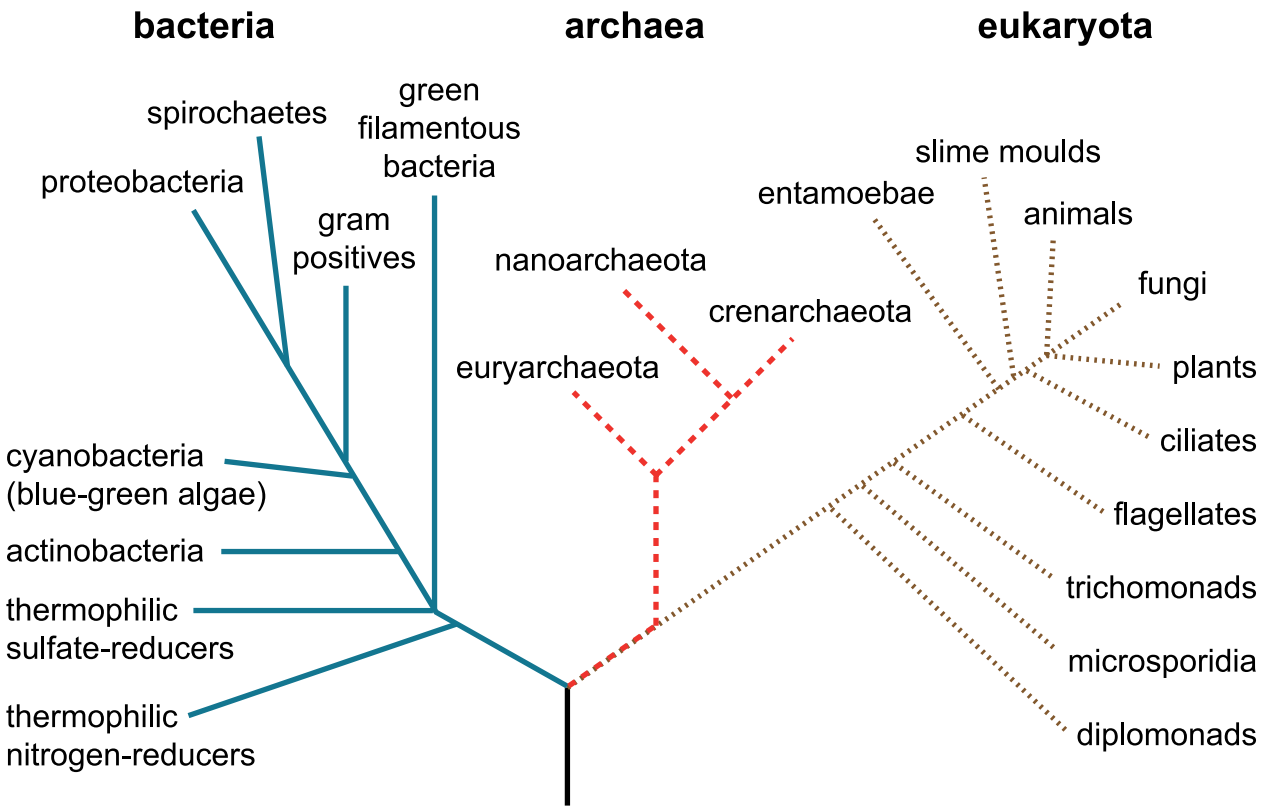
- describe the variety of microorganisms;
- explain their methods of metabolism and ecological range;
- learn how they can be exploited in industry;
- describe methods and conditions necessary to cultivate microbes;
- explain growth patterns.

6.1 Microorganisms

Microbiology is a specialised area of biology that studies organisms that are too small to be seen without magnification. These are microorganisms, or microbes. In today's world, microbiology makes up one of the largest and most complex biological sciences because it deals with microbe-human and microbe-environmental interactions. These interactions are relevant to disease (in both plants and animals), drug therapy, immunology, genetic engineering, industry, agriculture and ecology.

Microorganisms are capable of using a wide range of substrates for metabolism and are used to produce a variety of different products which are beneficial to mankind. As a result of their adaptability, microorganisms are found in a wide range of ecological niches and can be used for a variety of research and industrial uses because of their ease of cultivation and speed of growth.

Earlier classification systems described organisms as being prokaryote or eukaryote. While the term eukaryote still applies, prokaryotes are now described as **archaea** or bacteria. The significance of this is that the three domains archaea, bacteria and eukaryotes are thought to have evolved separately from an early common ancestor.

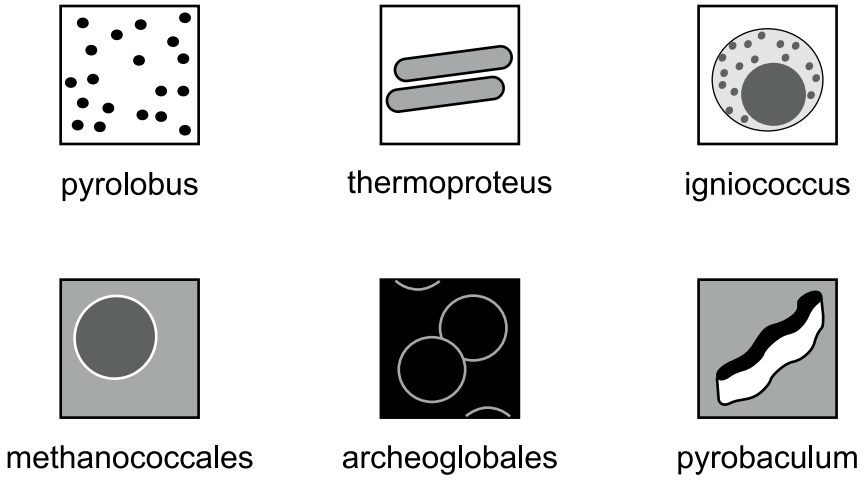


The three domains of life

6.1.1 Archaea

Archaea share some features with both bacteria and eukaryotes, yet are significantly different to merit their own grouping.

They are single-celled and have no nucleus or organelles. There are currently four or five major sub-groups, but this may alter with further research. While outwardly appearing similar to bacteria, several of the metabolic pathways are similar to eukaryotes. They are significantly different to both in the type and composition of the lipids in their membranes. Many would have been classified as extremophiles and the properties that allowed them to exploit these niches make them of potential use to industry.



Images of archaea

6.1.2 Bacteria

Bacteria can be classified into groups that contain organisms with similar characteristics. Bacteria are divided into three main groups. Each group is further divided until the species level is reached. The members of a bacterial species are similar to each other but can be distinguished from other species on the basis of several characteristics.

Occasionally, however, not all of the members are identical in what might be thought of as a 'pure' species culture. Different types within a species are called strains; that is, groups of different cells derived from a single cell. Strains may be identified by numbers, letters or names, for example, *E. coli* 0157 or *E. coli* 0111.

A variety of cell shapes (morphologies) and arrangements for bacteria exist as shown as follows.

Cocci

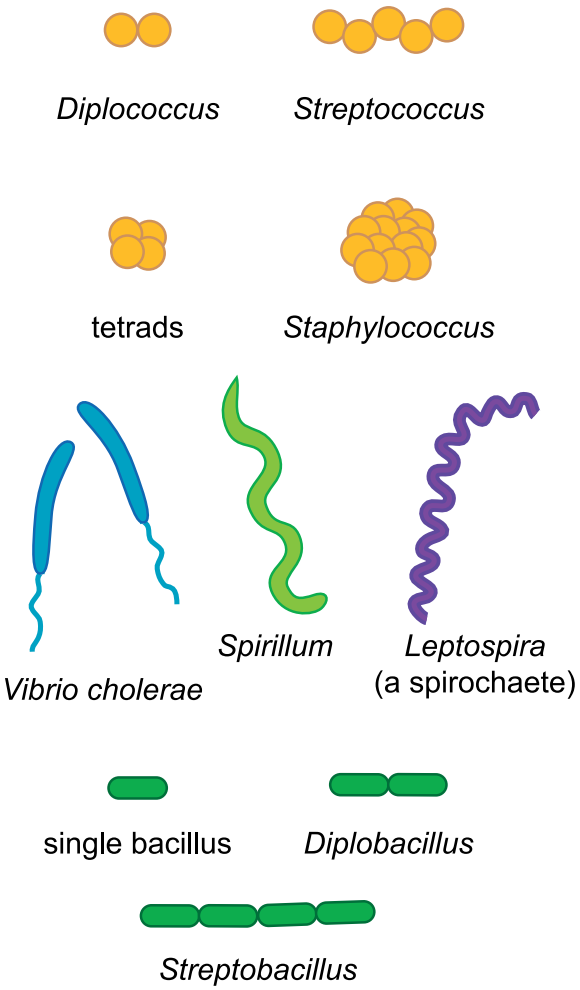
The number of planes in which the cells divide determines the final arrangement of the cells. Division may occur in a single plane (*Diplococcus* and *Streptococcus*), two planes (to produce tetrads), or in many planes (*Staphylococcus*).

Spirilla

There are many variations on this shape. Other shapes of bacteria do occur; *Stella*, for example, is star-shaped, and *Haloarcula* is square-shaped.

Bacilli

These rod-shaped bacteria can appear as single cells, in pairs or in chains.



Bacterial cell shapes and arrangements

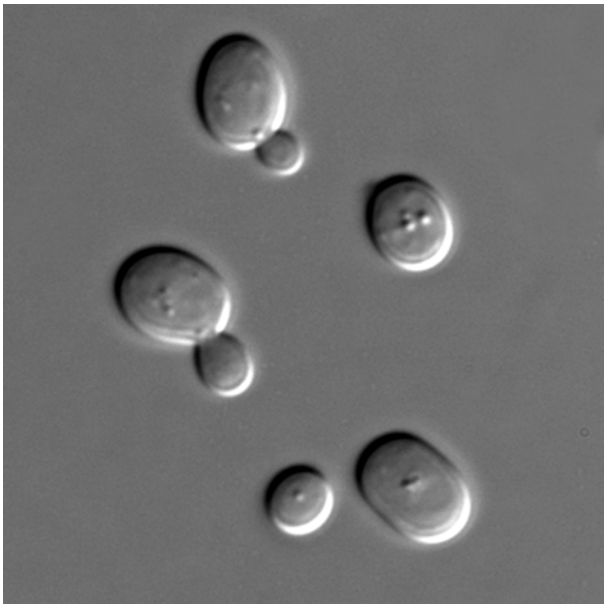
Many species of bacteria are of economic importance to humans. Bacteria are involved in the production of yoghurt, cheese, biofuels and many other products. Research into the properties of different bacterial species is also import because some cause disease. Research into bacterial species which have become resistant to antibiotics is of particular importance, i.e. MRSA.

6.1.3 Fungi

Fungi are eukaryotic cells and can be further sub-divided into yeasts and moulds. These differ with respect to their morphology. Fungi are of importance to humans because they can be both beneficial and harmful. Fungi act as decomposers, a role that is of great environmental significance.

Fungi are the major cause of plant disease. Over 5,000 species attack economically important crops and wild plants. Many animal diseases are also caused by fungi. Fungi are essential to many industrial processes that involve fermentation, such as bread, wine and beer production. They also play a major role in the manufacture of cheese and soy sauce. Fungi are essential for the commercial production of some organic acids, such as citric acid and gallic acid, and certain drugs, such as ergometrine and cortisol. They are also important in the manufacture of antibiotics (penicillin) and the immunosuppressive drug cyclosporin.

Yeasts are single cells, whereas moulds are multicellular. Generally, yeasts are larger than most bacteria and are usually oval in shape, although some may be elongated or spherical. They lack flagella and other means of locomotion. They form smooth, glistening colonies on an agar medium that are quite different from the spreading, furry, filamentous colonies formed by the moulds.

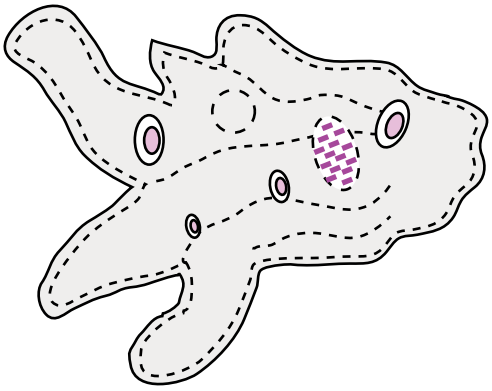


Scanning electron microscope image of yeast

6.1.4 Protozoa

Protozoa are eukaryotic cells that have a variety of shapes, such as oval, spherical or elongated. Some are polymorphic with different forms at different stages of the life cycle. Some are up to 2mm in length and, as such, are visible to the naked eye. Like animal cells, the protozoa lack cell walls, are motile at some stage of the life cycle, and are **heterotrophic**. Each individual cell is a complete organism containing all the organelles necessary to perform all the functions of an individual organism. Typical examples of protozoa are *amoeba*, *paramecium*, *euglena* and *plasmodium*.

Protozoa grow in a variety of moist habitats. They are susceptible to desiccation, so a moist environment is essential to their existence. Protozoa are predominantly free living and are found in both freshwater and marine environments. Terrestrial protozoa can be found in soil, sand and decaying matter. Many protozoa are parasites of animals or plants.

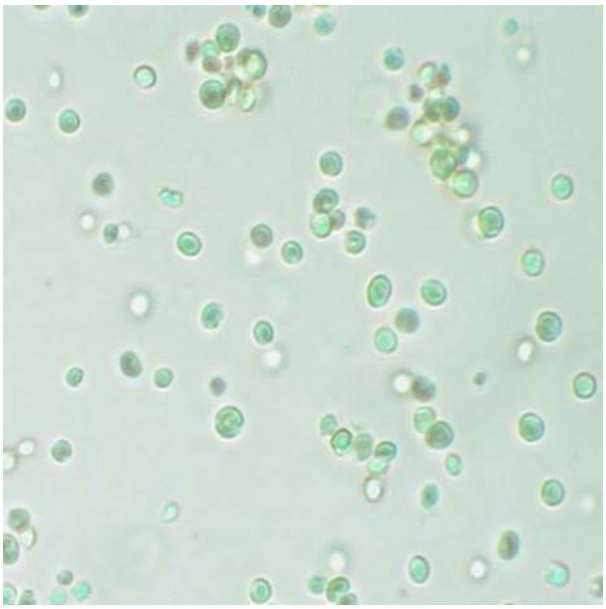


An amoeba

The cellular morphology of protozoan organisms is essentially the same as that of all eukaryotic multicellular organisms, but because all life processes occur within the single cell, there are some morphological and physiological features that are unique to protozoa.

6.1.5 Algae

Unicellular algae may be spherical, rod-shaped, club-shaped or spindle-shaped. Some are motile. Multicellular forms vary in shape and size, occurring sometimes in colonies, either as groups of single cells or a mix of different cell types with special functions. They also vary in the photosynthetic pigments present and their colour. Algae grow in a wide variety of habitats, from oceans to freshwater ponds. Algae may also be symbionts or parasitic, and they make a vital contribution to global productivity.



