Revision Guide

Biology - Unit 3

GCE A Level WJEC

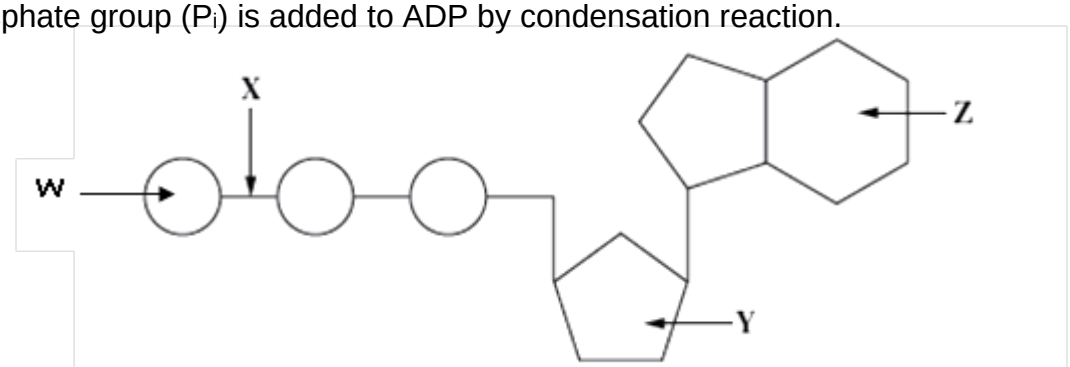
These notes have been authored by experienced teachers and are provided as support to students revising for their GCE A level exams. Though the resources are comprehensive, they may not cover every aspect of the specification and do not represent the depth of knowledge required for each unit of work.

Content

Section 3.1 - Importance of ATP

ATP – What you should already know

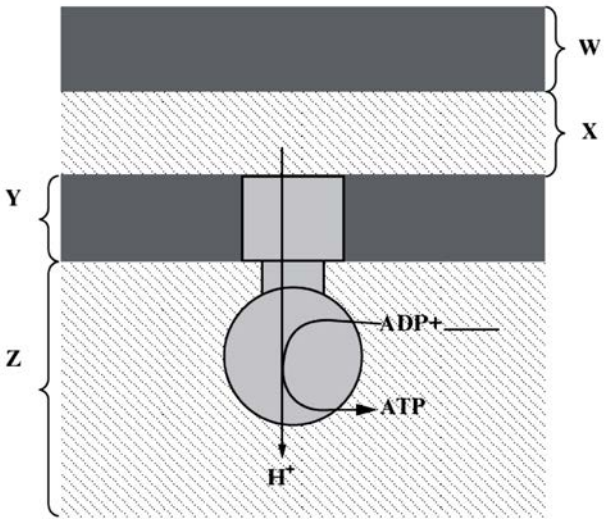
ATP is a **nucleotide** and the major energy currency of the cell – this means it's used for all reactions in all cells. It's inert, soluble, easily transported and releases energy efficiently. A single enzyme called **ATPase** hydrolyses (breaks) the terminal bond (shown as X below) between the last and middle phosphate group and this releases energy in useable amounts, so little is wasted as heat. ATP is easily reformed by **phosphorylation** when a phosphate group (P_i) is added to ADP by condensation reaction.



Look at the diagram above - **W** is a **phosphate group**, **Y** is a pentose sugar called **ribose** and **Z** is an organic base (containing nitrogen) called **adenosine**.

ATP synthesis

Most ATP synthesis takes place on the **internal membranes** of mitochondria and chloroplasts. Protons must pass through the enzyme **ATP synthetase,** which is found within the **stalked particles** of the inner membranes. This flow of protons generates an **electrochemical gradient**, which is a source of potential energy. This drives the phosphorylation of ADP (the addition of P_i) to form ATP (chemical energy). This whole process is called **chemiosmosis**.



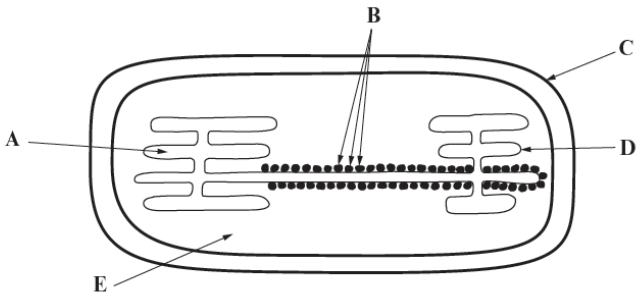
The diagram to the left shows a short section
of outer (**W**) and inner membrane (**Y**) of a mitochondrion. **X** is the intermembrane space.
Protons flow through ATP synthetase (shown
in light grey within the inner membrane)into the matrix (**Z**).

Chemiosmosis is the flow of protons down an electrochemical gradient, through ATP synthetase, which provides the potential energy necessary to synthesise ATP by phosphorylation.

Comparison of mitochondrial and chloroplast membranes

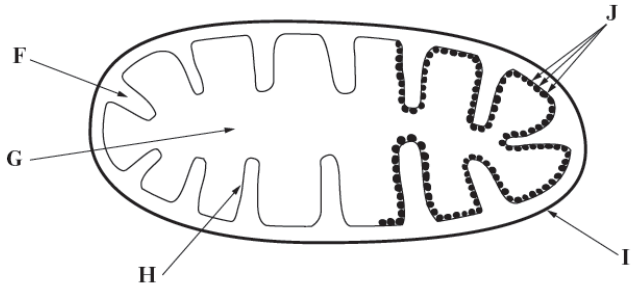
You must be able to make a like for like comparison and identify structures on diagrams of these organelles! In **mitochondria** protons flow from the intermembrane space into the matrix. In **chloroplasts** the protons flow across the thylakoid space into the stroma.

Chloroplast



A – Thylakoid space
B – Stalked particles containing ATP synthetase
C – Outer membrane
D – Thylakoid membrane (inner membrane)
E - Stroma

Mitochondrion



F – Intermembrane space
G – Matrix
H – Crista (inner membrane)
I – Outer membrane
J – Stalked particles containing ATP synthetase

Stage in ATP production	Chloroplast	Mitochondria
Protons are pumped across a membrane by proton pumps fuelled by electron energy	D	H
A high concentration of protons (hydrogen ions) build up	A	F
Protons flow down a concentration gradient to provide energy for ATP synthesis	B	J
Free electrons are taken up by a final electron acceptor	E	G

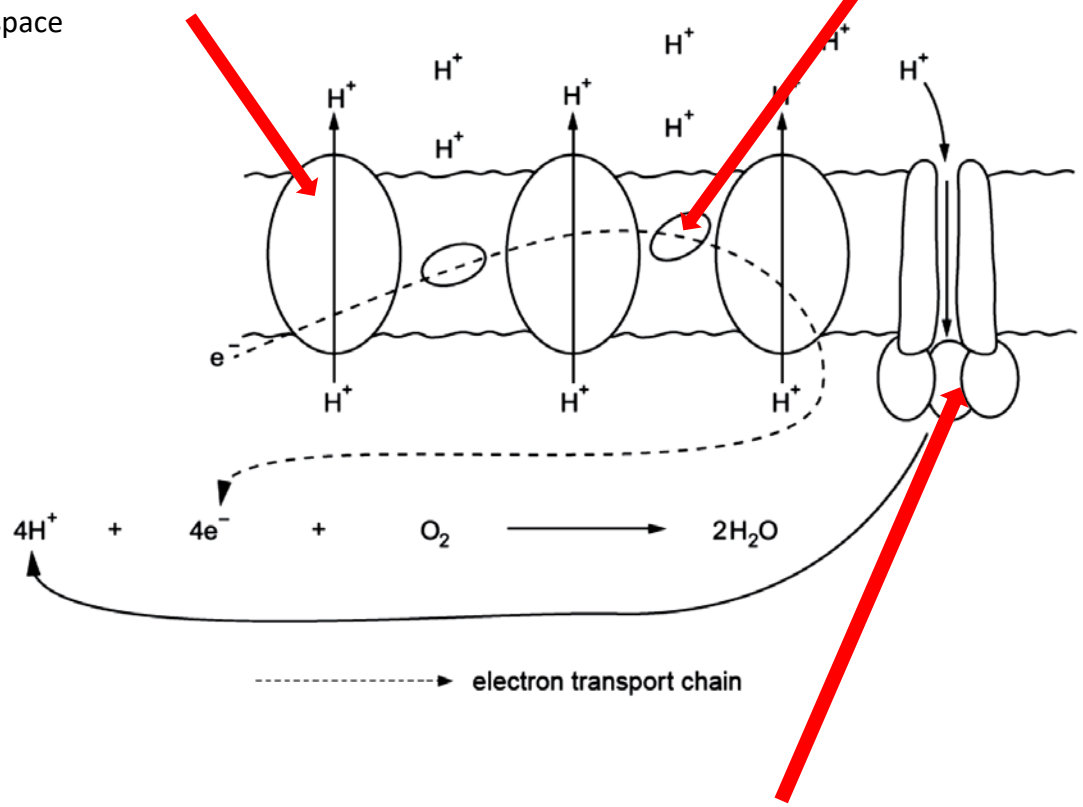
The electron transport chain and proton gradients

The diagram below shows the **electron transport chain** located within the inner membrane of the mitochondria or thylakoid membranes of the chloroplasts. **Proton pumps** and **electron carriers** alternate within the inner membranes and together generate a **proton gradient**.

Look at the diagram below - high energy electrons pass from electron carrier to electron carrier, providing electron energy to drive proton pumps. Protons are pumped into the space between the outer and inner membranes. The proton gradient generated allows protons to pass back into the matrix/stroma via **ATP synthetase**. The electrochemical gradient, and subsequent flow of charge, provides the energy necessary for phosphorylation of ADP to form ATP (chemiosmosis).

Proton pump transporting protons from the matrix/stroma to the intermembrane space or thylakoid space

Electron carrier passing electrons along the chain to provide electron energy to drive the proton pumps

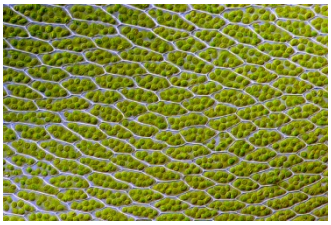


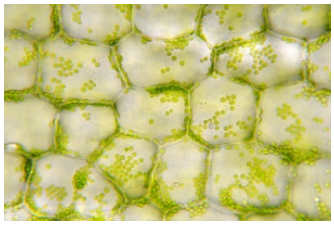
A flow of protons pass through ATP synthetase (down a concentration gradient, by facilitated diffusion), this generates the potential energy needed for ATP synthesis by **oxidative phosphorylation** – oxygen becomes the final electron acceptor allowing the flow of electrons to continue

Section 3.2 - Photosynthesis

Chromatography and R_f values

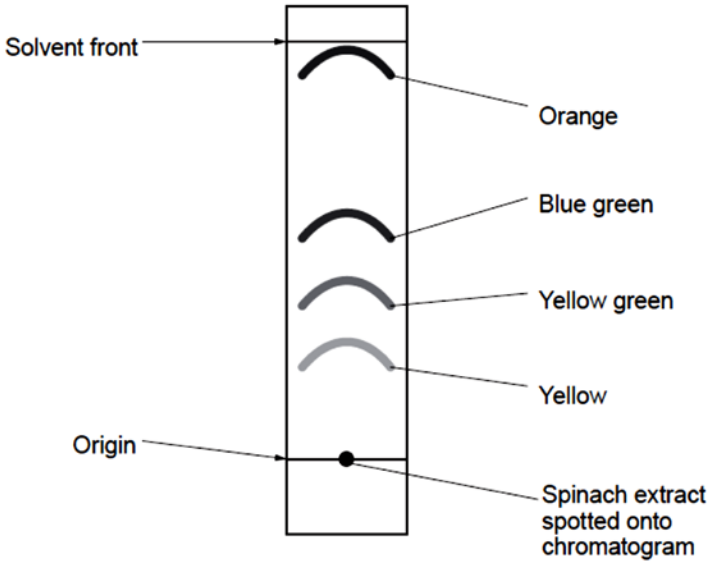
Photosynthesis takes place in chloroplasts. Chloroplasts contain photosynthetic pigments which **absorb light energy at particular wavelengths** of light. Examples of pigments include – chlorophyll a and b, carotene and xanthophylls. Chloroplasts are found in mesophyll tissues, predominantly in the palisade mesophyll cells. Chloroplasts orientate themselves to receive the maximum exposure to light.