Algae

6.2 Growth of microorganisms

Microorganisms can be cultured relatively easily in a laboratory. They must be given an appropriate growth medium and the environmental conditions must be carefully controlled to ensure successful growth.

Microorganisms are grown on nutrient material called culture media. A medium is any solid or liquid preparation made specifically for the growth, storage or transport of microorganisms, which generally supplies all the essential nutrients. The medium must also be at the correct pH and the organisms have to be grown in the correct gaseous environment. The medium must be sterile before use. Two types of media are commonly used:

- ***complex media*** contain one or more crude sources of nutrients and their exact chemical composition and components are unknown;
- ***defined media***, or synthetic media, are media in which the components of the medium are chemically known and are present in relatively pure form.

For the routine culture of microorganisms, complex media are generally used. Defined media are used in nutritional, genetic and physiological studies. Some varieties of media and the bacteria grown on them are summarised in the table below.

Type of medium	***Example***	***Extra constituents***	***Bacteria grown***
Complex media	nutrient agar	meat extracts, yeast extract	many bacteria will grow on this medium
Defined media	M9	M9 salts	*Escherichia coli*
Enriched media	blood agar	blood	*Streptococcus pyogenes*
Selective media	MacConkey agar	contains bile salts and crystal violet dye	inhibits the growth of Gram +ve bacteria and encourages the growth of Gram -ve bacteria

Growth media for bacteria

Microorganisms require an energy source (which may be chemical or light) and simple chemical compounds for biosynthesis. Many microorganisms can produce all of the complex molecules required for life, including amino acids for protein synthesis. Other microorganisms require more complex compounds to be added to the growth media, including vitamins and fatty acids.

In order to grow cells in culture, they must be supplied not only with the correct **nutrient medium** but also the correct environmental conditions, including:

- temperature (controlled using an incubator);
- pH (controlled by the use of buffers or addition of acid/alkali);
- gaseous environment (some microorganisms are anaerobic and will not grow in the presence of oxygen, others will require a good oxygen supply);
- light (if it is a photosynthetic microorganism).

Microorganisms are ubiquitous; any natural habitat will contain a diverse microbial population. All inanimate and living objects in the laboratory, including the atmosphere, carry large numbers of microorganisms, so special techniques are required to prevent such microorganisms from contaminating pure cultures. This is called aseptic technique and its function is three-fold:

1. to prevent contamination of cultures by unwanted organisms;
2. to prevent the organism that is being cultured from contaminating the environment (that is, the laboratory and the people in it);
3. to reduce competition with desired micro-organisms for nutrients and reduce the risk of spoilage of the product.

One way to ensure that equipment is sterile is to use heat sterilisation. During this process, all utensils (such as inoculating wires and loops) and media used to handle and grow pure cultures of

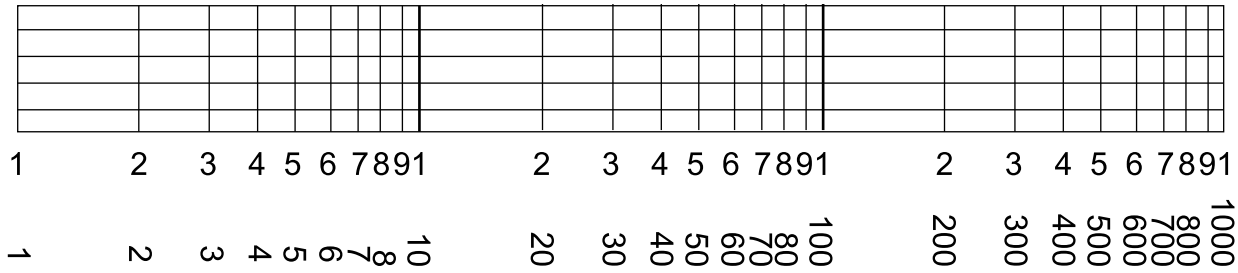
microorganisms are sterilised beforehand in order to eliminate all the organisms present in or on them.

Sterilisation is normally carried out by autoclaving or by dry heat (in an oven). An autoclave is shown below. The essential part of autoclaving is that the high temperature and pressure is maintained for at least 15 minutes. This is because the spores of some bacteria are only killed at these heat and pressure intensities. Microorganisms can be grown on sterile agar or sterile broth in flasks, bottles or tubes. In each case, the vessel is topped with a metal or plastic cap, a cotton wool bung, or a foam plug designed to exclude microorganisms.

A benchtop autoclave
(http://commons.wikimedia.org/wiki/File:Laboratory_autoclave.jpg?uselang=en-gb by Nadina Wiórkiewicz (pl.wiki: http://en.wikipedia.org/wiki/pl:user:Nadine90, commons: http://commons.wikimedia.org/wiki/User:Nadine90), licensed under http://creativecommons.org/licenses/by-sa/3.0 via http://commons.wikimedia.org/)

6.3 Patterns of growth

Under ideal conditions, some species of bacteria are capable of doubling in number every 20 minutes. If you were trying to plot bacterial growth on normal graph paper, you would either run out of space very quickly on the x axis, or the scale would be so reduced it would make plotting or reading with any accuracy almost impossible. The solution is to use semi-logarithmic graph paper. This has been printed in a specific way to allow data which has a very wide range to be plotted. This allows exponential relationships to be depicted. In microbial cases, time is usually plotted as the independent variable (x axis) and logarithmic growth of bacteria is plotted as the dependent variable (y axis).



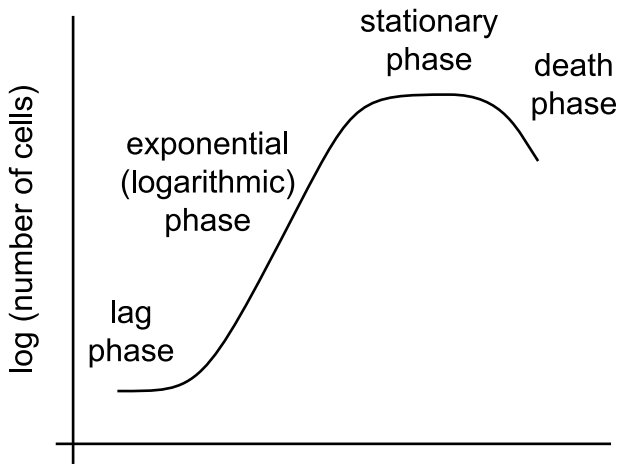
Logarithmic scale

The extent of growth of a microbial culture can be estimated by taking samples from the culture at certain time points and counting the number of cells present at each one. A count is also made at the time of inoculation so that the initial concentration of cells is known. The results of the timed samplings will give enough information to construct a growth curve.

The four main stages in growth are:

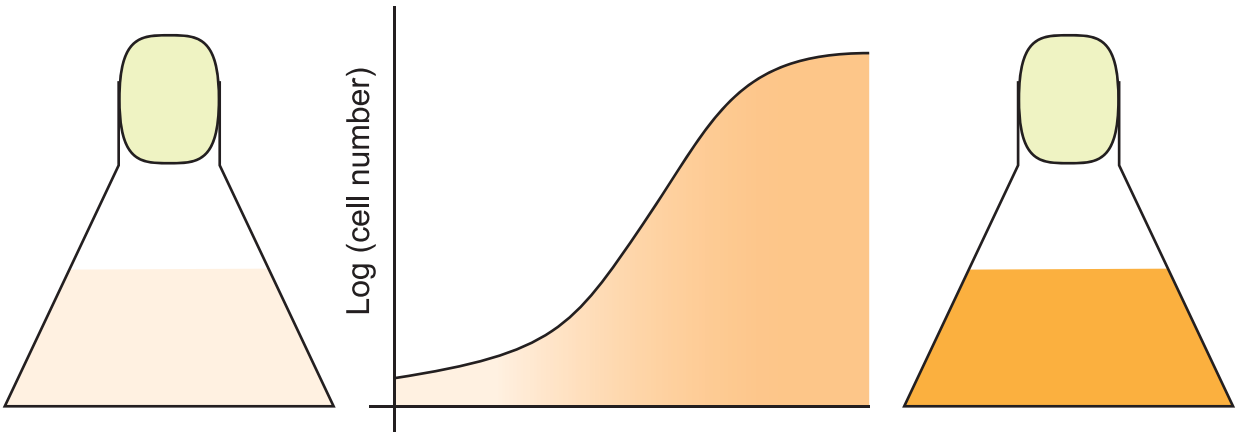
1. ***lag phase*** - where microorganisms adjust to the conditions of the culture by producing enzymes that metabolise the available substrates;
2. ***exponential (logarithmic) phase*** - during this phase the rate of growth is at its highest due to plentiful nutrients;
3. ***stationary phase*** - occurs due to the nutrients in the culture media becoming depleted and the production of toxic **metabolites**. Secondary metabolites are also produced, such as antibiotics. In the wild these metabolites confer an ecological advantage by allowing the micro-organisms which produce them to outcompete other micro-organisms;
4. ***death phase*** - occurs due to the toxic accumulation of metabolites or the lack of nutrients in the culture.

Note the use of a logarithmic scale on the vertical axis in the following graph.



Microbial growth curve

This following illustration shows the change in turbidity (optical density) of a culture as it grows.



Turbidity

Knowledge of microbial growth rates is essential to microbiologists because growth rate studies are relevant to fundamental research and also to applied situations, such as the industrial production of microorganisms. A knowledge of growth rates allows scientists to predict, and possibly control, the growth of any unicellular microbial species.

During the exponential phase of growth, a microorganism is dividing at constant intervals. The population will therefore double during a certain period of time, called the generation time. Not all species of microorganism have the same generation time. For example, *E. coli* grown in an appropriate medium has a generation time as low as 15 minutes. *Mycobacterium tuberculosis*, on the other hand, has a generation time of 13-15 hours. In addition, generation time for a species will vary if growth conditions change. For example, *E. coli* will take much longer to divide than in the example above if it is grown in a nutritionally poor medium. Examples of typical generation times for different types of microorganism are given in the following table.

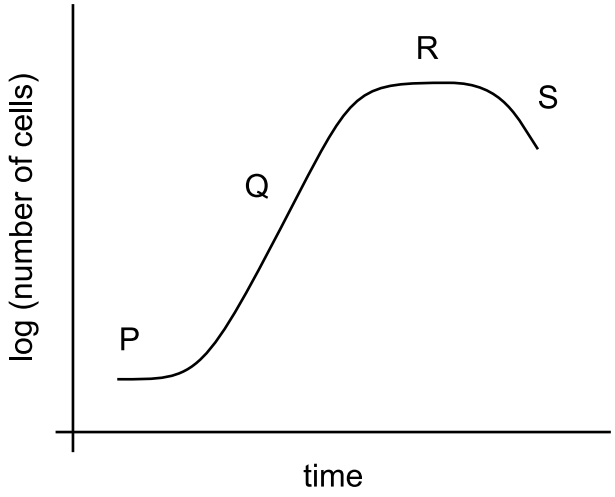
Microorganism	***Generation time (hours)***
Bacteria	
Escherichia coli	0.35
Bacillus subtilis	0.43
Clostridium botulinum	0.58
Algae	
Chlorella pyrenoidosa	7.75
Skeletonema costatum	13.1
Euglena gracilis	10.9
Protozoa	
Paramecium caudatum	10.4
Fungi	
Saccharomyces cerevisiae	2.0

Generation times for microorganisms

When cell counts are performed, they fall into one of two categories: total cell count or viable cell count. A total cell count involves a direct counting of the cells in culture whether dead or alive. A viable cell count is a method where only live cells are counted. Only viable cell counts show a death phase where cell numbers are decreasing.

Bacterial culture growth phases: Questions Go online

This is an analysis activity. Each letter represents a different phase of growth for a bacterial culture. Study the graph and then answer the questions that follow.



Bacterial culture growth phases

Q1: In which phase are the bacteria doubling at a constant rate?

a) P
b) Q
c) R
d) S

. . . . . .

Q2: In which phase does bacterial cell division equal bacterial death?

a) P
b) Q
c) R
d) S

. . . . . .

Q3: In which phase are the bacteria metabolically active but not dividing?

a) P
b) Q
c) R
d) S

. . . . . .

Q4: In which phase does bacterial cell death exceed cell division?

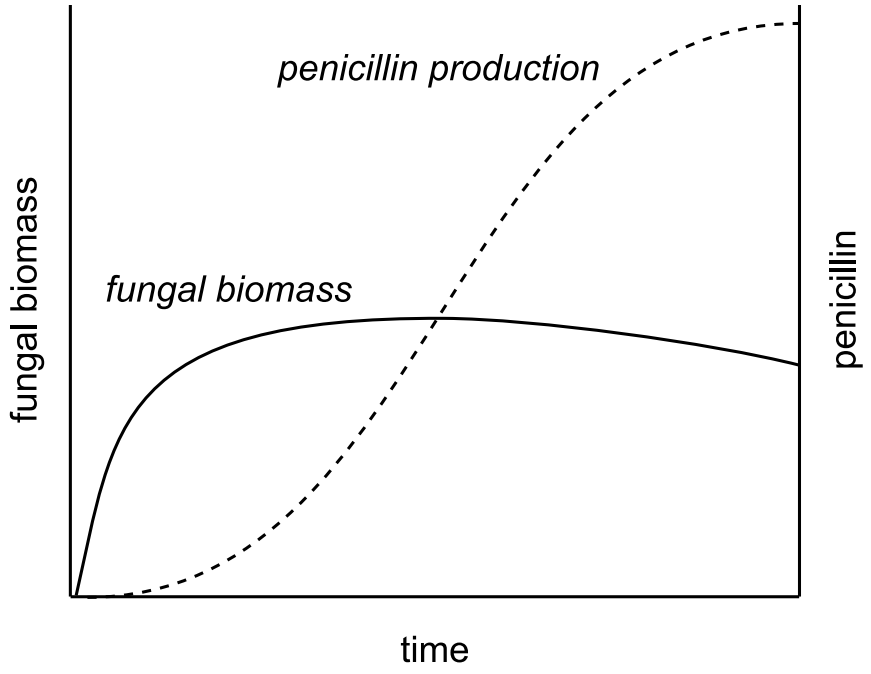
a) P
b) Q
c) R
d) S

6.4 Penicillin production

Many antibiotics are produced by microorganisms, predominantly by the actinomycetes *Streptomyces* spp. and some filamentous fungi. Environmental control is crucial to the production of antibiotics such as penicillin.

Penicillium chrysogenum is the fungus that produces penicillin. It is the control of the composition of the medium in which the *Penicillium* is grown that allows high yields of the product to be obtained. The organism is grown in stirred fermenters but the rapid growth of the cells, with glucose as a carbon source, does not necessarily lead to maximum antibiotic yields. The greatest yield occurs when the medium is provided with a combination of lactose, as a carbon source, with a limited nitrogen availability that stops growth. However, the same result can be achieved by using a slow continuous feed of glucose and this is the method used today.

It can be seen, therefore, that the manipulation of the growth medium or the organism itself can result in high yields of the product.



Penicillin production by Penecillium

6.5 Learning points

Summary

- Microbiology is the study of organisms that are too small to be seen by the naked eye.
- Microbes can be found in all three domains: archaea, bacteria and eukaryotes.
- As a result of their adaptability, microorganisms are found in a wide range of ecological niches, and can be used for a variety of research and industrial uses because of their ease of cultivation and speed of growth.
- Microorganisms require an energy source (chemical or light) and simple chemical compounds for biosynthesis.
- Many microorganisms can produce all the complex molecules they require. Other microorganisms require more complex compounds to be added to the growth media.
- Culture conditions include sterility, to eliminate any effects of contaminating microorganisms, control of temperature in an incubator, control of oxygen levels by aeration and control of pH by buffers or the addition of acid or alkali.
- Microbes grow in vast numbers; plotting growth requires the use of semi logarithmic scales.
- The growth cycles consists of distinct phases: lag, log, stationary and death.
- During the lag phase, microorganisms adjust to the conditions of the culture by producing enzymes that metabolise the available substrates.
- During the log phase, the rate of growth is at its highest due to plentiful nutrients.
- The stationary phase occurs due to the nutrients in the culture media becoming depleted and the production of toxic metabolites. Secondary metabolites are also produced, such as antibiotics. In the wild these metabolites confer an ecological advantage by allowing the micro-organisms which produce them to outcompete other micro-organisms.
- During the death phase, a lack of substrate and the toxic accumulation of metabolites cause death of cells.
- Secondary metabolites have no direct relationship to the synthesis of cell materials and normal growth; they may confer an ecological advantage.

6.6 Extended response question

The activity which follows presents an extended response question similar to the style that you will encounter in the examination.

You should have a good understanding of the patterns of growth shown by microbes before attempting the question.

You should give your completed answer to your teacher or tutor for marking, or try to mark it yourself using the suggested marking scheme.

Extended response question: The patterns of growth shown by microbes

Give a brief description of the patterns of growth shown by microbes. *(6 marks)*

6.7 Extension materials

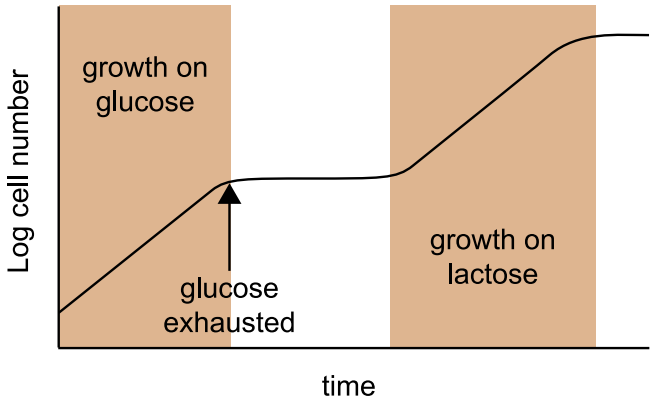
The material in this section is not examinable. It includes information which will widen your appreciation of this section of work.

Extension materials: Diauxic growth and catabolite repression

The mechanism of diauxic growth can be conveniently explained using the growth of *Escherichia coli* (*E. coli*) as an example. In a culture medium there may only be one source of sugar, such as glucose, for bacterial metabolism. However, when there is more than one sugar available, which sugar does the bacterium metabolise first or are they metabolised together? These are the questions that were asked in the 1940s by the distinguished scientist Jacques Monod.

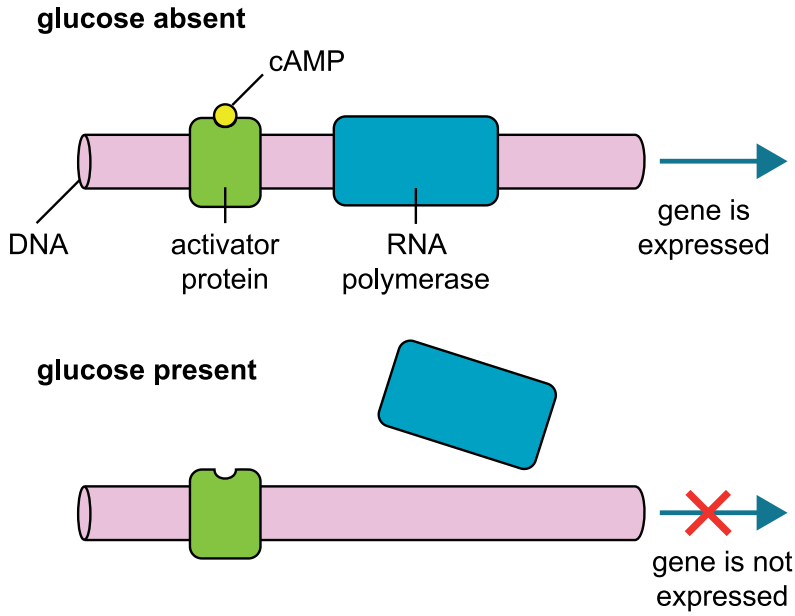
The answer is that bacteria use sophisticated mechanisms to use all of the available sugar efficiently. In a culture medium containing both glucose and lactose, for example, the glucose is always metabolised first. This is because it is metabolised much more efficiently than lactose. After the available glucose has been used up, the lactose is metabolised. This results in a two-step growth curve and this form of growth is called diauxic growth.

How does this happen? Glucose prevents growth on lactose by acting as a repressor in the synthesis of the enzymes of the lactose operon. This is called catabolite repression. It is known that glucose represses the synthesis of a large number of enzymes in many pathways and so it is considered to be a global repressor.



Diauxic growth by E. coli when grown on a mixture of glucose and lactose

The main features of glucose repression involve a reduction in the level of cyclic AMP (cAMP), and the inhibition of an enzyme (adenylate cyclase) involved in cAMP synthesis. cAMP forms a complex with an activator protein (called CAP, for cAMP Activated Protein) that binds to the promoter of glucose-repressible genes. When glucose is absent, cAMP levels are high, cAMP is bound to CAP, and there is no glucose repression of gene expression. When glucose is present, cAMP levels are low, CAP is inactive, and RNA polymerase does not readily bind to the promoter of glucose-repressible genes. Consequently, the level of gene expression is very low.



Catabolite repression

Why is catabolite repression important? It ensures that a cell uses the most readily metabolised source of energy in its environment. This will therefore support the most rapid growth of the cell. Since glucose is metabolised faster than lactose in *E. coli*, it is used first. Lactose will be metabolised only when glucose is depleted. This regulatory mechanism has one purpose and that is to allow bacteria to reproduce at their maximum rate in any environment.

6.8 End of topic test

End of Topic 6 test Go online

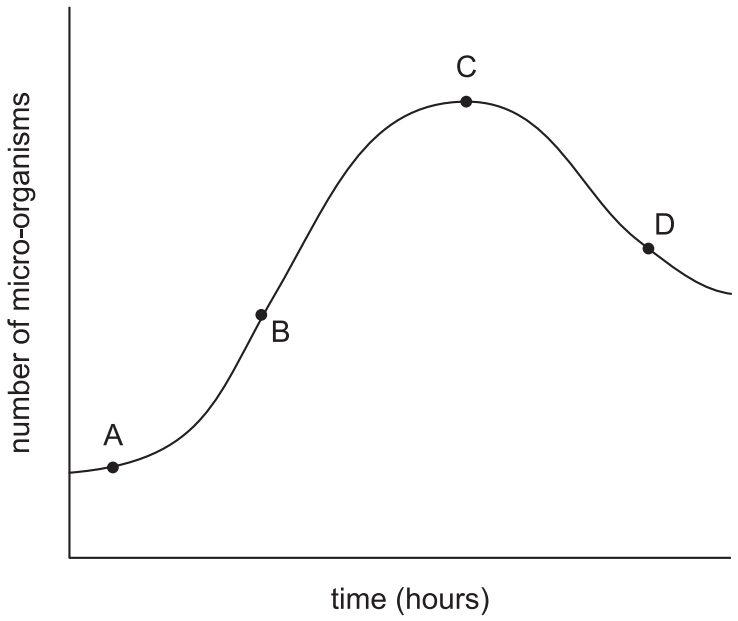
Q5: Living organisms are classified under three domains. Name these domains.

. . . . . .

Q6: List two environmental conditions that need to be controlled for microbes to grow successfully.

. . . . . .

Q7: The following graph shows a typical growth pattern for micro-organisms in culture.



At which lettered stage does the death rate exceed the rate of cell division?

. . . . . .

Q8: Arrange the processes in a growth curve into the correct order.

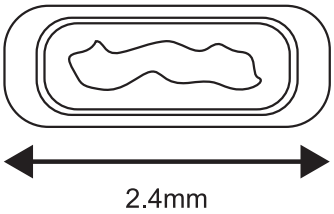
1. Lag phase -
2. Log phase -
3. Stationary phase -
4. Death phase -

Processes:

- decline of population due to exhaustion of nutrients and build-up of toxins;
- organisms acclimatising to their environment;
- organisms growing exponentially;
- organisms likely to be producing secondary metabolites.

. . . . . .

Q9: The following diagram shows a bacterial cell that has been magnified 800 times.

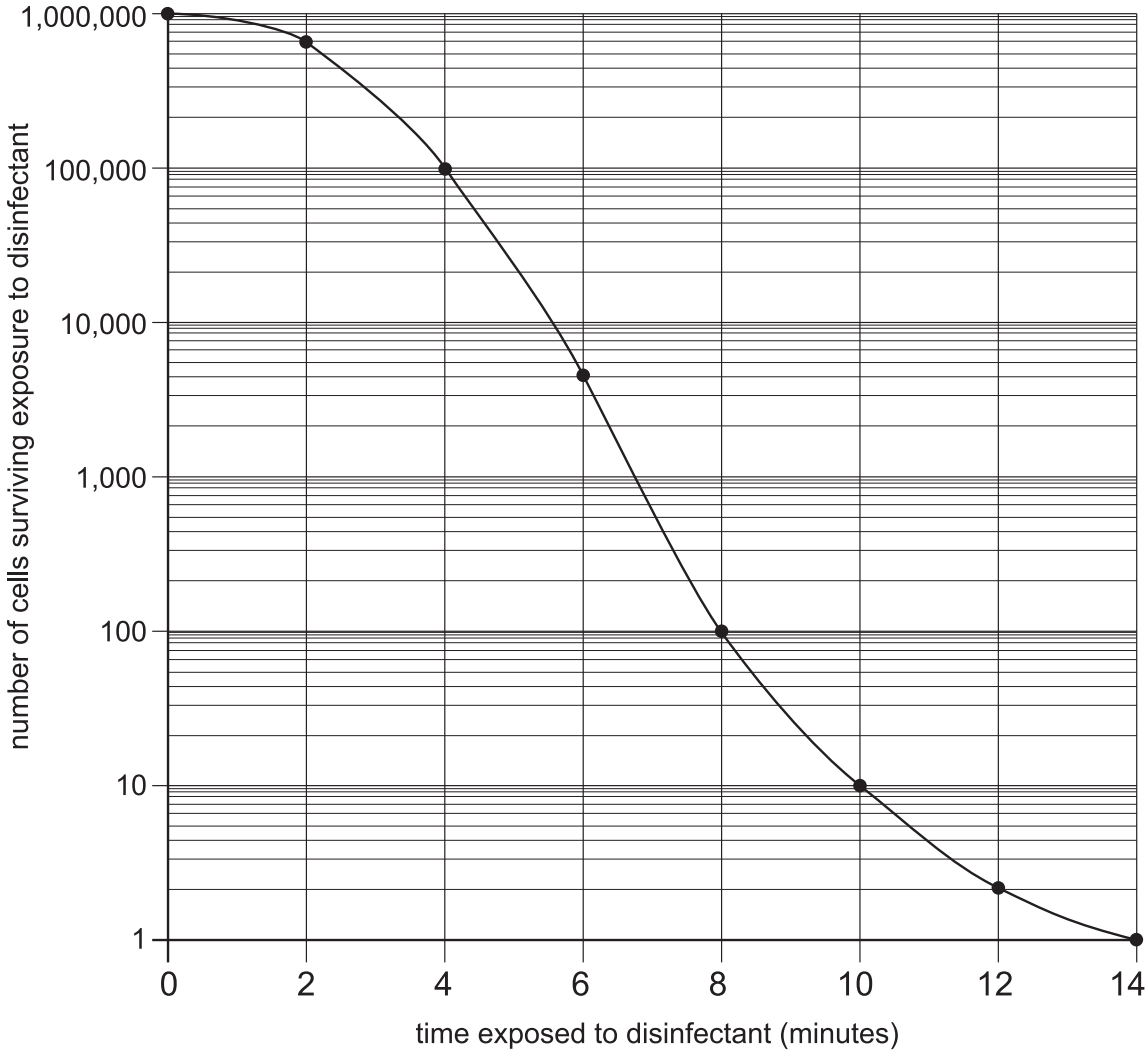


What is the actual length of the cell in μm?

a) 0.003
b) 0.03
c) 0.3
d) 3.0

. . . . . .

Q10: Bacterial cells were exposed to disinfectant for increasing lengths of time to determine the number of live cells left after treatment. The following graph shows the number of bacterial cells which survived.



How many cells survive after 6 minutes?

a) 1300
b) 4000
c) 5000
d) 5500

Topic 7

Genetic control of metabolism

Contents

7.1 Improving wild strains of microorganisms 89
7.2 Mutagenesis 89
7.3 Selective breeding 90
7.4 Recombinant DNA 90
7.5 Recombinant DNA technology 91
7.6 Bovine somatotrophin (BST) 94
7.7 Learning points 96
7.8 Extension materials 97
7.9 End of topic test 98

Prerequisites

You should already know that:

- a mutation is a random change to genetic material;
- mutations may be neutral, or confer an advantage or a disadvantage;
- mutations are spontaneous and are the only source of new alleles;
- environmental factors, such as radiation and chemicals, can increase the rate of mutation.

Learning objective

By the end of this topic, you should be able to:

- explain that wild strains of micro-organisms can be improved by mutagenesis, or recombinant DNA technology;
- explain that mutations can be induced by radiation or chemicals;
- explain that plant and animal genes can be transferred to microbes, causing them to express the donor gene products (proteins);
- know that yields can be increased by gene manipulation;
- know that safety mechanisms can also be introduced, such as introducing genes which prevent the growth of genetically modified organisms outside the laboratory environment;
- explain that extra chromosomal DNA, such as plasmids, can be transferred to microorganisms which contain a variety of genes with specific functions;
- describe the roles of restriction endonuclease and ligase enzymes;
- know that some plant and animal proteins may be produced more successfully in a recombinant yeast cell, rather than a recombinant bacterial cell.

7.1 Improving wild strains of microorganisms

When working with microorganisms in a lab, it may be necessary to improve the strain you are using, for example by enabling it to produce a desired product in large quantities. This can be achieved by one of two different methods:

- mutagenesis;
- recombinant DNA.

7.2 Mutagenesis

Mutations can, on very rare occasions, give rise to improved forms of any organism. The same is true for microorganisms. The major difference is that the generation time for many microbes is very short when compared to humans, for example. This means that, while mutation rates may be no greater, in a relatively short period of time in human terms, large numbers of mutants may arise within a population of microorganisms. When coupled to the vast numbers in which they are found, microbe mutants can be induced and isolated quite easily.