In low light conditions chloroplasts will distribute evenly throughout the cytoplasm to maximise absorption of the available light. In high light intensity they line up in vertical columns against the cell wall, side on to the light to prevent damage by over-exposure.

Photosynthetic pigments can be separated using **chromatography**, a chromatogram is shown below.



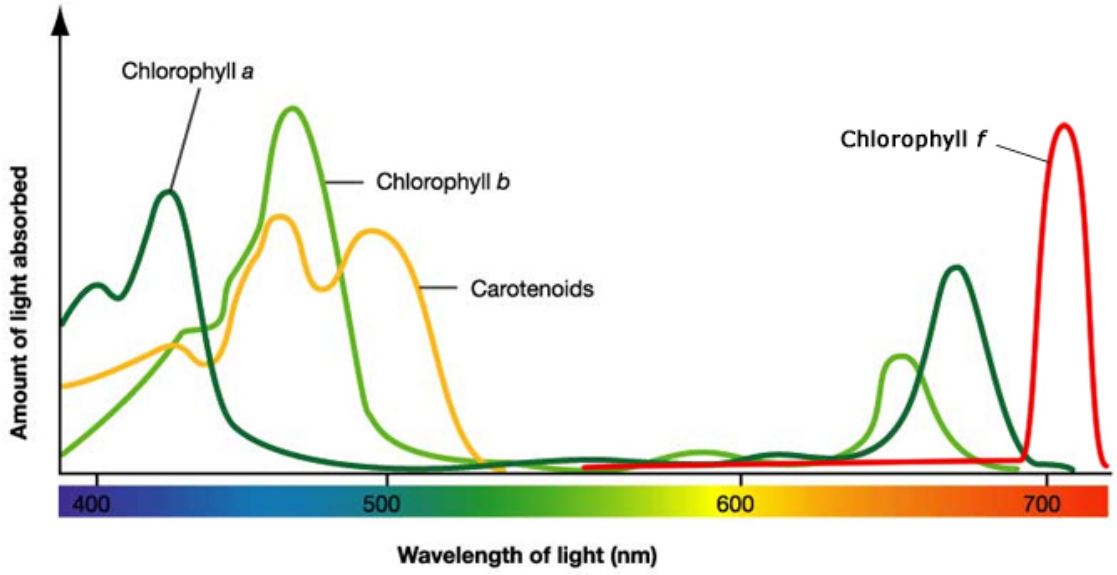
Pigments can be identified by using their relative positions on the chromatogram to calculate their **R_f values**. Your calculated R_f values can be compared to the R_f values of known pigments (this information will always be provided in the exam).

Top Tip – use a ruler to draw a line across the chromatogram at the tip of the curve (apex) of each pigment before you measure the distance between the origin and pigment.

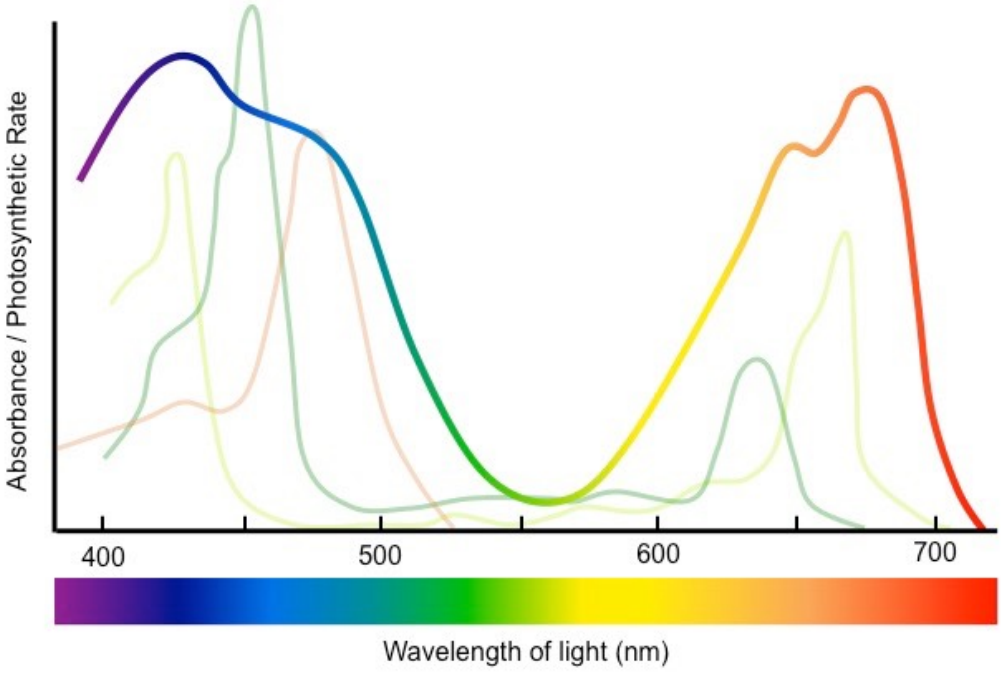
$$\mathbf{Rf} = \frac{\text{distance moved by pigment from origin}}{\text{distance moved by solvent front from origin}}$$

Absorption and action spectra

An **absorption spectrum** is a graph which shows how much light is absorbed by a pigment at different wavelengths of light. Look at chlorophyll a on the absorption spectrum below, it has absorption peaks at 435 nm (blue) and 670-80 nm (red).

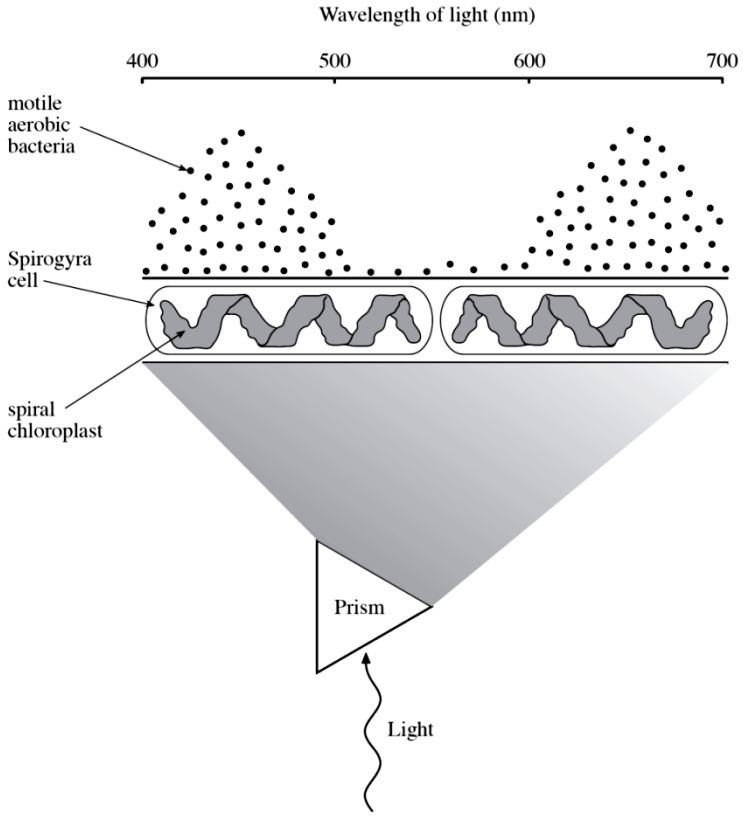


An **action spectrum** is a graph which shows the rate of photosynthesis at different wavelengths of light. If you overlay an action spectrum onto an absorption spectrum the peaks show a **very close correlation**. This suggests that the wavelengths of light absorbed, by the photosynthetic pigments, are actually used for photosynthesis.



Thomas Englemann experiment with spirogyra

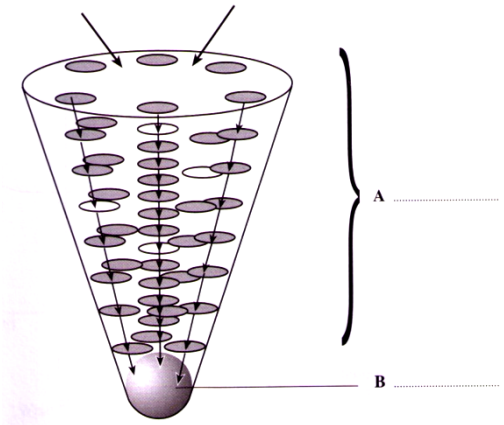
Thomas Englemann devised an experiment to determine which wavelengths of light were used most for photosynthesis. He placed the algae spirogyra in a suspension of motile aerobic bacteria. He used a prism to refract white light into its constituent rainbow colours.



Oxygen is a product of photosynthesis; the most oxygen will be produced at the wavelengths of light used most for photosynthesis. Aerobic bacteria need oxygen for respiration and migrate towards regions with the highest concentration of oxygen – this corresponds to the blue and red regions of the spectrum. Englemann concluded that the blue and red regions of the spectrum caused the most photosynthetic activity.

Light Harvesting

Photosynthetic pigments absorb light energy – this is a process called **light harvesting** and it is achieved by antenna complexes within the thylakoid membranes of the chloroplasts. The diagram to the right shows an antenna complex (**A**) with a reaction centre at its base (**B**). **Chlorophyll a is a primary pigment** and is found in the reaction centre. All other pigments e.g. carotenes, xanthophylls and chlorophyll b are accessory pigments found in the antenna complex – they allow a range of wavelengths to be absorbed.



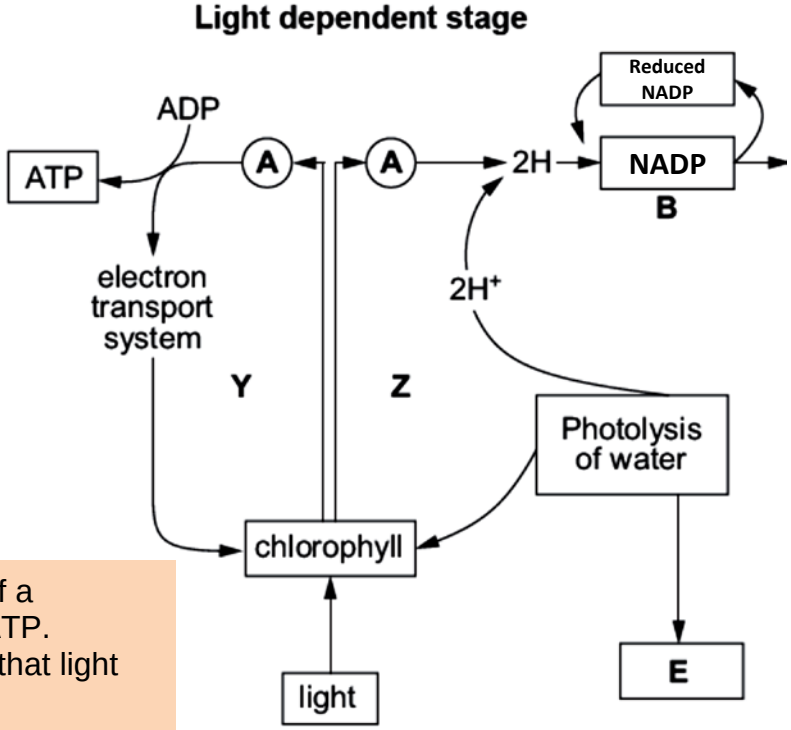
Light dependent stage

Light energy hits the top of the antenna complex and is passed from accessory pigment molecule to accessory pigment molecule towards the reaction centre at the base. Proteins associated with the antenna complex prevent the light energy escaping from the antenna complex.

The reaction centre contains two molecules of chlorophyll a (a primary pigment). The chlorophyll a molecules absorb light energy and emit **high energy electrons**. There are two types of reaction centre:

- ✓ Photosystem I (PSI) – with an absorption peak of 700nm (also called P700)
- ✓ Photosystem II (PSII) – with an absorption peak of 680nm (also called P680)

Photons of light passed to chlorophyll a in the reaction centre excite electrons and raise them to a higher energy level. These high energy electrons are passed to electron acceptors/donors (shown as **A** on the diagram to the right). These electrons will either be used for **ATP synthesis by photophosphorylation** or used to **reduce NADP**.



Phosphorylation is the addition of a phosphate group to ADP to form ATP. **Photophosphorylation** indicates that light energy is needed to achieve this.

Cyclic photophosphorylation involves PSI only (this is pathway **Y** on the diagram above). High energy electrons pass form PSI to an electron acceptor (**A**). The electron is donated to the **electron transport system** to help generate a proton gradient for chemiosmosis (look back at section 3.1). The electron (now at a lower energy state) is passed back to PSI. This is a cyclic pathway as the electron originates from PSI and returns to PSI.

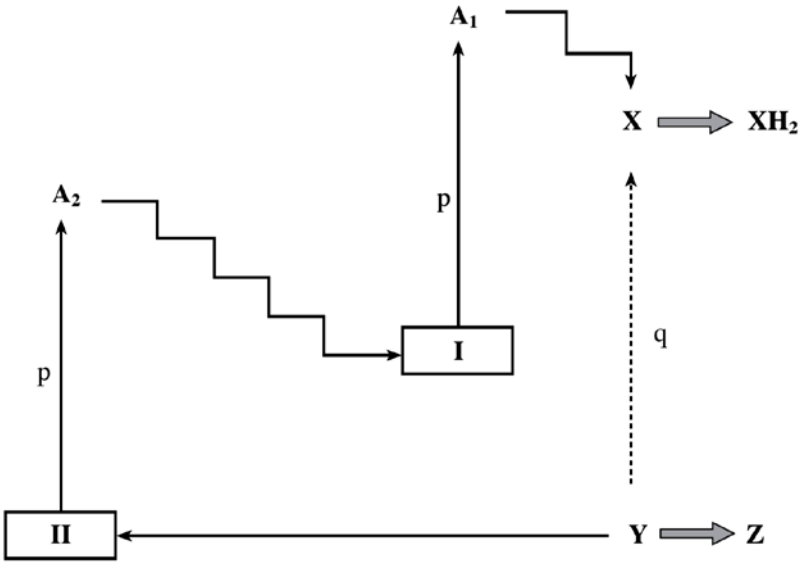
Non-cyclic photophosphorylation is an alternative pathway involving PSI and PSII. High energy electrons, from PSI, follow pathway **Z** and are passed to **NADP** forming **reduced NAPD** (two electrons are needed to reduce NADP). PSI is now an electron short and has a positive charge and is therefore primed to steal an electron form PSII. Simultaneously PSII has passed a high energy electron to the electron transport system to generate a proton gradient. After driving chemiosmosis this electron is passed to PSI, **not PSII**. It is a non-cyclic pathway as the electron does not return to its origin. PSII is now an electron short!

Photolysis and Z scheme

Photolysis provides an electron for PSII. Molecules of water, within the thylakoid space, absorb light energy and are split to form electrons, protons (hydrogen ions) and oxygen (**E** in the diagram on page 7). The electrons are passed to PSII to replace those lost during non-cyclic photophosphorylation. The protons are used to reduce NADP; each NADP molecule picks up two protons and two electrons to become reduced.

The products of the light dependent stage are **ATP** (energy source) and **reduced NADP** (reducing power), both are essential for the **light independent stage** or **Calvin cycle**, which occurs in the stroma of the chloroplast. The **oxygen** produced by photolysis diffuses out of the chloroplast as a waste product.

Non cyclic photophosphorylation can be illustrated as a **Z scheme** (as its shape looks like the letter Z).



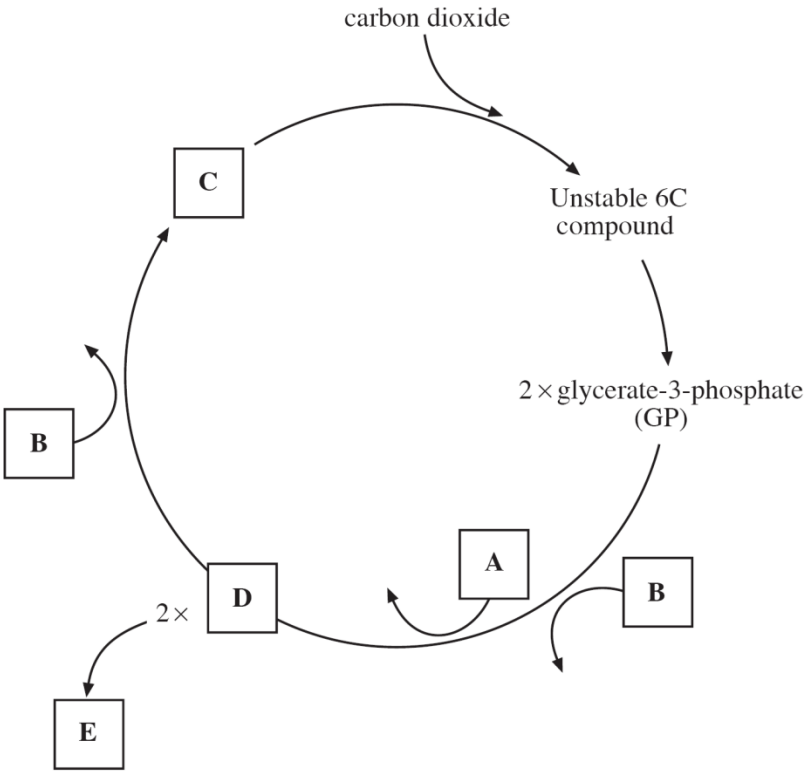
Look at the Z scheme above. The photosystems are shown as **I** and **II**, **p** represents high energy electrons passing to electron acceptors **A** (with become reduced when they accept an electron). The zig-zag arrow between **A_2** and PSI represents the electron transport system which will generate the proton gradient necessary for photophosphorylation and ATP synthesis**. X** and **XH_2** represent NADP and reduced NADP respectively. **Y** is water and **Z** is oxygen diffusing out of the chloroplast. The arrow between **Y** and PSII represent an electron passing to PSII and **q** is a proton (hydrogen ion) passing to NADP.

Photolysis is the splitting of water by light, producing protons (hydrogen ions), electrons and oxygen. The water molecules used for photolysis are found in the thylakoid space.

Top Tip – Remember **ATP** (energy source) and **reduced NADP** (reducing power) are needed to drive the **Calvin cycle** or **light independent stage**. Without these products the Calvin cycle will stop and carbon dioxide will not be fixed into carbohydrate. The plant will die as there will be no glucose for respiration.

Light independent stage (Calvin cycle)

The **light independent stage** or **Calvin cycle** fixes carbon dioxide into carbohydrate. Carbon dioxide is taken up by **5C ribulose bisphosphate** (RUBP) to form an unstable 6C compound and then two molecules of **3C glycerate-3-phosphate** (GP) – this is catalysed by the enzyme **rubisco**. ATP and reduced NADP (from the light dependent stage) reduce GP to **3C triose phosphate** (TP). TP is converted into glucose and then starch. Most of the TP is converted into RUBP (using energy from ATP) – this regeneration of RUBP allows the light independent stage to continue.



Top Tip – **RUBP** will always be found just before carbon dioxide enters the cycle. Remember that **ATP is needed twice**, but **reduced NADP only once**, this should help you decide which is A and which is B in the diagram to the left. Remember also that ATP will be hydrolysed into ADP a P_i. Reduced NADP will be oxidised to form NADP. ADP and NADP will return to the light dependent stage (in the thylakoid membranes) to reform ATP and reduced NAPD.

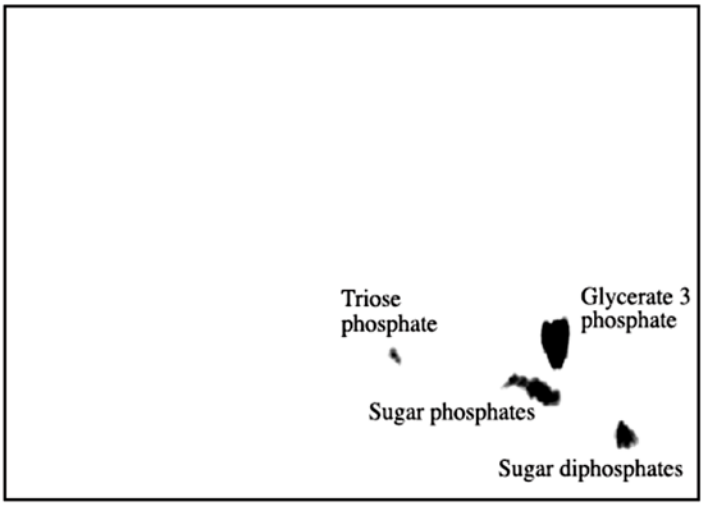
Look at the diagram above. **C** must be RUBP as it combines with carbon dioxide to form an unstable 6C compound and then two molecules of GP (remember rubisco catalyses this). GP needs the energy from ATP (**B**) and the reducing power of reduced NADP (**A**) to form two molecules TP (**D**). Most TP is used to regenerate RUBP (ATP is needed for this again). Some TP is converted into glucose (**E**) and then starch.

Lipids, proteins and carbohydrates can be made from the products of the Calvin cycle. The first carbohydrate made is **glucose**, which can be converted into **starch** by condensation reaction. Acetyl coenzyme A can be synthesised from GP (formed during the Calvin cycle) and converted into **fatty acids**. TP can be converted into **glycerol**. Condensation reactions between glycerol and fatty acids form **triglycerides**. GP can also be converted into **amino acids** for **protein** synthesis although nitrogen atoms are needed to form the amino group of the amino acid – nitrogen is derived from ammonium and nitrate ions absorbed by root hair cells by active transport.