Mutation rates in humans and bacteria

Normal mutation rate is given as approximately one in one hundred million. If the population of the USA is roughly 310 million, this would give rise to only three people having a mutation for a particular trait. Assuming a world population of seven thousand million (7 billion) and the same rate of mutation, this would suggest only 70 people in the world would exhibit this mutation. However, within the human gut, any one millilitre may contain 10 billion bacteria. This greatly increases the number of mutations for any given characteristic simply because the numbers of bacteria are so much greater.

Mutations can occur naturally and are mostly harmful to an organism. Those which are beneficial are very rare and, even more rarely, they can revert to **wild-type**, i.e. normal. Mutation rates are greatly increased by exposing the DNA to certain mutagenic agents. These have the effect of altering the DNA sequence (the genome) and, as a result, there may be a change in the proteins produced.

The process of creating mutants is known as mutagenesis. By exposing microorganisms to mutagens, for example UV light, the rate of mutagenesis can be increased. Scientists can expose microorganisms to various mutagens and then screen them for a specific desired characteristic, for example the ability to grow on cheap nutrients. These mutants can then be cultured and used in the lab.

Although this seems a relatively straightforward way to produce a desirable characteristic in a microorganism, mutant strains tend to be genetically unstable. This means they are likely to revert back to their wild type (normal) phenotype.

7.3 Selective breeding

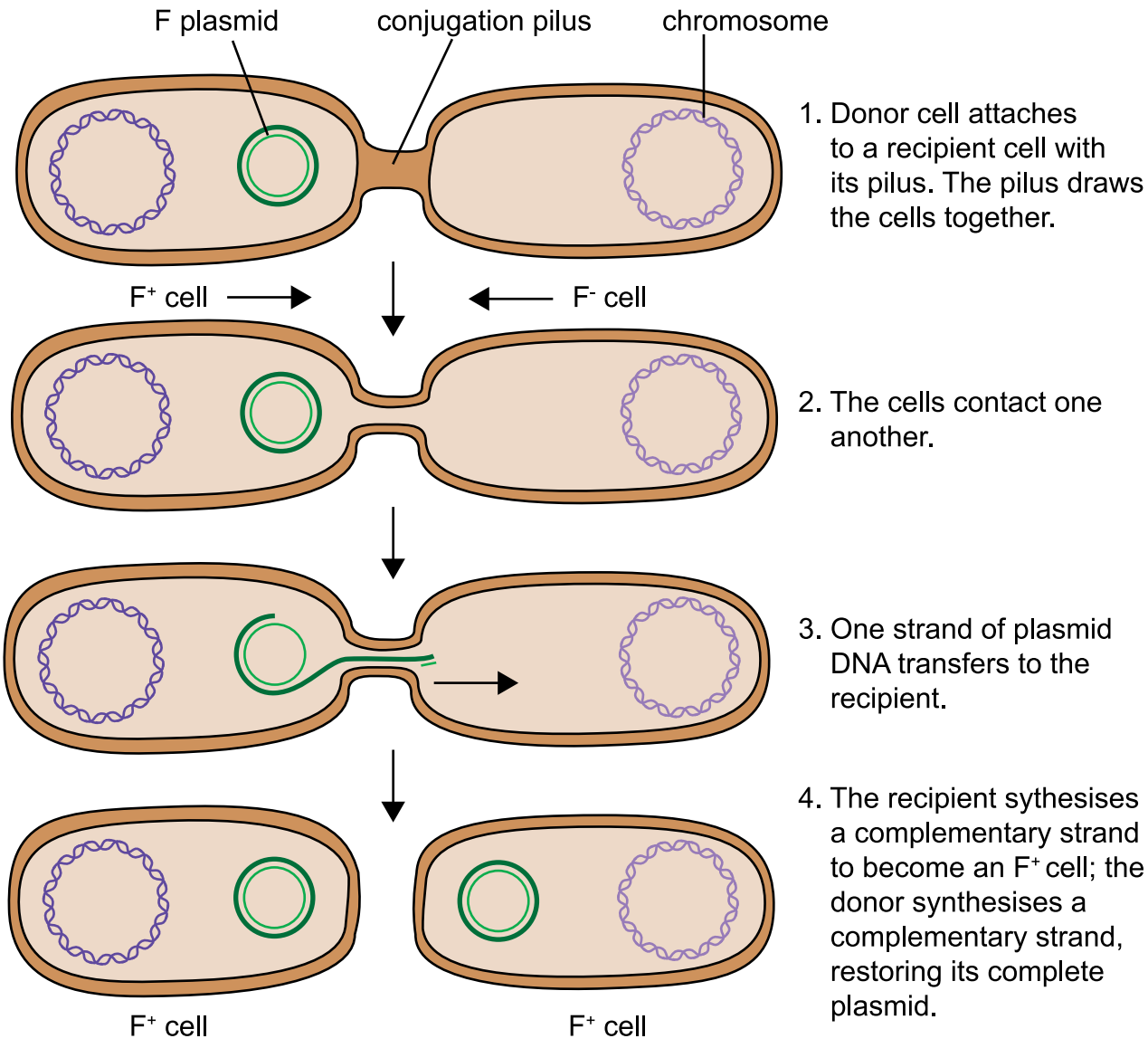
Some microorganisms (such as fungi) are capable of both asexual and sexual reproduction. Asexual reproduction produces offspring that are identical to the parent which produced them. Sexual reproduction produces offspring which have different characteristics from their parents, in other words they show variation.

Selective breeding involves sexual reproduction between two strains of microorganism, each with a desirable characteristic. The aim of selective breeding is to produce offspring which have a new genotype and that show both desirable characteristics. Selective breeding can take a long time to achieve the desired result as it is also likely that undesirable characteristics will combine and show up in the phenotype of the offspring.

7.4 Recombinant DNA

For the greater part of their existence, bacteria reproduce asexually by binary fission, resulting in clones. However, there are occasions when genetic material is exchanged horizontally, i.e. between members of the same generation. This can result in new strains of bacteria which show variation from the original strain.

Some bacteria are capable of transferring **plasmids** or pieces of chromosomal DNA between one another, whilst others are capable of taking up DNA from their environment and incorporating it into their genome. Scientists can take advantage of this to attempt to produce new strains of bacteria with desirable characteristics.



Transfer of a plasmid between bacterial cells

7.5 Recombinant DNA technology

Recombinant DNA technology refers to the ability of scientists to manipulate the genome of an organism by introducing new gene sequences. New gene sequences can be transferred between individuals of the same species or from one species to another. Using recombinant DNA technology, it is possible to artificially improve a strain of microorganism, for example allowing scientists to increase the yield of a desired product by introducing genes that remove inhibitory controls or amplify specific metabolic steps in a pathway. As a safety mechanism, genes are often introduced that prevent the survival of the microorganism in an external environment.

In order to introduce a gene into an organism, scientists must use a vector. A vector is a DNA molecule used to carry foreign genetic information into another cell and both **plasmids** and artificial chromosomes are used as vectors during recombinant DNA technology. Recombinant plasmids and artificial chromosomes share many features, but plasmids are only capable of carrying relatively small quantities of DNA whereas artificial chromosomes can carry longer DNA sequences.

Recombinant plasmids and artificial chromosomes contain the following features which allow them to operate effectively:

- selectable markers - such as antibiotic resistance genes protect the micro-organism from a selective agent (antibiotic) that would normally kill it or prevent it growing. Selectable marker genes present in the vector ensure that only micro-organisms that have taken up the vector grow in the presence of the selective agent (antibiotic);
- restriction sites - contain target sequences of DNA where specific restriction endonucleases cut;
- origin of replication - allows self-replication of the plasmid/artificial chromosome;
- regulatory sequences - control expression of the inserted gene as well as other genes found on the vector.

The manipulation of DNA involves the use of two types of enzyme: **restriction endonuclease** and **ligase**.

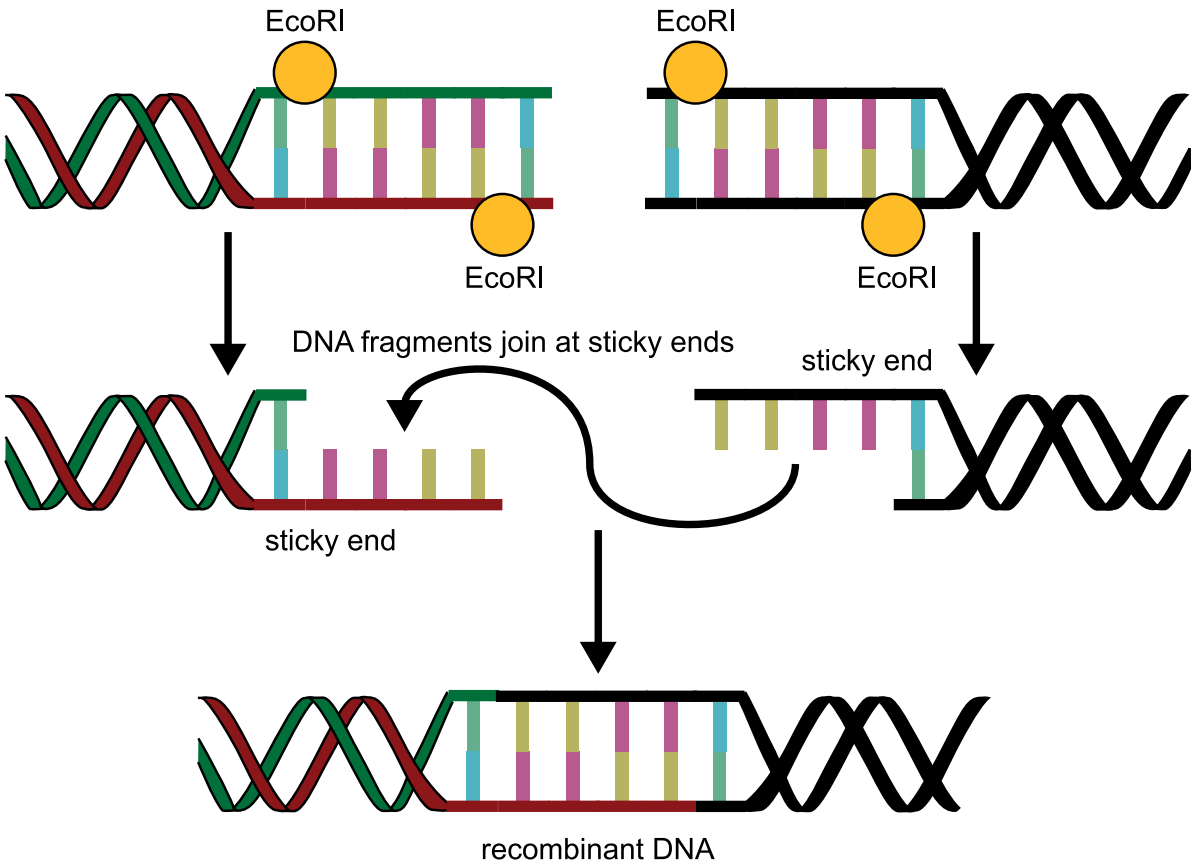
Restriction endonucleases are enzymes that cut specific target sequences of DNA. Typically, there is a sequence of four to six nucleotide bases which the enzyme will recognise and cut. There will be complementary sequences opposite on the other strand of DNA, which is also cut by the enzyme.

For example, the enzyme EcoR1 binds at the sequence *GAATTC* and breaks the chain between the G and first A, leaving a small section of single-stranded DNA *AATTC* - a so called 'sticky end'. It is called this because the same happens on the opposite strand of the same double-stranded DNA molecule.

These ends could re-join by base-pairing with each other or with similar sticky ends from another source, for example a plasmid as shown below. If base-pairing occurs between two sticky ends of DNA, the backbone can be re-joined by an enzyme called ligase.

Recombinant DNA: Steps Go online

The following shows the steps involved in recombinant DNA.



It can be difficult to express animal or plant (eukaryotic) genes successfully in bacterial cells. Bacterial proteins do not undergo the same post-transcriptional and post-translational modifications which eukaryotic proteins do. Expression of plant or animal genes in bacteria may result in polypeptides that are folded incorrectly and are therefore non-functional. In cases where this happens, the eukaryotic proteins may be produced more successfully in a recombinant yeast cell.

7.6 Bovine somatotrophin (BST)

Increasingly, farm animals are being treated with products made by recombinant DNA technology. These include vaccines, antibodies and growth hormones. In the USA, dairy cattle are routinely injected with a growth hormone called bovine somatotrophin, or BST, which increases milk production by about 10%. BST also improves weight gain in beef cattle.

To produce pure BST from the cloned gene, the following steps were taken:

- a **restriction endonuclease** enzyme was used to isolate the BST gene on a small fragment of DNA;
- **plasmid** DNA was isolated from *E. coli*;
- the same restriction endonuclease enzyme that was used to cut out the BST gene was also used to cut the plasmid DNA;
- the BST gene was inserted, or ligated, into the bacterial plasmid;
- the recombinant plasmid was used to transform *E. coli* cells.

The production of large quantities of BST requires the following:

- propagation of the transformed *E. coli* expressing the BST gene in large vats containing nutrient broth;
- purification of the BST secreted by the *E. coli* into the nutrient broth.

Some countries (including the UK) continue to ban the import of milk produced by cows injected with BST. The concern is that the milk could contain minute quantities of BST and may have potential side-effects.

Bovine somatotrophin: Questions Go online

Q1: Bacterial plasmids are described as vectors. What is meant by the term 'vector'?

. . . . . .

Q2: What is a 'recombinant plasmid'?

. . . . . .

Q3: _____ are enzymes that cut up DNA into fragments.

a) Ligases
b) Restriction endonuclease enzymes

. . . . . .

Q4: Bacterial plasmids are cut open using _____.