Interpreting autoradiographs

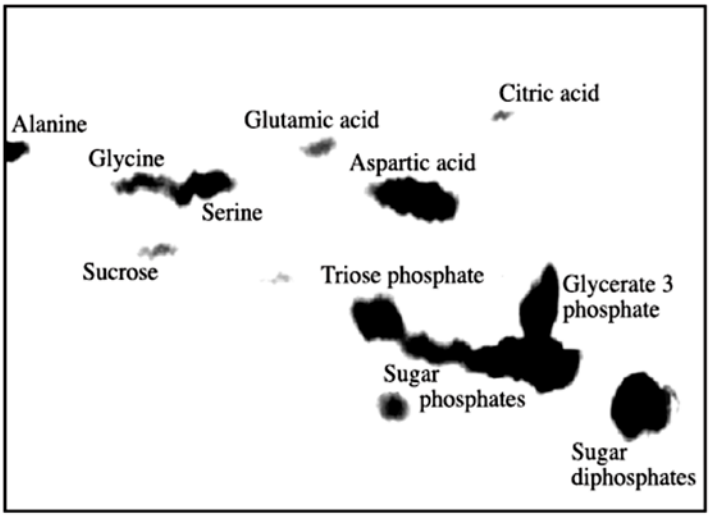
You may be asked to interpret an **autoradiograph** of products derived from the Calvin cycle. The alga chlorella was exposed to $^{14}CO_2$. At 5 second time intervals a sample of chlorella was placed into hot ethanol to stop all enzymatic reactions. The radioactive compounds were separated by chromatography.

After 5 seconds:



The darker and larger the spot the more of that compound is present. Initially only the early products of the Calvin cycle are observed as they are produced first. GP is formed before TP in the cycle, therefore there is more present. Small amounts of sugar phosphates and sugar diphosphates have also been formed at this early stage.

After 30 seconds:



After 30 seconds more GP and TP have formed as the spots are darker and larger. There has also been sufficient time for other molecules to form e.g. amino acids (formed from GP) such as alanine and glycine and the disaccharide sucrose (formed from sugar phosphates).

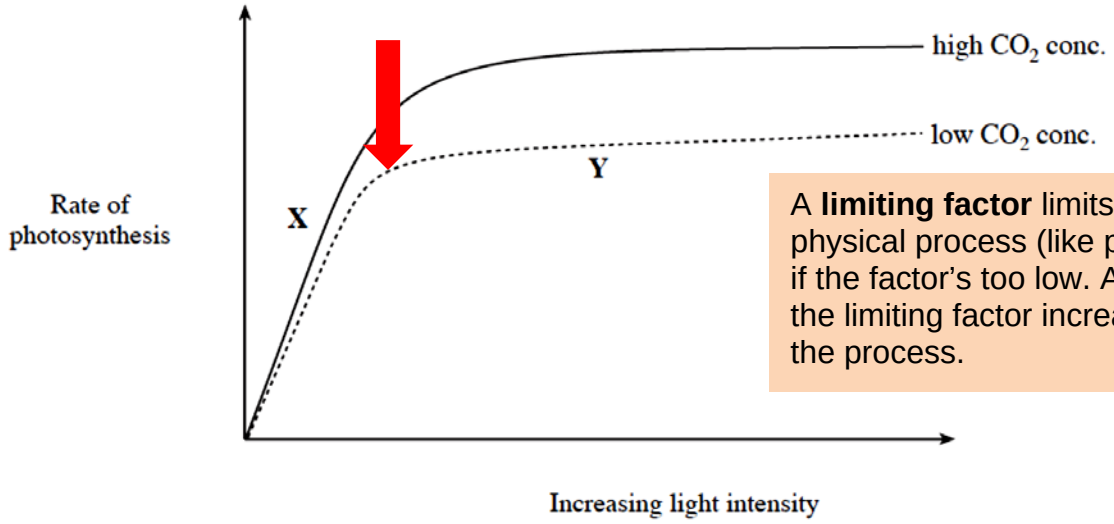
Subsequent samples taken later would contain more complex, larger molecules such as starch, lipids and proteins.

Top Tip – Remember to **describe** what you see in each autoradiograph clearly and logically, also **compare** as fully as you can e.g. there is more GP present after 30 seconds as the spot is darker and larger or serine, alanine and glycine are present after 30 seconds, but were absent after 5 seconds as amino acids take longer to form (GP must be formed first). Just say what you see!

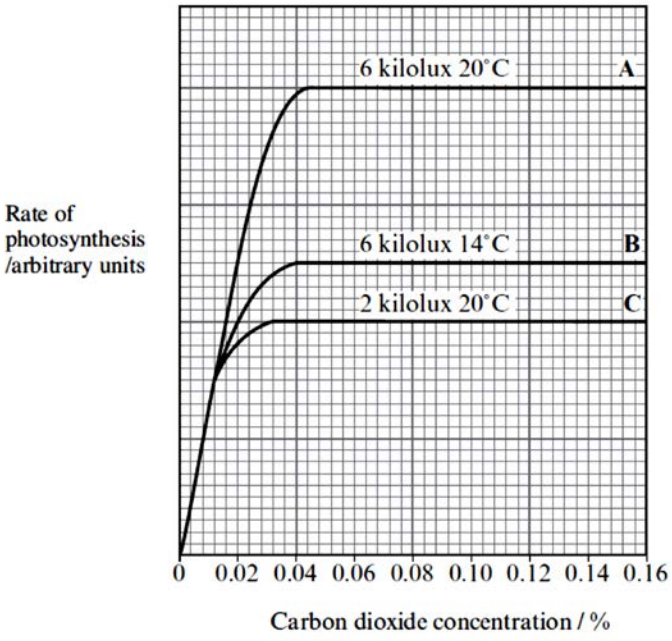
Limiting factors

To achieve efficient photosynthesis plants need a suitable environment. Plants need carbon dioxide and water as reactants, sufficient light intensity at suitable wavelengths (for the light dependent stage) and an optimal temperature for enzyme activity. If any of these factors become too low the rate of photosynthesis will decrease – they become **limiting factors**!

If you increase a limiting factor the rate of photosynthesis will increase. Look at curve **Y** on the graph below, as light intensity increases the rate of photosynthesis increases until it reaches the saturation point (indicated by the red arrow); beyond the **saturation point** the rate of photosynthesis plateaus (levels off). A further increase in light intensity will not increase the rate of photosynthesis as another factor has now become limiting. In this example the new limiting factor must be carbon dioxide concentration, as you can see by looking at curve **X,** the rate of photosynthesis increases further at a higher carbon dioxide concentration.



A **limiting factor** limits the rate of a physical process (like photosynthesis) if the factor's too low. An increase in the limiting factor increases the rate of the process.



Temperature affects kinetic energy and therefore rates of transport and enzyme activity.

Light intensity is a key factor; light is needed to excite electrons in the photosystems during the light dependent stage.

Limiting factors can combine – the actual rate of photosynthesis will be controlled by the factor that is nearest its minimum value. Look at curve **C** to the left, at high CO_2 concentrations light intensity is limiting the rate of photosynthesis not temperature as light is nearest its minimum value.

Mineral nutrition

Inorganic ions are needed by the plant, and may themselves become limiting factors if they are in short supply. **Macronutrients** are needed in substantial quantities and include sodium, magnesium, calcium, nitrate and phosphate. **Micronutrients** are needed in tiny amounts and include manganese and copper.

Nitrogen is absorbed by the roots as nitrates (by active transport). It is transported as nitrates in the xylem and as amino acids in the phloem. It is used for the synthesis of amino acids, nucleic acids and other nucleotides such as ATP. Symptoms of nitrogen deficiency are **reduced growth of all organs** and chlorosis, a **yellowing of the leaves** due to inadequate chlorophyll production; chlorosis first appears in older leaves.

Magnesium is absorbed as Mg^{2+} and transported in the xylem. Its function is chlorophyll production and activation of ATPase. Magnesium is needed by all tissues, especially the **leaves**. Magnesium forms a part of the chlorophyll molecule. Magnesium deficiency causes **chlorosis**, which begins between the veins of older leaves. This is because existing magnesium in the plant is mobilised and transported to newly formed leaves.

Section 3.3 - Respiration & Glycolysis

Respiration

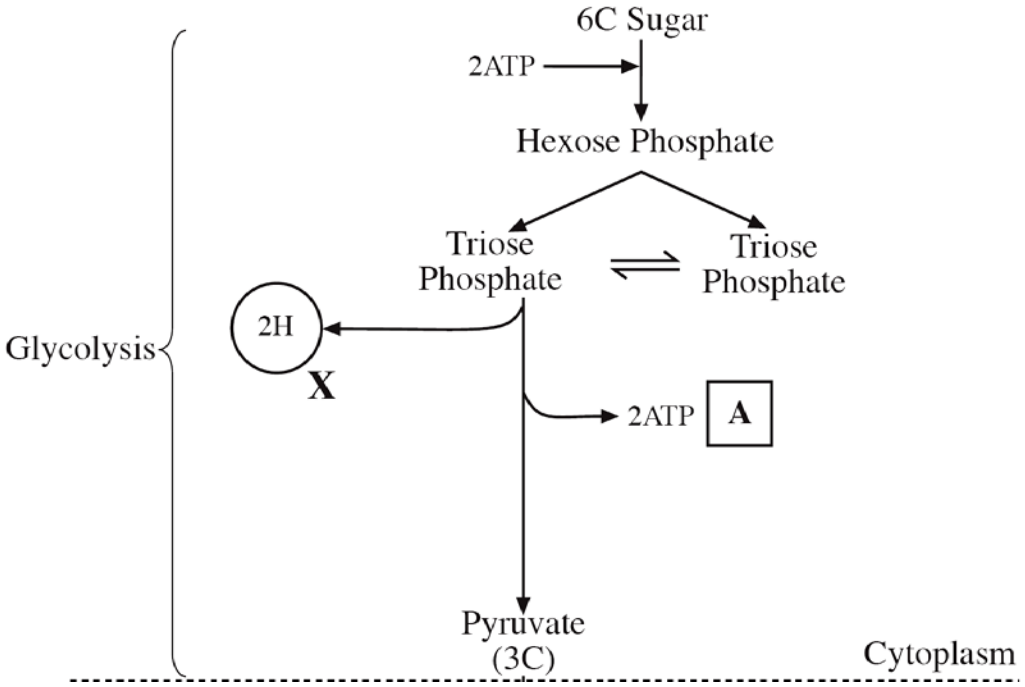
During respiration high energy bonds, in energy rich molecules such as glucose and fatty acids, are broken e.g. the bonds between C-C, C-H and C-OH. The energy released is used to produce **ATP by phosphorylation** (when P_i is attached to ADP). Respiration is catalysed by **enzymes**.

Aerobic respiration involves the complete breakdown of glucose and requires oxygen as the final electron acceptor. Large amounts of energy are released to produce a large number of ATP molecules by oxidative phosphorylation.

Anaerobic respiration involves the incomplete breakdown of glucose in the absence of oxygen, releasing relatively little energy and making a small number of ATP molecules by substrate level phosphorylation.

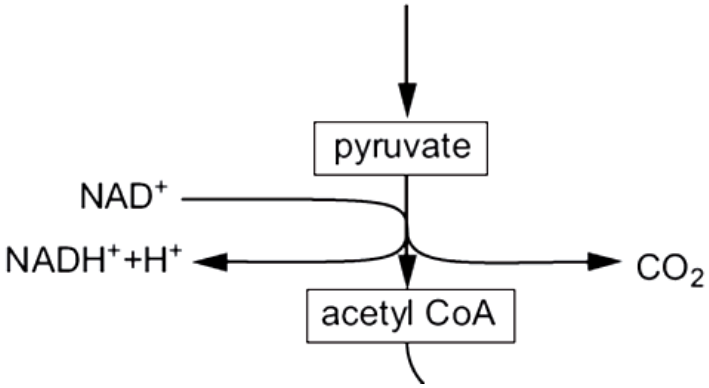
Glycolysis

Glycolysis takes place in the cytoplasm and is anaerobic (requires no oxygen). 2ATP molecules are needed for the phosphorylation of glucose, forming hexose phosphate. Hexose phosphate is unstable and forms two molecules of triose phosphate (TP). Each TP molecule loses two hydrogen atoms by **dehydrogenation**, catalysed by the enzyme **dehydrogenase**. The hydrogen atoms released are picked up by the nucleotide NAD (**X** on the diagram below) forming reduced NAD. Two molecules of ATP are also produced by substrate level phosphorylation (**A**) - pyruvate (3C) is formed. The products formed by glycolysis are pyruvate, reduced NAD and two molecules of ATP (net).



The Link Reaction

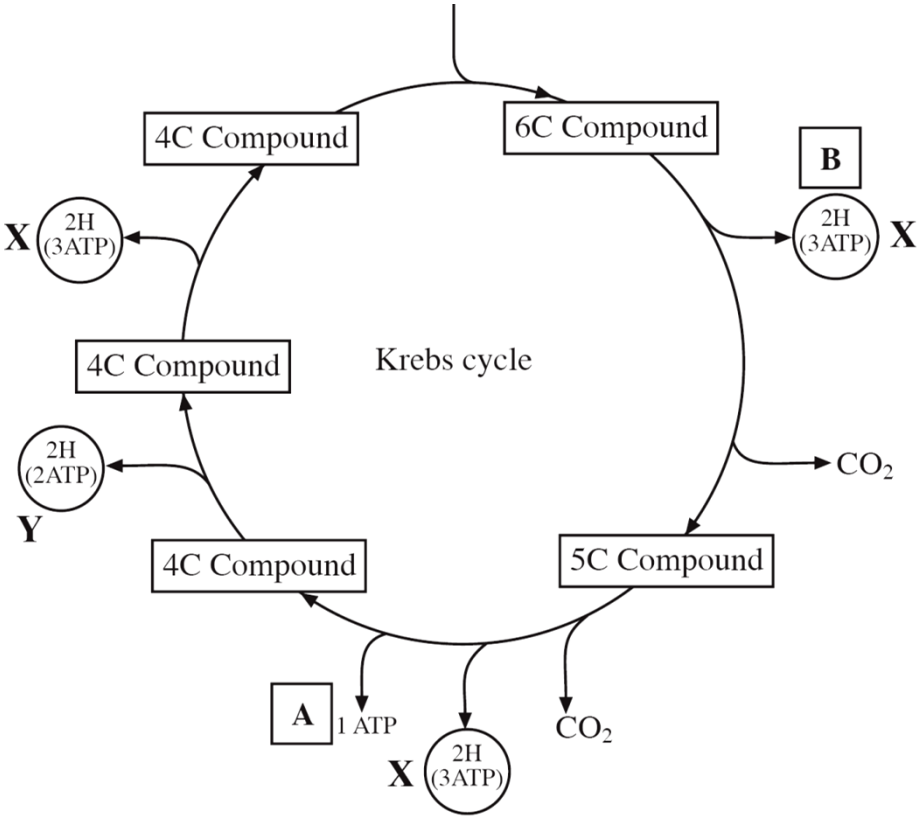
Pyruvate diffuses down its concentration gradient into the mitochondrial matrix. Here pyruvate is **decarboxylated** by the enzyme **decarboxylase**, releasing carbon dioxide, and dehydrogenated by dehydrogenase – releasing two hydrogen atoms to form 2C acetate or acetyl. The acetate/acetyl attaches to coenzyme A to form acetyl CoA. Once again the hydrogen atoms released are picked up by NAD to form reduced NAD.



Dehydrogenation is the removal of one or more hydrogen atoms from a molecule, catalysed by the enzyme dehydrogenase. **Decarboxylation** is the removal of a carboxyl group from a molecule, releasing carbon dioxide.

The Krebs Cycle

Acetyl CoA enters the Krebs cycle (also in the mitochondrial matrix) and combines with a 4C compound to form a 6C compound. CoA is regenerated. On the diagram below **X** is NAD, **Y** is FAD and **A** represents substrate level phosphorylation.



The **Krebs cycle** is a series of enzyme controlled oxidation-reduction reactions. The 6C compound is dehydrogenated, releasing two hydrogen atoms to reduce NAD; decarboxylation releases carbon dioxide and forms a 5C compound. The 5C compound undergoes dehydrogenation and decarboxylation to form a 4C compound. An ATP molecule is also produced by substrate level phosphorylation. Two further dehydrogenation reactions take place to regenerate the 4C compound at the beginning of the cycle and reduce more NAD and a FAD molecule. Failure to regenerate this 4C compound would lead to the build-up of acetyl CoA and the Krebs cycle would stop.

Remember there are two Krebs cycles per glucose molecule. By the end of the Krebs cycle the original glucose molecule has been completely oxidised (broken down). Here is a list of the products made by the Krebs cycle and their functions:

Krebs cycle products	Function
2 CoA	Regenerated and returns to the link reaction
2 4C molecules	Regenerated to allow the Krebs cycle to continue
4 CO_2	Waste product, diffuses out of the cell
2 ATP	Produced by substrate level phosphorylation
6 Reduced NAD	Pass to the inner membrane of the mitochondria (cristae) and donate electrons and protons to the electron transport chain
2 Reduced FAD	Pass to the inner membrane of the mitochondria (cristae) and donate electrons and protons to the electron transport chain

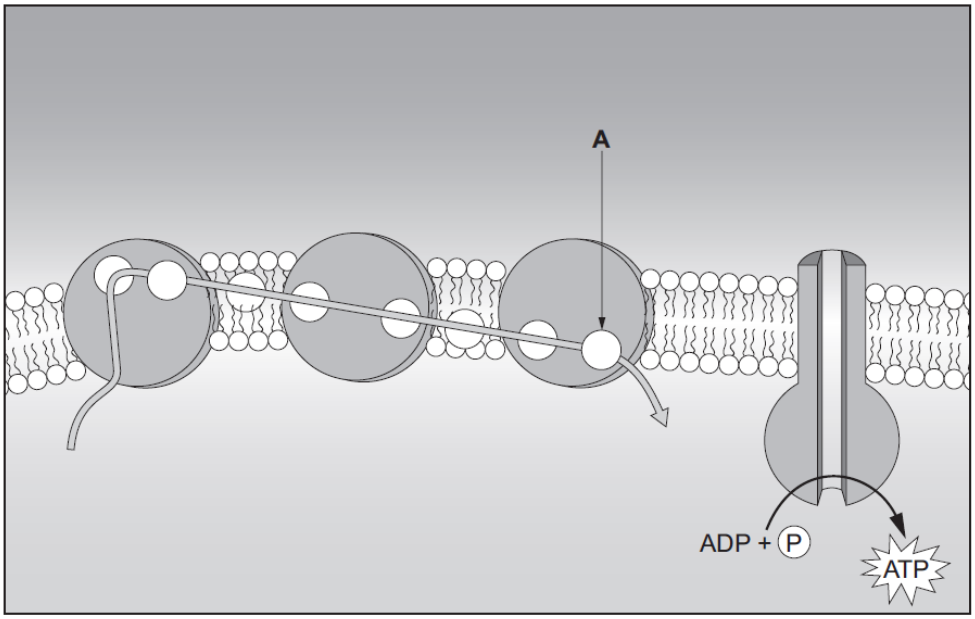
The Electron Transport Chain (ETC)

Reduced NAD and reduced FAD transport pairs of hydrogen atoms to the **electron transport chain** (ETC) located within the inner membrane (cristae) of the mitochondria – NAD and FAD are coenzymes. Reduced NAD is associated with three ETC proton pumps and therefore generates 3 ATP molecules, reduced FAD is associated with two ETC proton pumps and generates 2 ATP molecules by oxidative phosphorylation.

Reduced NAD and reduced FAD are oxidised as they release their hydrogen atoms; the electrons and protons derived from these hydrogen atoms drive the process of chemiosmosis (see Unit 3.1).

At the end of the ETC the electrons and protons combine with oxygen to form water. Oxygen is the **final electron acceptor** and is essential as it removes electrons and protons from the matrix, preventing accumulation. An accumulation of protons in the matrix would destroy the proton gradient and ATP synthetase would not function. ATP would not be produced and the cell would die quickly.

Cyanide is a non-competitive respiratory inhibitor of the final electron carrier (**A** on the diagram below); it prevents electrons and protons passing to oxygen to form water. This would prevent the proton gradient forming between the inter-membrane space and matrix and chemiosmosis would stop.



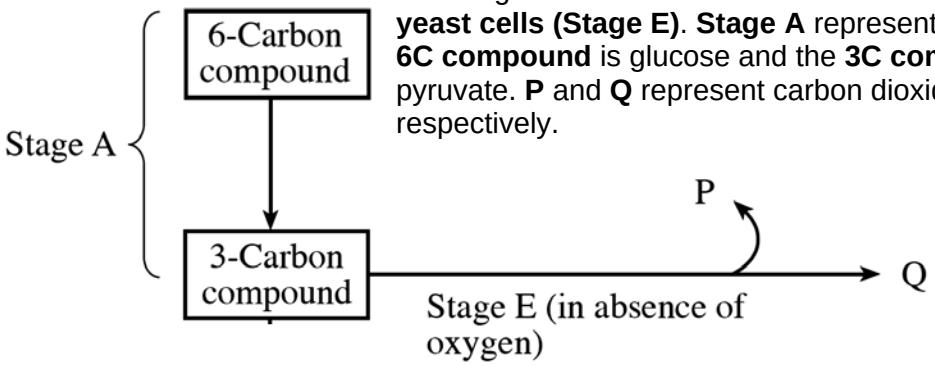
Anaerobic respiration

Without oxygen as the final electron acceptor the link reaction and Krebs cycle stop as NAD and FAD cannot be re-oxidised and are not available to pick up more hydrogen atoms.

In animals – glycolysis still continues in the cytoplasm, but reduced NAD must pass its hydrogen atoms to pyruvate; pyruvate becomes the final electron acceptor. Lactate or lactic acid is formed.

In yeast – pyruvate is decarboxylated, forming ethanal and releasing carbon dioxide (**P**). Reduced NAD passes hydrogen atoms to ethanal to form ethanol (**Q**).

ATP is produced by substrate level phosphorylation, a net production of 2ATPs per glucose molecule (2% efficiency). This is much less efficient than oxidative phosphorylation in the presence of oxygen.



The diagram the left illustrates **anaerobic respiration in yeast cells (Stage E)**. **Stage A** represents glycolysis. The **6C compound** is glucose and the **3C compound** is pyruvate. **P** and **Q** represent carbon dioxide and ethanol respectively.

Energy Budget

Aerobic Respiration – 38 ATPs per glucose molecule.

- ✓ **Glycolysis** – 2ATPs by substrate level phosphorylation (net production)
- ✓ **Krebs Cycle** – 2ATPs by substrate level phosphorylation (remember there are two cycles per glucose molecule)
- ✓ **Chemiosmosis at the inner membranes of the mitochondria** – 34 ATPs by oxidative phosphorylation

Remember the 10 reduced NAD molecules each produce 3 ATPs (3x10 = 30 ATPs) and reduced FAD molecules produce 2 ATPs each (2x2 = 4 ATPs). Add the 34 ATPs from oxidative phosphorylation to the net production by substrate level phosphorylation (glycolysis and Krebs), which is 4 ATPs, and you get your total of 38 ATP molecules.