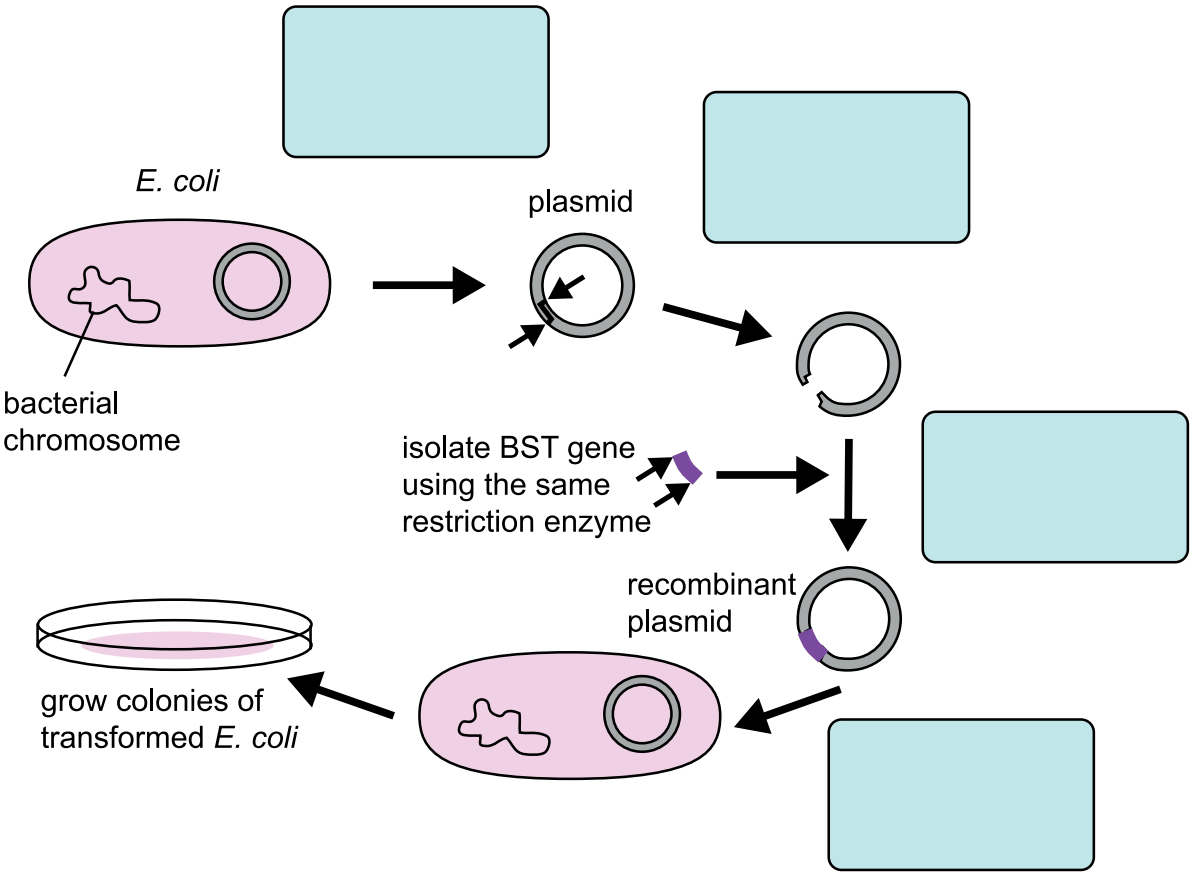
a) Ligases
b) Restriction endonuclease enzymes

. . . . . .

Q5: _____ are enzymes that are used to seal DNA fragments into plasmids.

a) Ligases
b) Restriction endonuclease enzymes

Q6: Complete the diagram that shows the cloning of the bovine somatotrophin gene using the labels provided.



lyse cells and isolate plasmid | digest with restriction enzyme | DNA ligase | transformation

7.7 Learning points

Summary

- Wild strains of micro-organisms can be improved by mutagenesis, or recombinant DNA technology.
- Exposure to UV light and other forms of radiation or mutagenic chemicals results in mutations, some of which may produce an improved strain of micro-organism.
- Recombinant DNA technology involves the use of recombinant plasmids and artificial chromosomes as vectors.
- A vector is a DNA molecule used to carry foreign genetic information into another cell and both plasmids and artificial chromosomes are used as vectors during recombinant DNA technology.
- Artificial chromosomes are preferable to plasmids as vectors when larger fragments of foreign DNA are required to be inserted.
- Restriction endonucleases cut open plasmids and specific genes out of chromosomes, leaving sticky ends.
- Complementary sticky ends are produced when the same restriction endonuclease is used to cut open the plasmid and the gene from the chromosome.
- Ligase seals the gene into the plasmid.
- Recombinant plasmids and artificial chromosomes contain restriction sites, regulatory sequences, an origin of replication and selectable markers.
- Restriction sites contain target sequences of DNA where specific restriction endonucleases cut.
- Regulatory sequences control gene expression and origin of replication allows self-replication of the plasmid/artificial chromosome.
- Selectable markers such as antibiotic resistance genes protect the micro-organism from a selective agent (antibiotic) that would normally kill it or prevent it growing.
- Selectable marker genes present in the vector ensure that only micro-organisms that have taken up the vector grow in the presence of the selective agent (antibiotic).
- As a safety mechanism, genes are often introduced that prevent the survival of the micro-organism in an external environment.
- Recombinant yeast cells may be used as plant or animal recombinant DNA expressed in bacteria may result in polypeptides being incorrectly folded.

7.8 Extension materials

The material in this section is not examinable. It includes information which will widen your appreciation of this section of work.

Extension materials: Restriction endonuclease EcoR1

In 1973, Cohen and Boyer demonstrated that frog DNA which coded for ribosomal RNA could be transferred into a bacterium. They constructed a plasmid (pSC101) which contained two specific features. One was a single site for the attachment of the restriction endonuclease EcoR1. The other was a gene conferring resistance to the antibiotic tetracycline.

By incubating the frog DNA with EcoR1, it was cut at specific points into fragments with sticky ends. Similarly, the plasmids were digested by the EcoR1, revealing the complementary sticky ends. The frog DNA fragments were mixed with the opened plasmids and recombination took place.

Various fragments of frog DNA were incorporated into the plasmids and some would include the ribosomal RNA gene. The enzyme ligase was introduced to join the phosphodiester bonds and link the DNA into the plasmids, which were in turn reintroduced to *E.coli* bacteria by transformation. These *E.coli* were sensitive to tetracycline and, as a result, only those bacteria carrying the plasmid with gene for resistance would survive when grown in a medium containing the antibiotic. Of the surviving colonies, those containing rRNA gene were selected.

7.9 End of topic test

End of Topic 7 test Go online

Q7: Most mutations are beneficial. True or false?

. . . . . .

Q8: Mutagenic agents can be roughly placed into two groups. Name them.

. . . . . .

Q9: What name is given to an agent which can carry DNA into a cell?

. . . . . .

Q10: In recombinant DNA technology, name the enzyme which cuts DNA leaving sticky ends.

. . . . . .

Q11: Name the enzyme which joins sticky ends of DNA together.

. . . . . .

Q12: Describe one feature which must be present on a plasmid to allow it to act as an effective vector.

. . . . . .

Q13: If an animal protein is expressed in a bacterial cell, what problems could arise with its production?

. . . . . .

Q14: If an animal protein is not expressed successfully in a bacterial cell, what type of cell could it be expressed in more successfully?

Topic 8

End of unit test

End of Unit 2 test Go online

Metabolic pathways

Q1: Metabolic pathways are regulated by _____.

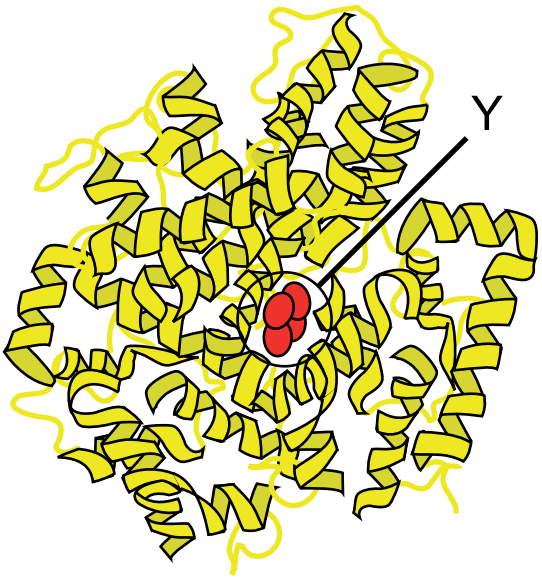
. . . . . .

Q2: Reactions which build up large molecules from small molecules are known as _____.

. . . . . .

Q3: Reactions which break down large molecules into smaller molecules are known as _____.

The enzyme shown in the following diagram controls one step of a metabolic pathway.



Q4: In the diagram, what is represented by the letter Y?

. . . . . .

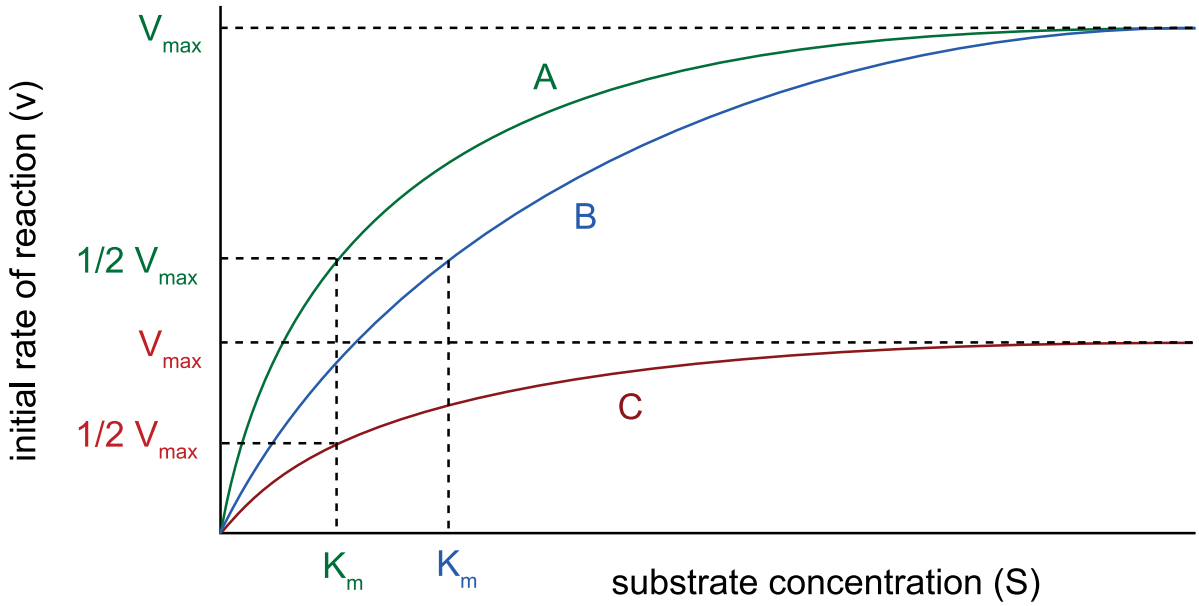
Q5: Explain the term 'induced fit'.

. . . . . .

Q6: Explain the difference between a competitive inhibitor and non-competitive inhibitor.

. . . . . .

Q7: The following diagram shows the effects of enzyme inhibition on reaction rate. The curve labelled A shows the rate of reaction in the absence of an inhibitor.



Which line shows competitive inhibition?

. . . . . .

Q8: Which line shows non-competitive inhibition?

Cellular respiration

Q9: Name the enzyme which produces ATP.

. . . . . .

Q10: Name the enzyme which removes hydrogen ions and electrons from substrates.

. . . . . .

Q11: Name the first stage of respiration.

. . . . . .

Q12: Where is the first stage of respiration located?

. . . . . .

Q13: Name the coenzyme which accepts hydrogen ions and electrons.

. . . . . .

Q14: During the citric acid cycle, _____ combines with an acetyl group to form _____; this is gradually turned back into _____ by a series of _____-controlled reactions.

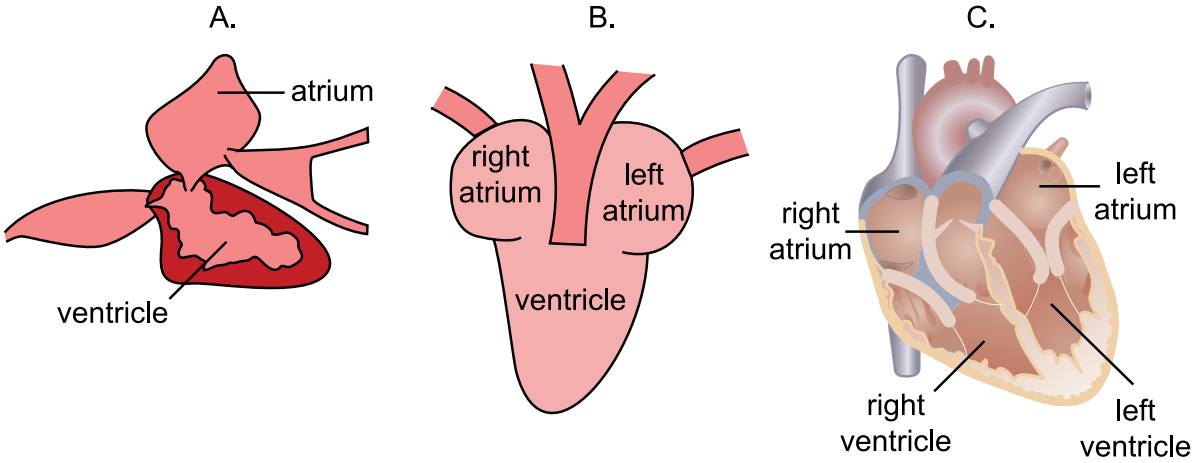
Metabolic rate

Q15: Which of the following measurements do not allow metabolic rate to be calculated?

a) Calorie intake
b) Carbon dioxide production
c) Heat generation
d) Oxygen consumption

. . . . . .

Q16: The following diagram shows the structure of three different types of heart.



What is the correct order of organisms to match with the hearts in the order as labelled A-C?

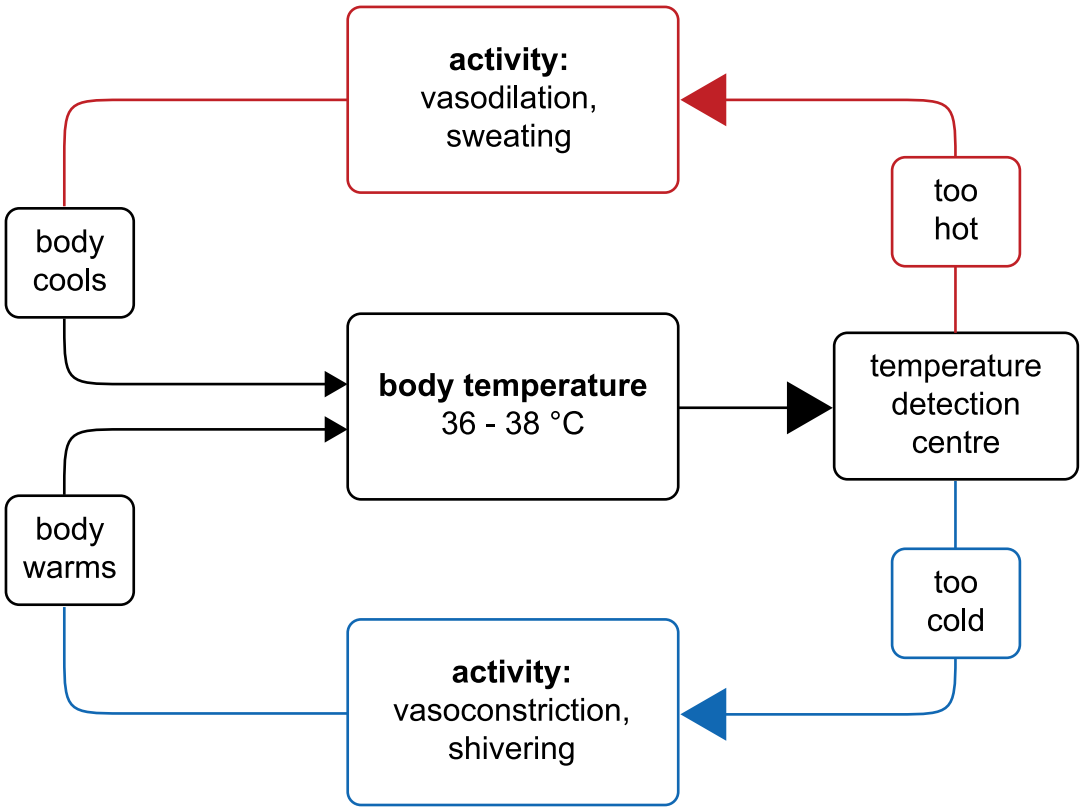
a) Amphibian, fish, mammal
b) Amphibian, mammal, fish
c) Fish, amphibian, mammal
d) Mammal, fish, amphibian

Metabolism in conformers and regulators

Q17: Name an external abiotic factor which can affect the ability of an organism to maintain its metabolic rate.