Anaerobic Respiration in Mammalian Muscle Tissue – 2ATPs per glucose molecule (net production)

- ✓ Glycolysis – 2ATPs by substrate level phosphorylation (net production)

Alternative Respiratory Substrates

When glucose molecules have been completely depleted, lipids and amino acids can be used as **alternative respiratory substrates**.

Lipids are hydrolysed into fatty acids and glycerol by the enzyme lipase. Glycerol is converted into the 3C compound triose phosphate and enters via glycolysis. Fatty acids are very long hydro-carbon chain which are split into 2C acetate molecules and feed into the Krebs cycle ac acetyl CoA.

Proteins are hydrolysed into their constituent amino acids, which are deaminated (amino group removed) in the liver forming a keto acid and ammonia. Some keto acids are fed into glycolysis via pyruvate and some are fed into the Krebs cycle via acetyl CoA. Amino acids are only used as an energy source in severe circumstance e.g. starvation.

Respiratory Quotients

The number of carbon dioxide molecules produced and the number of oxygen molecules consumed can be used to calculate the **respiratory quotient (RQ)** of a particular respiratory substrate. Glucose has a RQ of 1 as equal volumes of oxygen and carbon dioxide are consumed and produced respectively.

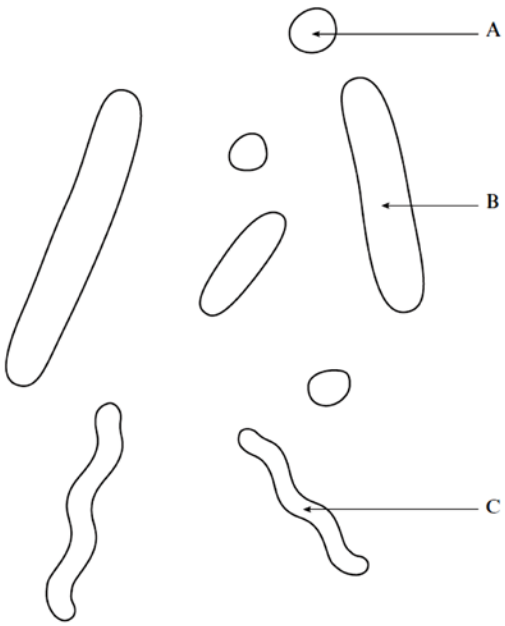
$$RQ = \frac{\text{number of molecules of } CO_2 \text{ produced}}{\text{number of molecules of } O_2 \text{ consumed}}$$

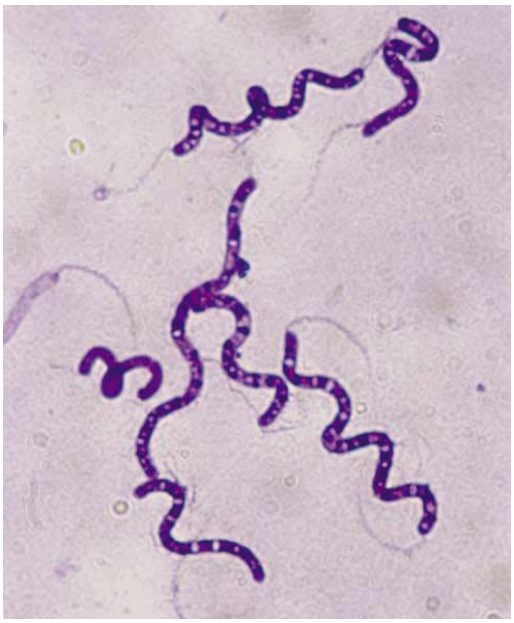
Respiration of lipid generates **more energy per unit mass** than the respiration of glucose derived from glycogen in the liver and muscle cells. Less oxygen is consumed and less carbon dioxide produced when glucose is the respiratory substrate – which is why energy is stored as glycogen in the liver and not fat. However, lipids do generate more **metabolic water** during respiration which is essential for some desert organisms.

Section 3.4 - Microbiology

Classification of bacteria according to their shape

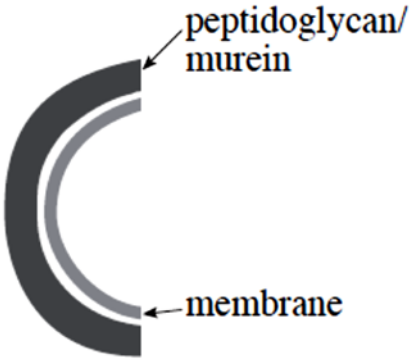
Bacteria can be classified according to their shape. **A** is a spherical **Coccus**, **B** is a rod shaped **Bacillus** and **C** is a spiral or corkscrew shaped **Spirillum**.





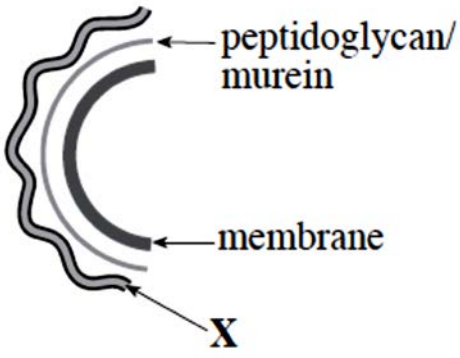
Classification by staining

Bacteria can also be classified by their reaction to the **Gram stain**. Gram positive bacteria will be stained **purple**, Gram negative bacteria will stain **red** – this is due to the different chemical composition and structure of their cell walls.

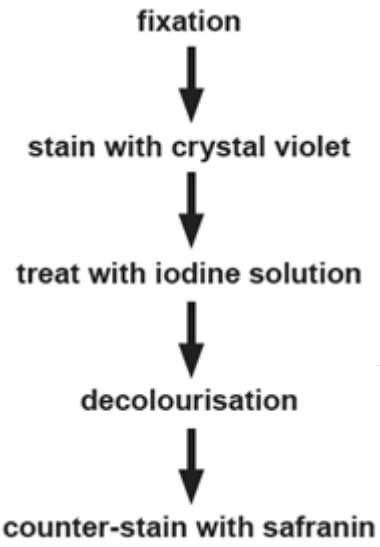


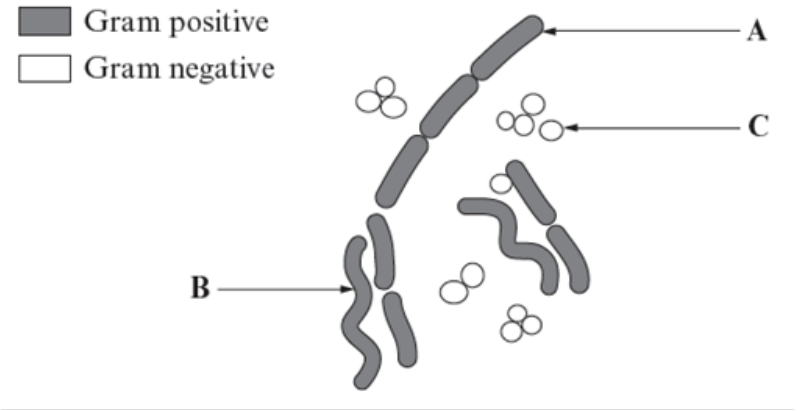
Gram positive bacteria have a **thicker outer wall** (around 8 nm) made of peptidoglycan (murein) with no outer lipopolysaccharide layer. This allows the crystal violet/iodine complex to be retained within the cell – staining the cells **purple**.

Gram negative bacteria have a thinner peptidoglycan cell wall (2nm) and an outer lipopolysaccharide membrane (**X** on the right). On treatment with alcohol, the Gram negative cell walls lose the outer lipopolysaccharide membrane, and the thin peptidoglycan wall allows the purple stain complex to be washed away. Gram negative cells are not stained by the Gram stain (they remain colourless), but stain **red** after counterstaining with safranin.



Gram stain procedure





A – Gram positive Bacillus
B – Gram positive Spirillum
C – Gram negative Coccus

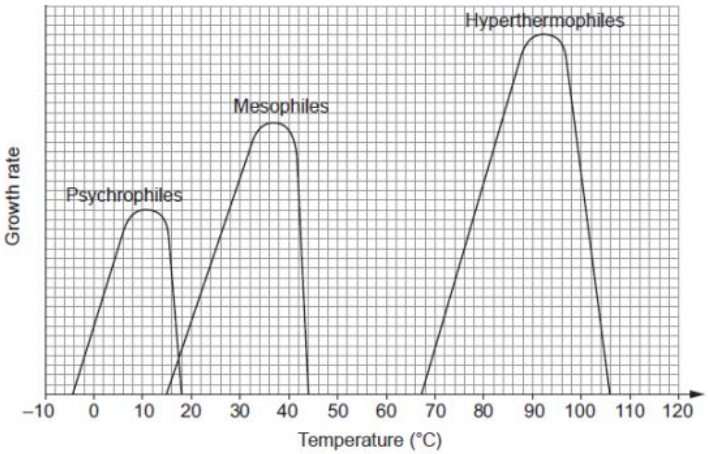
Culturing bacteria in the laboratory

Bacteria can be cultured on a nutrient rich agar jelly or in a nutrient broth. These growth media must include:

- ✓ A suitable **carbon source**, usually glucose or lactose
- ✓ A suitable **nitrogen source** such as ammonium or amino acids
- ✓ A source of sulphur and phosphorous
- ✓ Vitamins, minerals and growth factors
- ✓ A **suitable pH**
- ✓ The growth media should be incubated at a **suitable temperature** – 25^{o}C in the school laboratory

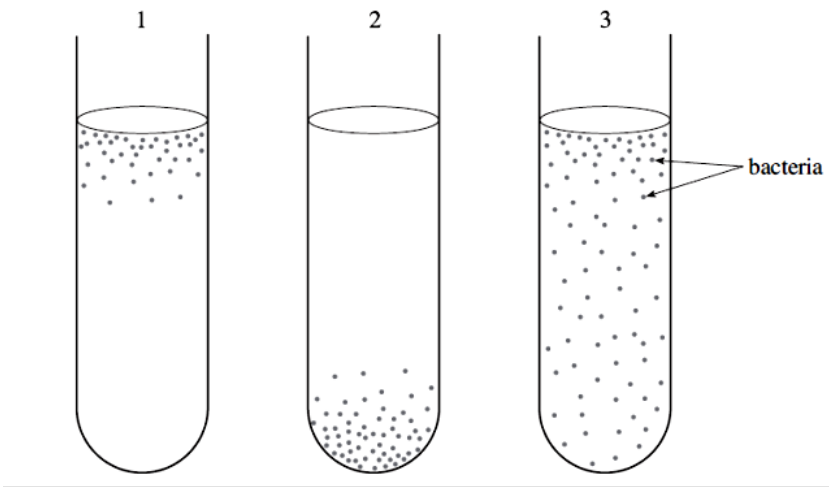
Different species vary in their requirements and usually grow over a range of temperature and pH values, with an optimum within the range.

Top Tip – To work out the optimal temperature **range** for each of these types of bacteria use a ruler to draw a line across the top of each peak. In this example the **Psychrophiles** have an optimal range of 9-12 ^{o}C (top of mountains and Arctic soil), **Mesophytes** 36-38 ^{o}C (inside human body) and **Hyperthermophiles** 91-94^{o}C (thermal vents and volcanoes).



Bacteria have different oxygen requirements

Obligate aerobe growth is inhibited in the absence of oxygen.
Obligate anaerobe growth is inhibited in the presence of oxygen.
Facultative anaerobes grow best in the presence of oxygen, but can respire anaerobically if they need to.



Top Tip – Look at the diagram above. First you should **describe** the distribution of bacteria, and then **explain** why.
Tube 1 – Bacteria are clustered at the top of the tube as they need oxygen for respiration and growth (oxygen diffuses into the growth medium from the air). Their **growth is inhibited in the absence of oxygen**, they must be obligate aerobes.
Tube 2 – Bacteria are only found at the bottom of the tube, away from the source of oxygen, they must be obligate anaerobes.
Tube 3 – Bacteria are spread throughout the tube, but are mainly found towards the top. These bacteria **can grow in the absence of oxygen, but grow best with oxygen,** they are facultative anaerobes.

Aseptic technique

Aseptic techniques are used to prevent:

- ✓ **Contamination of the environment by the microorganisms being handled.**
- ✓ **Contamination of the bacterial cultures by unwanted microorganisms from the environment.**

All equipment and growth media which come into contact with microorganisms being cultured (grown) must be **sterile**. Examples of sterilisation include:

- ✓ Passing metal transfer tools (such as inoculating loops) through a roaring/blue Bunsen flame until they **glow red**.
- ✓ Using pre-sterilised petri dishes.
- ✓ Sterilising any glassware used under high pressure and high temperature (121^{o}C) in a special piece of apparatus called an **autoclave** for 15 minutes.
- ✓ Heating the nutrient agar used for the plates in an autoclave to sterilise it before pouring it into the plates and letting it set.

Pouring a sterile agar plate using aseptic technique

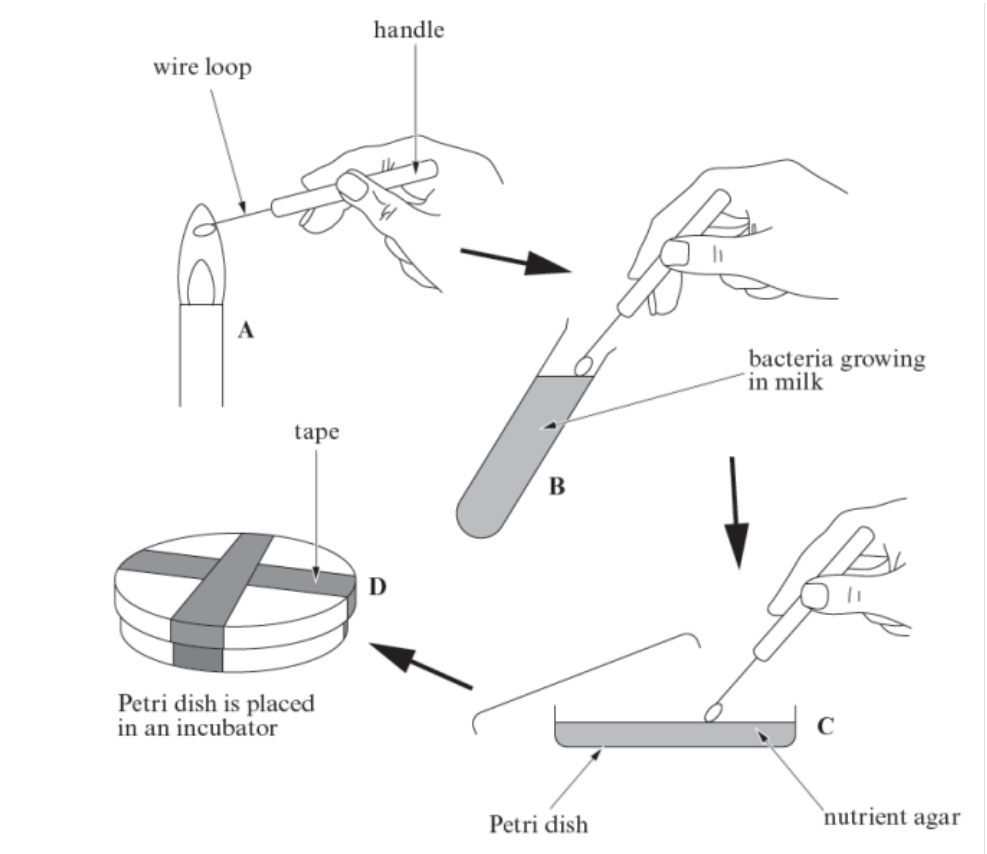
To prepare a sterile agar plate you would need a sterile Petri dish, molten nutrient agar which has been autoclaved, Bunsen burner and tape.

- ✓ Open the culture bottle cap using your little finger and do not place the cap or bottle on the bench.
- ✓ Flame the neck of the bottle in a blue Bunsen flame.
- ✓ Work close to the Bunsen flame as the updraft helps prevent contamination.
- ✓ Open the sterile Petri dish lid at an angle.
- ✓ Pour in the molten nutrient agar and close lid immediately. Swirl gently to remove air bubbles.
- ✓ Secure lid with tape.

Inoculating a set nutrient agar plate

This is how you inoculate a nutrient agar plate with bacteria growing in milk:

A – Sterilise the inoculating loop in a roaring blue Bunsen flame until it glows red.
B – Dip the inoculating loop into the milk sample.
C – Hold the Petri dish lid at an angle to reduce contamination by microorganisms in the air. Use the loop to spread the droplet of milk across the surface of the agar in a zig-zag pattern while rotating the plate.
D – Tape the lid shut to secure it in place; the lid should not be completely sealed as anaerobic conditions encourage pathogen growth. Incubate the plate at 25ºC – never 37ºC as this would also encourage pathogen growth.



Counting bacteria and estimating population size

Bacteria grown in liquid culture (nutrient broth) can be **counted directly** (by counting each cell) or **indirectly** by measuring turbidity (cloudiness of the culture medium) with a colorimeter.

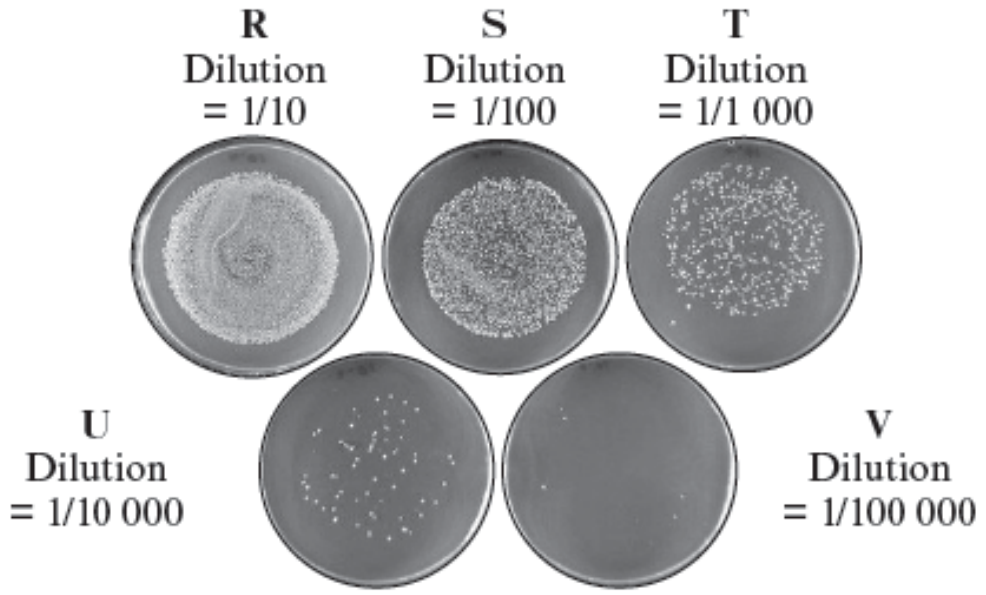
Direct counts are either total counts or viable counts.

- ✓ **Total counts** include both living and dead cells.
- ✓ **Viable counts** only count living or actively growing cells and therefore underestimates population size.

Viable count method

Viable counts count cells which are able to grow into visible colonies on an agar plate. Population density of the sample may be too high to count; if this is the case a dilution technique is used; this is called **serial dilution or dilution plating**. Here is the method:

- ✓ Fill 5 test tubes with 9 cm^3 sterile water using a sterile pipette.
- ✓ Add 1 cm^3 of your sample to the first tube; this is a 1 in 10 dilution or 10^{-1}.
- ✓ Mix the 10^{-1} dilution thoroughly and pipette 1 ml into the second test tube; this is a 1 in 100 or 10^{-2} dilution.
- ✓ Repeat this process until you reach a 1 in 10 000 dilution (10^{-4}).
- ✓ Plate each dilution on nutrient agar using aseptic techniques.



Look at the diagram above.

- ✓ On plates **R** and **S** it is impossible to distinguish individual colonies (colonies have merged together) and therefore it's impossible to count them.
- ✓ Plate **T** has too many colonies to count reliably.
- ✓ Plate **U** has enough distinct colonies, which are easy to count. This plate will give the most reliable results.
- ✓ Plate **V** does not have enough colonies for a reliable estimate.

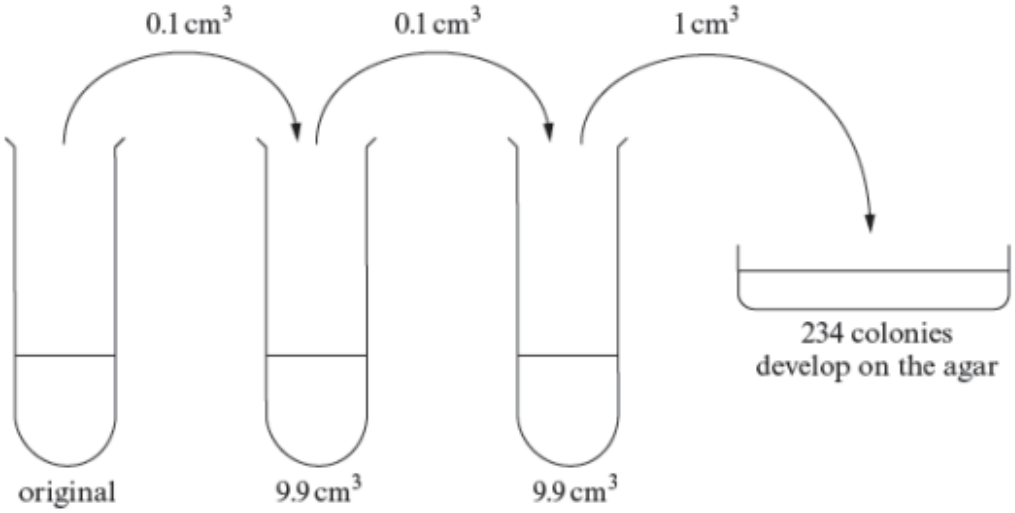
Estimating population size using dilution plating

On plate U **69 colonies were counted** (this is usually given in the question). The dilution factor for this plate is **1 in 10,000 or 10^{-4}** (you may have to calculate the dilution factor yourself).

To estimate the number of bacteria present in the original sample (before dilution) you need to multiply the **number of colonies counted** by the **dilution factor**. This is 69 colonies x 10,000 = 69,000 colonies in 0.5cm^3 of sample. Then multiply your answer by 2 to estimate the number of bacteria present in 1 cm^3 of the original sample.

This number is usually an **underestimate** as **it does not include dead or non-viable bacteria** and also we **cannot be sure that each colony has grown from a single bacterium** (bacterial cells may have been clumped together).