. . . . . .

Q18: The following diagram shows the control of body temperature in a mammal.



What is the location of the temperature detection centre?

. . . . . .

Q19: What name is given to the corrective mechanism shown in the diagram?

. . . . . .

Q20: Why is it important for mammals to control their body temperature?

. . . . . .

Q21: A _____ has an internal environment which is dependent on the external environment. They have _____ metabolic costs and inhabit a _____ range of ecological niches.

. . . . . .

Q22: A _____ uses energy to control its internal environment. They have _____ metabolic costs and inhabit a _____ range of ecological niches.

Maintaining metabolism during environmental change

Q23: Some animals avoid extreme or hostile environments by _____. *(Choose from 'hibernation' or 'migration'.)*

. . . . . .

Q24: Successful migration depends on a combination of _____ and _____ behaviours.

. . . . . .

Q25: What name is given to dormancy which occurs after the onset of adverse environmental conditions?

. . . . . .

Q26: What name is given to dormancy which occurs before the onset of adverse environmental conditions?

Environmental control of metabolism

Q27: Name the three domains of life.

. . . . . .

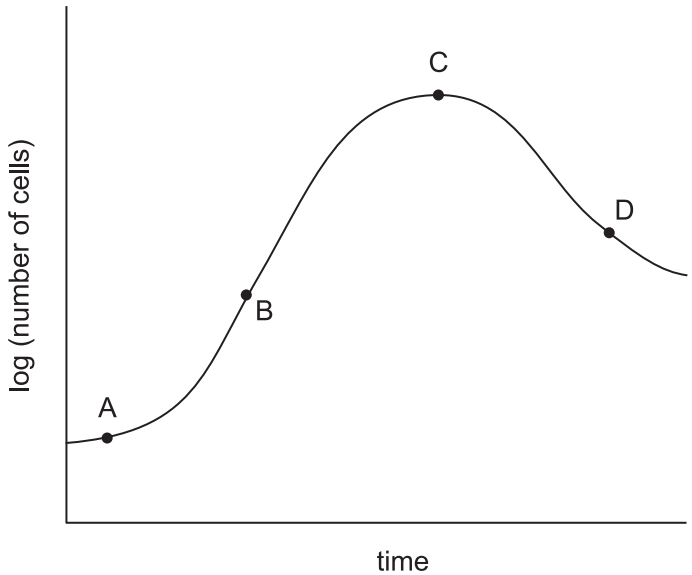
Q28: As a result of their adaptability, microorganisms are found in a _____ range of ecological niches.

. . . . . .

Q29: List two environmental conditions that need to be controlled for microbes to grow successfully.

. . . . . .

Q30: The following diagram shows a typical growth curve.



Name each of the stages shown in the graph.

Genetic control of metabolism

Q31: Name one way by which wild strains of microorganisms can be improved.

. . . . . .

Q32: Some bacteria can transfer plasmids or pieces of chromosomal DNA to each other, resulting in the production of new strains of bacteria. What is this type of transfer known as?

. . . . . .

Q33: In recombinant DNA technology, name the enzyme which cuts DNA leaving sticky ends.

. . . . . .

Q34: Name the enzyme which joins sticky ends of DNA together.

. . . . . .

Q35: When working with _____ in a lab, as a safety mechanism, genes are often introduced that prevent the survival of the _____ in an _____ environment.

. . . . . .

Q36: Plant or animal recombinant DNA in bacteria may result in polypeptides that are folded incorrectly. How can these polypeptides be expressed more successfully?

Problem solving

The tables below show the growth of *E. coli* in nutrient broth containing different sugar substrates. Nutrient broth was prepared with one of two different sugars, inoculated with *E. coli*, and the number of viable cells was calculated every hour for 12 hours.

Time (hours)	Viable cell number (millions per cm^3)
0	12
1	12
2	12
3	17
4	44
5	75
6	91
7	92
8	93
9	94
10	94
11	94
12	94

Table 1
Nutrient broth containing glucose

Time (hours)	Viable cell number (millions per cm^3)
0	10
1	10
2	10
3	10
4	10
5	12
6	34
7	60
8	87
9	91
10	92
11	92
12	92

Table 2
Nutrient broth containing lactose

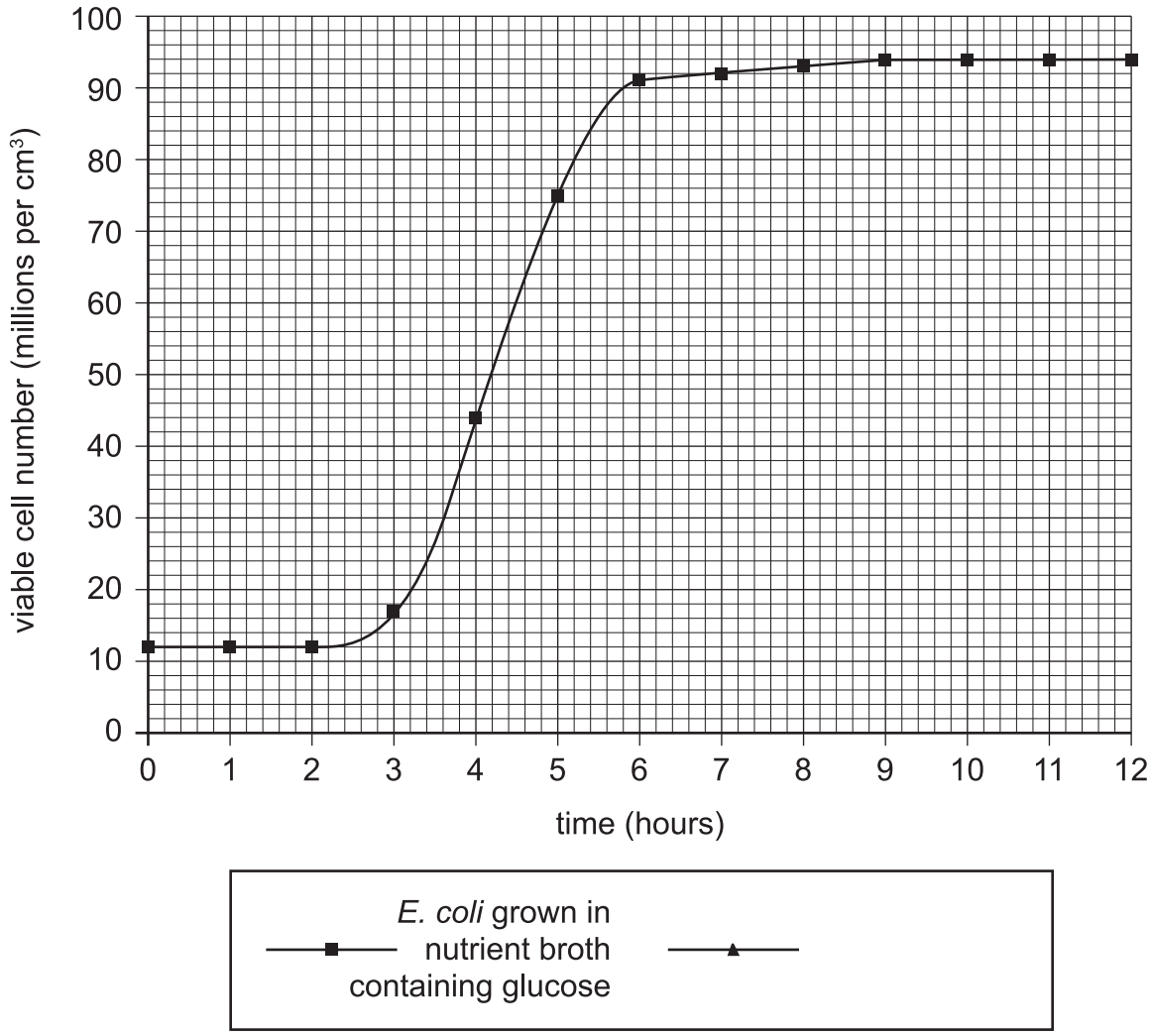
Q37: Give a reason why it is important to use separate syringes when preparing the nutrient broth containing different sugars.

. . . . . .

Q38: Name one factor which must be kept constant when setting up this experiment.

. . . . . .

Q39: Complete the graph and key to show the results for *E. coli* grown in nutrient broth containing lactose.



. . . . . .

Q40: Using viable cell numbers gives more reliable results than using total cell numbers. Explain why.

. . . . . .

Q41: How much longer does it take the culture to reach 60 million viable cells per cm^3 when using lactose as a respiratory substrate rather than glucose?

. . . . . .

Q42: In terms of phases of growth, give two conclusions from the results of this experiment.

Glossary

Activation energy

the minimum energy required by reactants to allow reaction to occur

Active site

the region of an enzyme molecule where the enzyme acts on the substrate

Aestivation

dormancy in response to high temperature or drought

Anabolic

a reaction which requires energy and builds up molecules

Archaea

a group of single-celled microorganisms

ATP synthase

an enzyme which produces ATP

Biological catalysts

catalysts made of protein that are only found in living cells

Calorimeter

a piece of equipment used to measure heat generation from an organism to allow metabolic rate to be calculate

Catabolic

a reaction which releases energy and breaks down molecules

Citric acid cycle

the second stage of respiration, where acetyl CoA and oxaloacetate join to form citrate and a series of reactions which return citrate to oxaloacetate

Competitive inhibition

competitive inhibition of enzyme activity occurs when an inhibitor, resembling the structure of the substrate, binds to the active site of the enzyme and blocks the binding of the substrate

Daily torpor

a period of reduced activity in organisms with high metabolic rates

Dehydrogenase

an enzyme which removes hydrogen ions and electrons from substrates

Dormancy

a condition of biological rest or inactivity characterised by cessation of growth or development and the suspension of many metabolic processes

Effector

cells, muscles or glands which perform responses to stimuli

Electron transport chain

the final stage of respiration where high energy electrons and hydrogen ions are used to synthesise ATP

Feedback inhibition

regulation of enzyme activity where the first enzyme of a metabolic pathway is inhibited by the reversible binding of the final product of the pathway

Fermentation

a type of respiration which takes place in the absence of oxygen

Glycolysis

the first stage of respiration where glucose is broken down into pyruvate

Heterotrophic

an organism which gains energy by consuming other organisms

Hibernation

an inactive state resembling deep sleep in which certain animals living in cold climates pass the winter

Hypothalamus

part of the brain which monitors and regulates temperature

Induced fit model

a model of an enzyme-substrate reaction that causes a conformational change in the active site of the enzyme that allows the substrate to fit perfectly

Ligase

an enzyme which joins fragments of DNA together

Metabolites

the intermediates and products of metabolic reactions that take place in organisms

Migration

a process which avoids metabolic adversity by expending energy to relocate to a more suitable environment

Mitochondria

a structure in the cell responsible for producing energy

NAD

a co-enzyme which easily attaches to hydrogen ions, but releases them when they are required

Negative feedback

homeostasis; the process by which an increase in one factor causes a decrease in another factor, thereby maintaining equilibrium around a set point (norm)

Non-competitive inhibition

a molecule binds to part of the enzyme away from the active site, and causes a conformational change in the active site of the enzyme, thereby inhibiting the binding of the appropriate substrate molecule

Nutrient medium

a mixture of nutrients (including carbon and nitrogen sources) required for growth

Plasmid

a circular, self-replicating DNA molecule that carries only a few genes

Receptor

cells which monitor changes in environment

Respirometer

a piece of equipment used to measure the rate of respiration

Restriction endonuclease

an enzyme that cuts specific target sequences of DNA

Selectively permeable

a property of a membrane which means that substances do not freely pass through it; the membrane allows the passage of certain small molecules, but excludes many other molecules

Vasoconstriction

contraction in diameter of a blood vessel, thus reducing blood flow

Vasodilation

enlargement in diameter of a blood vessel, thus increasing blood flow

Wild-type

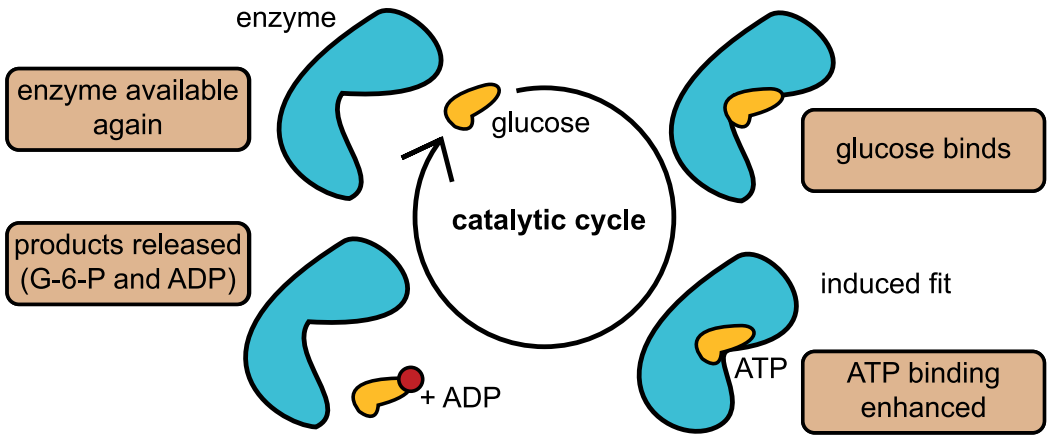
describes the phenotype of the typical form of a species as it occurs in nature

Answers to questions and activities

Topic 1: Metabolic pathways

Enzyme properties: Question (page 8)

Q1:



Competitive inhibition: Questions (page 11)

Q2: The active site is a small area of the enzyme, approximately 20 amino acids in length, where (depending on the reaction catalysed by the enzyme) one or more substrate molecules are bound, and where the reaction occurs.

Q3: Both the competitive inhibitor and the substrate are structurally similar, so that the inhibitor is able to bind to the active site.

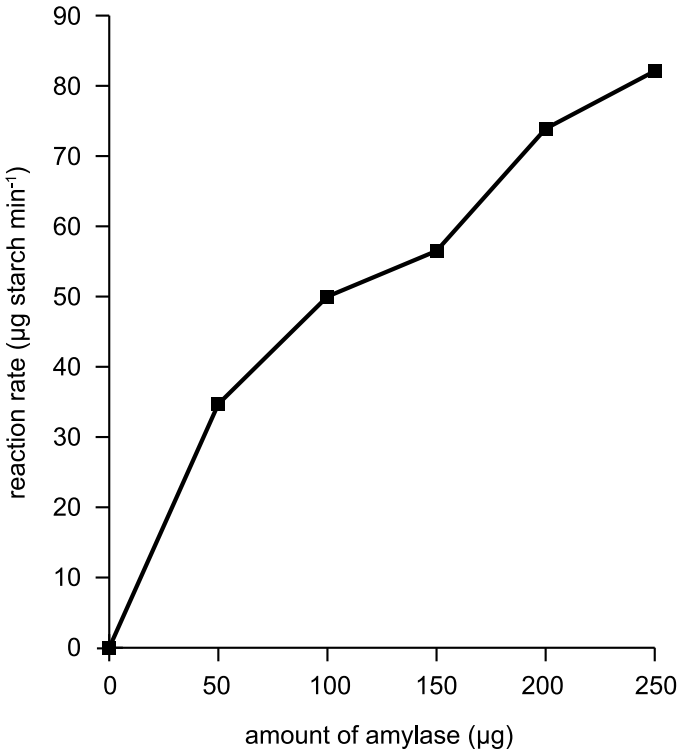
Q4: The addition of more substrate to the experimental reaction increases the chances of the substrate molecule colliding with the enzyme and thereby overcoming competitive inhibition.

Feedback inhibition: Question (page 13)

Q5: It is energetically efficient as it avoids the excessive (and wasteful) production of the intermediates of a pathway.

Answers from page 14.

Q6:



Q7: As the amount of enzyme increases, the rate of reaction also increases.

Q8: The graph indicates that the rate of reaction is not directly proportional to the amount of amylase. If it were, the result would be a straight line.

Q9: These tubes are experimental controls. Tube 6 shows that when there is no enzyme added the reaction does not occur. Tube 7 shows that when no substrate is added the reaction does not occur.

Q10: The reaction rate should continue to increase as the amount of amylase is increased. In practice, the increase in the reaction rate will become less and less as the amount of amylase is increased. This is because there is a finite amount of substrate present and once that has been converted to product (maltose) the enzyme becomes ineffective.

Extended response question: The control of the enzyme activity by inhibition (page 16)

Suggested marking scheme

Each line represents a point worth one mark. The concept may be expressed in other words. Words which are bracketed are not essential. Alternative answers are separated by a solidus (/); if both

such answers are given, only a single mark is allocated. In checking the answer, the number of the point being allocated a mark should be written on the answer paper. A maximum of eight marks can be gained.

1. Competitive inhibition. . .
2. . . . is where an inhibitor competes with the substrate for the active site of an enzyme.
3. Competitive inhibition can be reversed by an increase in the concentration of the substrate.
4. Non-competitive inhibition. . .
5. . . . is where an inhibitor binds to an enzyme away from the active site.
6. The shape of the active site is altered and the activity of the enzyme is reduced.
7. This type of inhibition may or may not be reversible.
8. Feedback inhibition. . .
9. . . . is used in the control of metabolic pathways.
10. The end-product of the pathway inhibits the activity of the first enzyme in the pathway.

End of Topic 1 test (page 16)

Q11: A mutation in gene 4 would result in enzyme E4 not being produced (or not functioning properly). This means that metabolite D would build up.

Q12: All the reactions that take place in an organism.

Q13: Reactions which release energy are said to be **catabolic** and reactions which require energy are described as **anabolic**.

Q14: Enzymes

Q15: They allow molecules to pass across the plasma membrane.

Q16: d) Competitive inhibitor, enzyme, non-competitive inhibitor.

Q17: d) When the substrate binds to the active site, the shape of the active site is changed.

Q18: Enzymes **lower** the activation energy and release products with a **low** affinity for the active site.

Q19: a) Enzyme 1

Q20: a) 0 to 5 minutes from the start of the reaction.

Q21: c) a non-competitive inhibitor

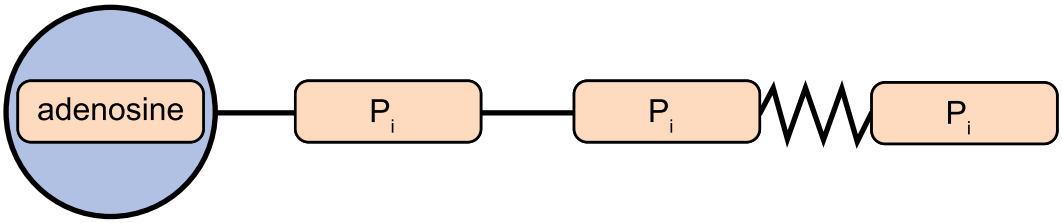
Q22: **Competitive** inhibitors decrease the activity of an enzyme by binding to the active site.

Q23: b) Feedback inhibition

Topic 2: Cellular respiration

The role of ATP: Questions (page 24)

Q1:



Q2: A chemical reaction that uses energy to build up complex molecules from simple molecules.

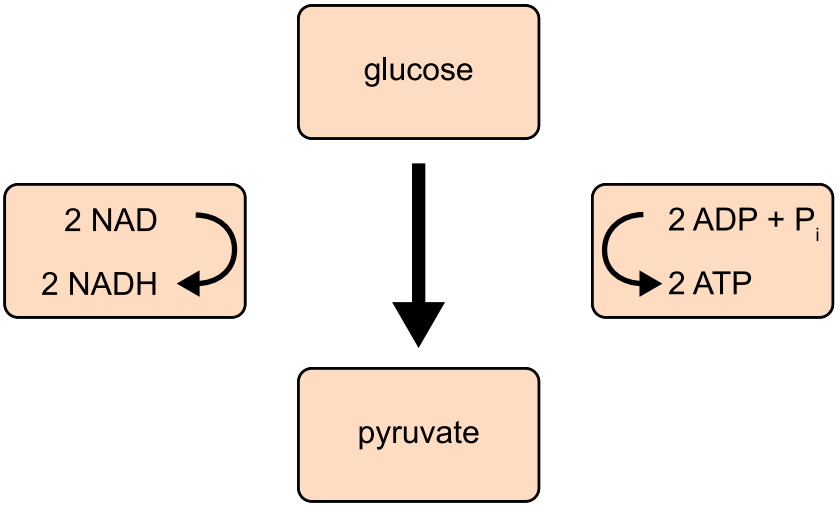
Q3: The synthesis of starch from glucose units / protein synthesis from amino acids.

Q4: An energy-releasing reaction that breaks down complex molecules to form simpler ones.

Q5: The breakdown of glycogen to glucose / the breakdown of glucose to carbon dioxide and water.

Glycolysis: Questions (page 25)

Q6:



Q7: Cytoplasm

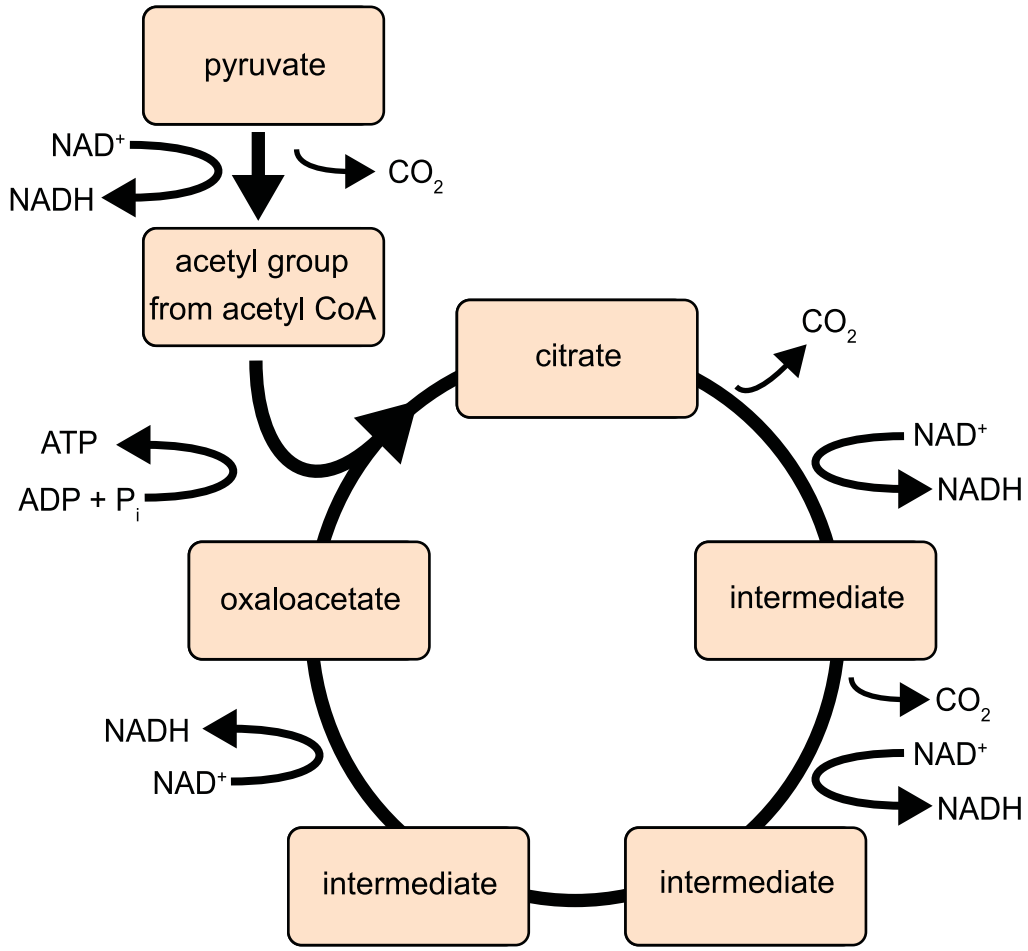
Q8: b) 2

Q9: No

Q10: Pyruvate

Citric acid cycle: Questions (page 27)

Q11:



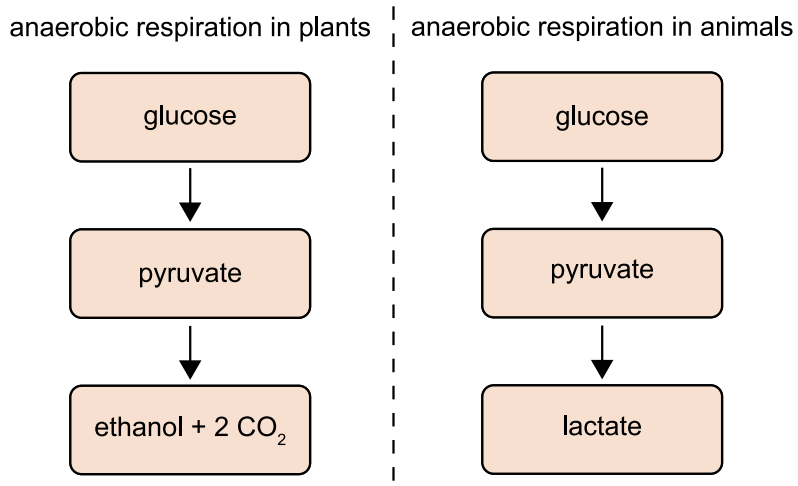
Q12: Citrate

Q13: c) Mitochondrion

Q14: Aerobic

Anaerobic respiration: Questions (page 29)

Q15:



Measuring the rate of respiration (page 30)

Q16: c) 0.3 ml

Q17: d) 0.6

Extended response question: The stages of cellular respiration (page 32)

Suggested marking scheme

Each line represents a point worth one mark. The concept may be expressed in other words. Words which are bracketed are not essential. Alternative answers are separated by a solidus (/); if both such answers are given, only a single mark is allocated. In checking the answer, the number of the point being allocated a mark should be written on the answer paper. A maximum of eight marks can be gained.

A) Glycolysis *(maximum of 4 marks)*:

1. Glycolysis is an anaerobic process / does not require oxygen.
2. It occurs in the cytoplasm.
3. Glucose is split into pyruvate.
4. The hydrogen ions and electrons released during glycolysis bind to the coenzyme NAD to form NADH.
5. Dehydrogenase enzymes remove H ions and electrons, which are passed to the coenzyme NAD.
6. Two molecules of ATP are required to convert glucose to pyruvate; this is an energy investment phase.
7. Four molecules of ATP are produced by glycolysis (a net gain of two ATP molecules).

B) Citric acid cycle *(maximum of 4 marks)*:

i. The citric acid cycle is an aerobic process that occurs in the matrix of the mitochondrion.
ii. Pyruvate is converted to acetyl CoA.
iii. Acetyl group binds to oxaloacetate to form citrate.
iv. Citrate is converted, through a series of enzyme-catalysed reactions, back into oxaloacetate.
v. In the process, both carbon (in the form of carbon dioxide) and hydrogen ions with electrons are released.
vi. Hydrogen ions and electrons become bound to NAD to form NADH.
vii. Dehydrogenase enzymes remove H ions and electrons, which are passed to coenzymes NAD. *(cannot be used again here if used in the glycolysis answer)*

End of Topic 2 test (page 32)

Q18: The production of ATP from ADP and P_i is called **phosphorylation**.

Q19: The breakdown of ATP releases **energy**, some of which is used in the synthesis of complex molecules.

Q20: b) It is a catabolic reaction that does not require oxygen.

Q21: d) Pyruvate

Q22: d) In the cytoplasm.

Q23: The citric acid cycle occurs in the **matrix** of the mitochondrion.

Q24: During the citric acid cycle **oxaloacetate** combines with an acetyl group to form **citrate**, this is gradually turned back into **oxaloacetate** by a series of **enzyme** controlled reactions.

Q25: c) Oxygen

Q26: ATP synthase

Q27: NAD

Topic 3: Metabolic rate

End of Topic 3 test (page 40)

Q1: Respirometer

Q2: a) Heart rate

Q3: 1642.5

Q4: A fish heart has **two** chambers.
An amphibian heart has **three** chambers.

Q5: It improves the efficiency of oxygen delivery / separating oxygenated and deoxygenated blood.

Q6: A mammal has a **double** circulatory system whereas fish have a **single** circulatory system.

Topic 4: Metabolism in conformers and regulators

Regulating body temperature: Question (page 52)

Q1:

Decrease in body temperature	*Increase in body temperature*
inactive sweat glands	active sweat glands
increase in metabolic rate	decrease in metabolic rate
hair erector muscles relaxed	hair erector muscles contracted
vasoconstriction	vasodilation

Q2: Hypothalamus

Q3: d) Shivering and vasoconstriction

Q4: Both sweating and flushing are mechanisms used by the body to cool itself down. When you sweat, the evaporation of the water from your skin cools you down. The skin flushes because the blood vessels have become vasodilated. This increases the amount of heat lost from the body by enabling more blood to flow to the skin surface.

Extended response question: Internal body temperature regulation in mammals (page 54)

Suggested marking scheme

Each line represents a point worth one mark. The concept may be expressed in other words. Words which are bracketed are not essential. Alternative answers are separated by a solidus (/); if both such answers are given, only a single mark is allocated. In checking the answer, the number of the point being allocated a mark should be written on the answer paper. A maximum of seven marks can be gained.

1. The hypothalamus is the temperature monitoring centre.
2. Information is communicated by electrical impulses through nerves to the effectors.
3. Effectors bring about corrective responses to return temperature to normal.
4. Vasodilation - increased blood flow to the skin increases heat loss. OR Vasoconstriction - decreased blood flow to skin decreases heat loss.
5. Increased temperature / body too hot leads to (increase in) sweat production OR converse.
6. Body heat used to evaporate water in the sweat, cooling the skin.
7. Decrease in temperature causes hair erector muscles to raise / erect hair traps (warm) air OR forms insulating layer.
8. Decrease in temperature causes muscle contraction / shivering which generates heat/raises body temperature.
9. Temperature regulation involves / is an example of negative feedback.

10. When body temperature increases, metabolic rate is decreased so less heat produced. OR When body temperature decreases, metabolic rate is increased so more heat produced.

End of Topic 4 test (page 56)

Q5: a) Light intensity

Q6: Enzyme activity works around an optimum range, above it becomes denatured, below it is inactive.

Q7: The internal environment of **conformers** is dependent upon the external environment.

Regulators control their internal environment.

Q8: Advantage: low metabolic requirements.

Disadvantage: narrow niche range.

Q9: Hypothalamus

Q10: Vasoconstriction

Q11: a) Decreased metabolic rate, and d) Vasodilation

Q12: Nerves *or* electrical impulses

Topic 5: Maintaining metabolism

Dormancy: Questions (page 61)

Q1:

Term	*Definition*
Hibernation	Period of long-term inactivity in animals
Aestivation	Dormancy in response to hot, dry conditions
Daily torpor	Period of short-term inactivity in animals

End of Topic 5 test (page 64)

Q2: b) Consequential dormancy

Q3: a) aestivation

Q4: c) hibernation

Q5: a) consequential dormancy.

Q6: c) both predictive and consequential dormancy.

Q7: 95

Q8: Successful migration depends on a combination of **innate** and **learned** behaviours.

Q9: *Any from:*

- capture and release;
- direct observation;
- radio tracking;
- tagging.

Topic 6: Environmental control of metabolism

Bacterial culture growth phases: Questions (page 78)

Q1: b) Q

Q2: c) R

Q3: a) P

Q4: d) S

Extended response question: The patterns of growth shown by microbes (page 81)

Suggested marking scheme

Each line represents a point worth one mark. The concept may be expressed in other words. Words which are bracketed are not essential. Alternative answers are separated by a solidus (/); if both such answers are given, only a single mark is allocated. In checking the answer, the number of the point being allocated a mark should be written on the answer paper. A maximum of six marks can be gained.

1. Growth of microorganisms can be followed on a growth curve.
2. Growth phases are lag, log, stationary and death.
3. During the lag phase microorganisms adjust to the conditions of the culture. . .
4. . . . by producing enzymes that metabolise the available substrates.
5. During the log phase the rate of growth is at its highest.
6. During the stationary phase the culture medium becomes depleted. . .
7. . . . and secondary metabolites are produced.
8. During the death phase a lack of substrate and the toxic accumulation of metabolites cause death of cells.
9. Primary metabolites are produced in the early phases.
10. Secondary metabolites are produced later.

End of Topic 6 test (page 83)

Q5: Archaea, bacteria and eukaryotes.

Q6: *Any two from:*

- gaseous environment;
- light;
- ph;
- temperature.

Q7: D

Q8:

1. Lag phase - organisms acclimatising to their environment.
2. Log phase - organisms growing exponentially.
3. Stationary phase - organisms likely to be producing secondary metabolites.
4. Death phase - decline of population due to exhaustion of nutrients and build-up of toxins.

Q9: d) 3.0

Q10: b) 4000

Topic 7: Genetic control of metabolism

Bovine somatotrophin: Questions (page 94)

Q1: Cloning vectors are DNA molecules that can transfer foreign DNA into a bacterial cell and replicate there.

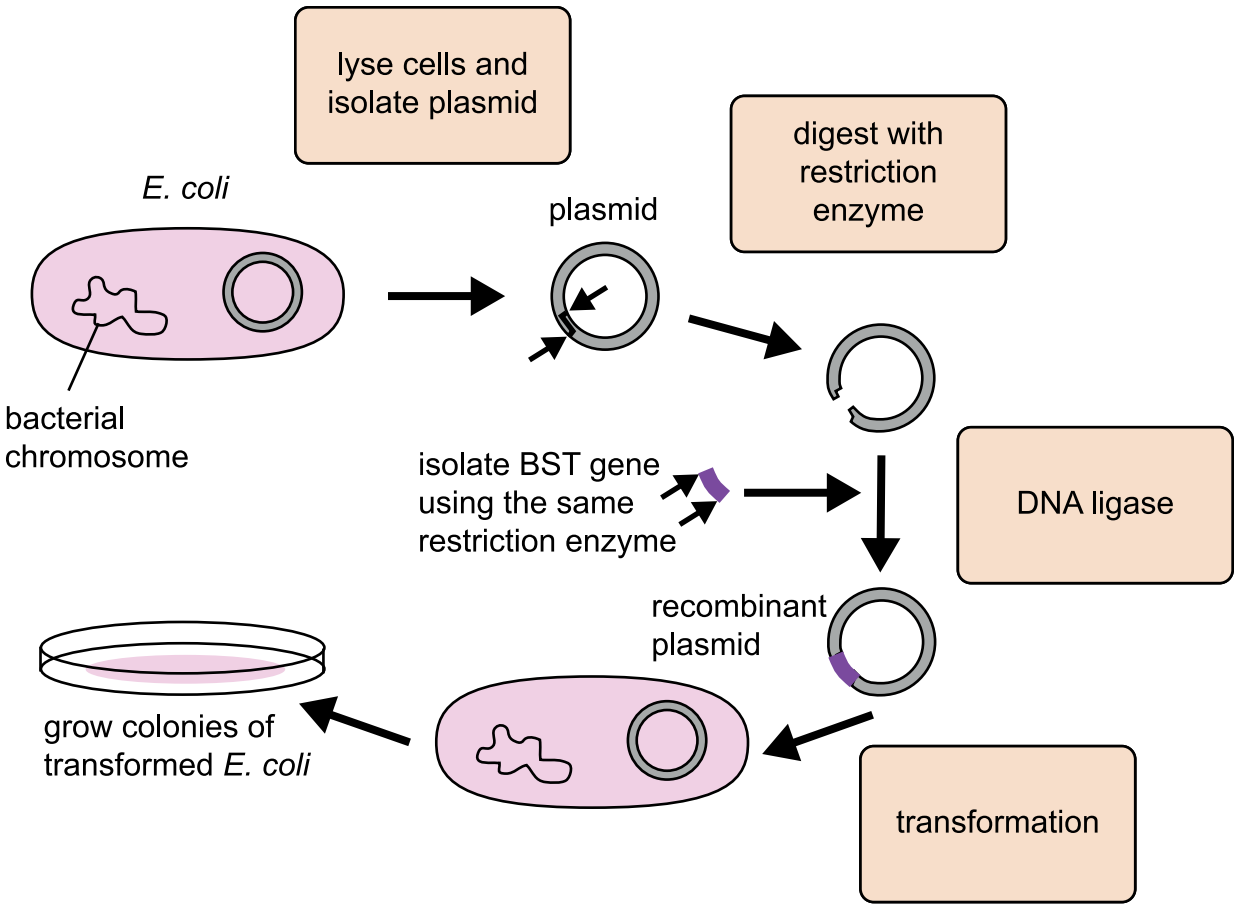
Q2: A plasmid containing foreign DNA.

Q3: b) Restriction endonuclease enzymes

Q4: b) Restriction endonuclease enzymes

Q5: a) Ligases

Q6:



End of Topic 7 test (page 98)

Q7: False

Q8: Chemical and radiation.

Q9: Vector

Q10: Restriction endonuclease

Q11: Ligase

Q12: *Any one from*:

- marker genes;
- restriction site;
- genes for self-replication;
- regulatory sequences.

Q13: It may be folded incorrectly.

Q14: Recombinant yeast cell.

Topic 8: End of unit test

End of Unit 2 test (page 100)

Q1: enzymes

Q2: anabolic

Q3: catabolic

Q4: Substrate

Q5: When a substrate binds the active site on the enzyme changes shape.

Q6: Competitive inhibitors bind at the active site, non-competitive inhibitors bind away from the active site.

Q7: B

Q8: C

Q9: ATP synthase

Q10: Dehydrogenase

Q11: Glycolysis

Q12: Cytoplasm

Q13: NAD

Q14: During the citric acid cycle, **oxaloacetate** combines with an acetyl group to form **citrate**; this is gradually turned back into **oxaloacetate** by a series of **enzyme**-controlled reactions.

Q15: a) Calorie intake

Q16: c) Fish, amphibian, mammal

Q17: Salinity / pH / temperature

Q18: Hypothalamus

Q19: Negative feedback

Q20: To maintain optimum temperature for enzymes.

Q21: A **conformer** has an internal environment which is dependent on the external environment. They have **low** metabolic costs and inhabit a **narrow / small** range of ecological niches.

Q22: A **regulator** uses energy to control its internal environment. They have **high** metabolic costs and inhabit a **wide / large** range of ecological niches.

Q23: Some animals avoid extreme or hostile environments by **migration**.

Q24: Successful migration depends on a combination of **innate** and **learned** behaviours.

Q25: Consequential

Q26: Predictive

Q27: Archaea, bacteria, eukaryotes

Q28: As a result of their adaptability, microorganisms are found in a **wide / large** range of ecological niches.

Q29: *Any two from*:

- gaseous environment;
- light;
- pH;
- temperature.

Q30:

A) Lag.
B) Log / exponential.
C) Stationary.
D) Death.

Q31: Any from:

- mutagenesis;
- recombinant DNA.

Q32: Horizontal

Q33: Restriction endonuclease

Q34: Ligase

Q35: When working with **microorganisms** in a lab, as a safety mechanism, genes are often introduced that prevent the survival of the **microorganism** in an **external** environment.

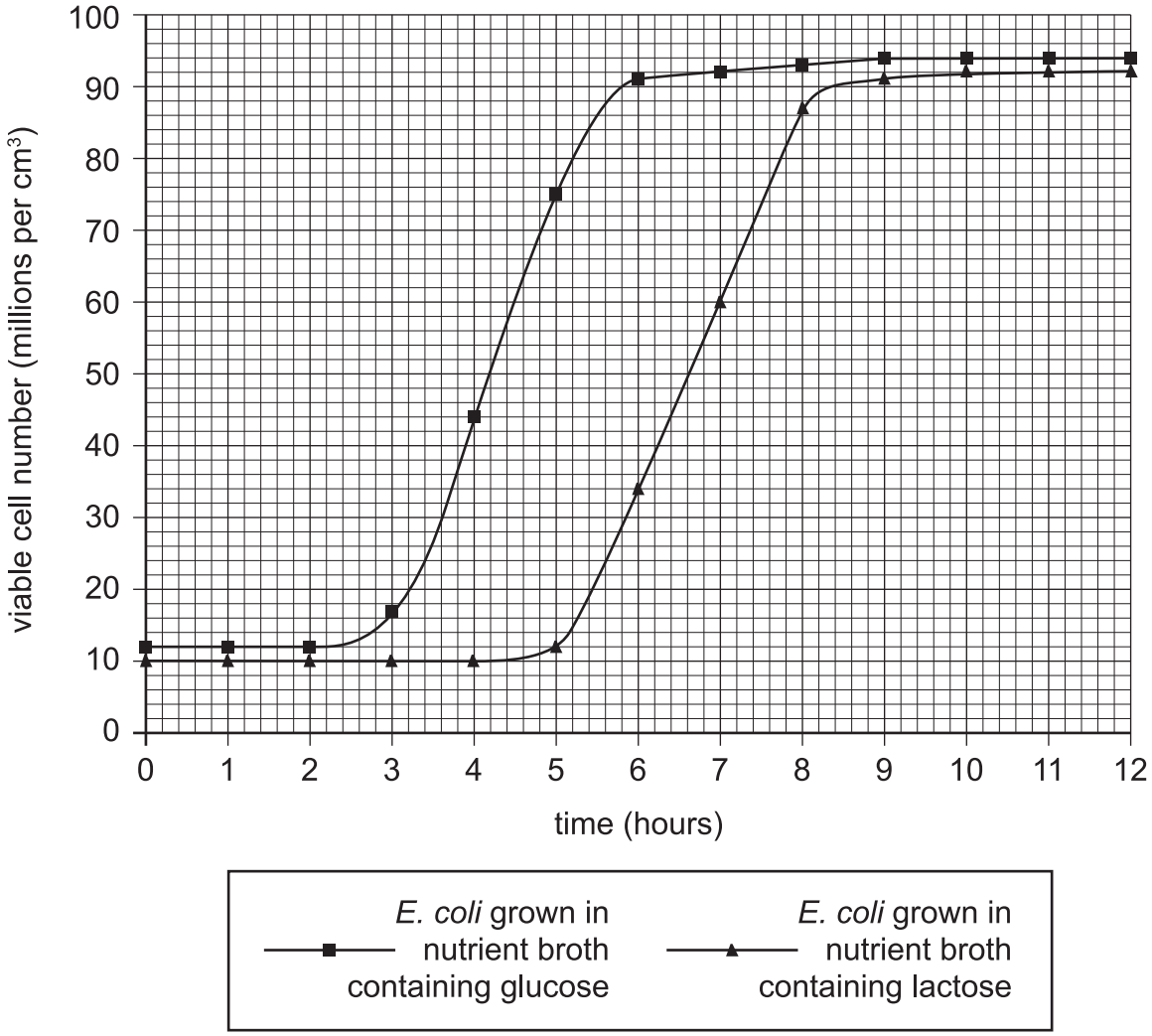
Q36: Using a recombinant yeast cell.

Q37: To prevent cross-contamination.

Q38: Any from:

- mass of sugar added to the nutrient broth;
- volume of nutrient broth;
- type of nutrient broth;
- pH of nutrient broth;
- strain of *E. coli*.

Q39:



(*1 mark for plotting the points correctly and connecting them with a ruler; 1 mark for filling in the key correctly)*

Q40: The total cell numbers includes dead cells.

Q41: 2.5 hours / 150 minutes

Q42: *Any two from:*

- The lag phase is shorter when glucose is used as a respiratory substrate / the lag phase is longer when lactose is used as a respiratory substrate.
- The log phase begins earlier when glucose is used as a respiratory substrate / the log phase begins later when lactose is used as a respiratory substrate.
- The log phase lasts longer when glucose is used as a respiratory substrate / the log phase is shorter when lactose is used as a respiratory substrate.
- The stationary phase begins earlier when glucose is used as a respiratory substrate / the stationary phase begins later when lactose is used as a respiratory substrate.