Here's another example. This time you need to calculate the dilution factor.



Each dilution this time is a factor of 100. The middle tube is a 1 in 100 dilution and the 3rd tube is a 1 in 10 000 dilution. Multiply the number of colonies (234) by the dilution factor (10 000).

234 x 10 000 = 2 340 000 colonies per 1 cm^3 of original sample

Top Tip – You must read each question carefully. Text and diagrams are there to help you. Keep your exam paper open as a double page spread and refer back to the question often.
The **assumption made during dilution plating** is that each colony has arisen from a single bacterium – this may not be the case due to clumping.

A **colony** is a cluster of cells (all clones) arising from a single bacterium or fungal spore.
A **pathogen** is a microorganism that causes disease in its host.
Aseptic technique is laboratory practice that maintains sterility of apparatus and prevents contamination.

Section 3.5 - Population size and ecosystems

Factors affecting population size

A **population** is a group of organisms of a single species interbreeding and occupying a particular area. The following factors determine the size of a population:

- ✓ **Birth rate** – number of new individuals produced by sexual or asexual reproduction per unit time.
- ✓ **Death rate** – number of individuals dying per unit time.
- ✓ **Immigration** – new individuals joining a population
- ✓ **Emigration** – individuals leaving a population

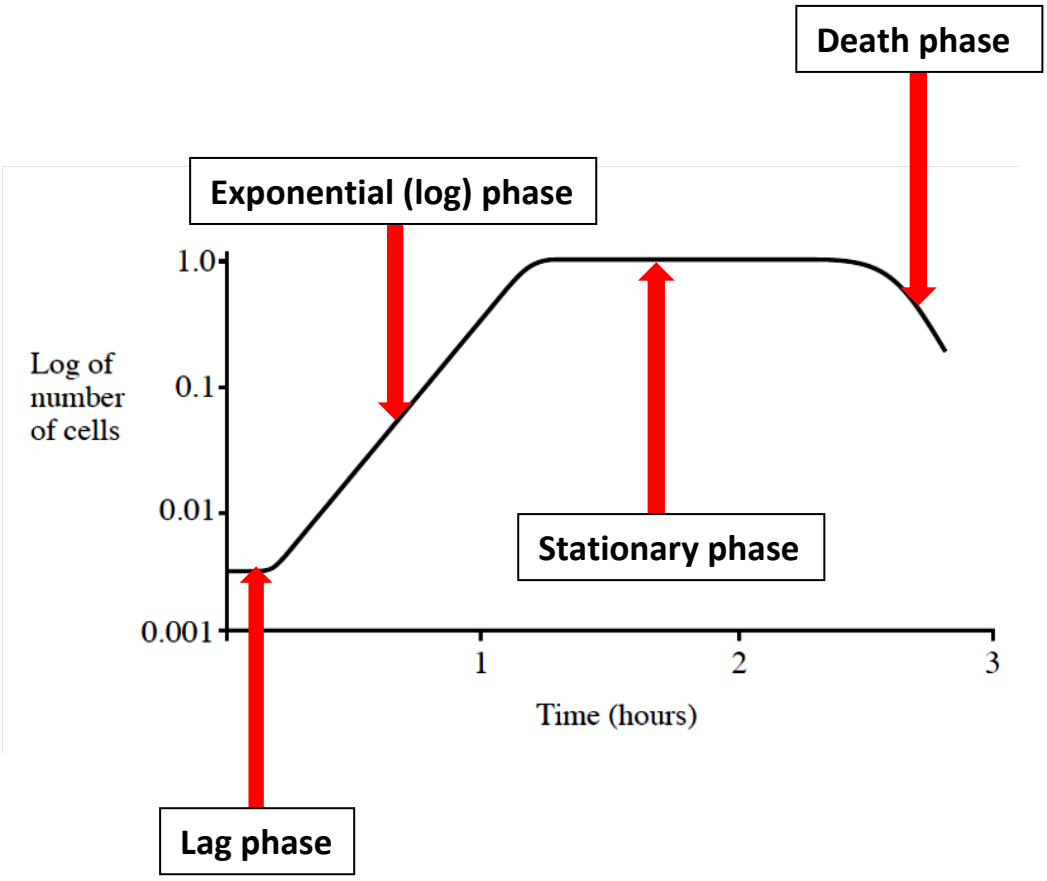
An **increase in birth rate and immigration** will increase the size of a population.
An **increase in death rate and emigration** will decrease the population size.

Strategies for population growth

Fugitive species cannot tolerate competition. To increase in numbers they reproduce rapidly and have effective dispersal (spreading) mechanisms. They are able to invade new environments rapidly – algae and weeds are great examples of fugitive species.

Equilibrium species control their population by competition within a stable habitat. Their usual pattern of growth is a sigmoid (S-shaped) curve called a **one-step growth curve**. Bacteria and rabbit populations show this kind of growth.

Population growth in a bacterial population



Lag phase – Bacteria adjust to their new environment and prepare for growth by synthesising enzymes.
Exponential (log) phase – Bacteria cells replicate exponentially (double per unit time). There are no limiting factors.
Stationary phase – Bacterial growth levels off as cell death equals the number of new cells produced by cell division. Factors such as nutrient supply have become limiting and waste products accumulate.
Death phase – Cell death exceeds cell division. Nutrients are now depleted and waste products have reached toxic levels which inhibit growth.

Top Tip – When describing a one-step growth curve you should be mindful of the type of organism you're discussing. Never use the term birth rate to describe any phase of bacterial growth; bacterial populations increase due to cell division, but rabbits give birth.

Environmental resistance

Environmental resistance include all the factors that may limit population growth. Some factors will be biotic (living) and some will be abiotic (non-living).

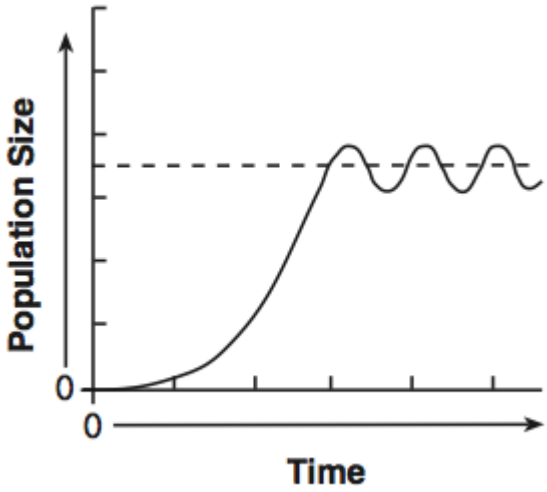
- ✓ **Abiotic factors** include temperature, pH, light intensity and water availability.
- ✓ **Biotic factors** include competition for limited resources between members of the same species (intraspecific competition) or competition between organisms of different species (interspecific competition). New diseases, parasitism and an increase in predator numbers are also biotic factors which limit population size.

Predator prey interactions

The number of prey can limit the population growth of a predator population. The number of predators can limit the population growth of a prey population. Predator-prey interactions cause both populations to oscillate due to **negative feedback mechanisms**.

Carrying capacity

The **carrying capacity** is the maximum population size of a species that an environment can sustain. On the bacterial growth curve the carrying capacity corresponds to the stationary phase. The **carrying capacity set point** is shown as a dashed line on the graph below.

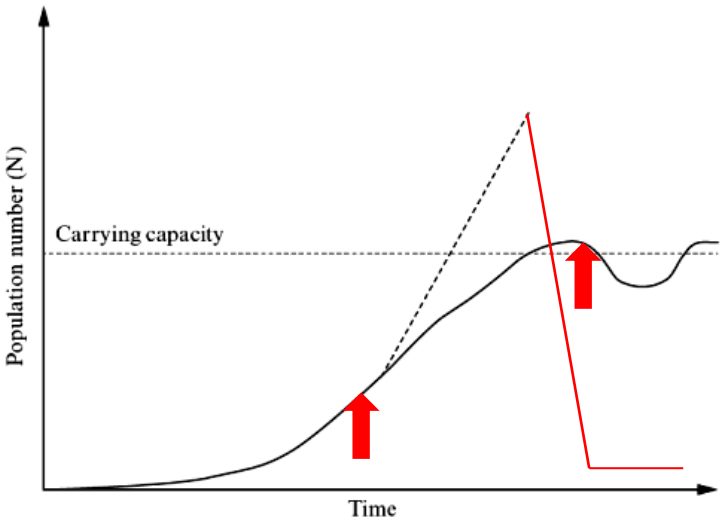


Population crash

A **population crash** is a sudden dramatic decrease in population number; it occurs when a population greatly exceeds its carrying capacity. After a population crash the carrying capacity set point for that environment will be much lower; possibly due to damage caused to the environment by overpopulation.

The growth curve for more complex organisms, such as mammals, can be represented by the following word equation:

$$Population\ growth = (Birth + Immigaration) - (Death + Emigration)$$



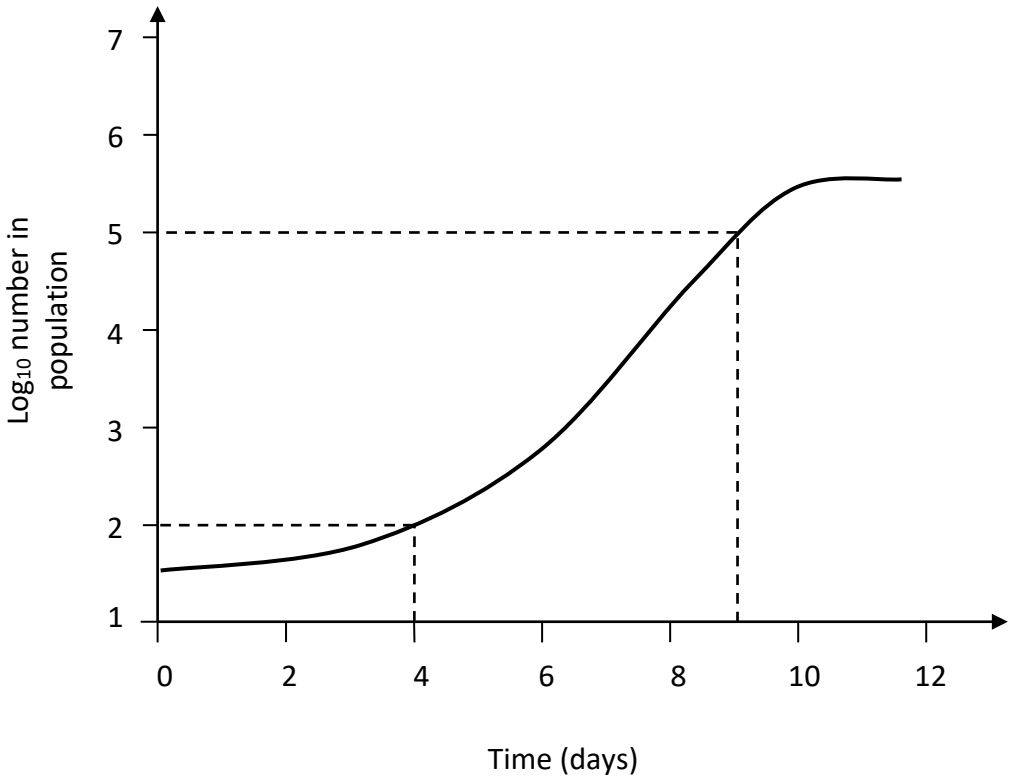
Look at the solid black line on the graph to the left. Arrow Nº 1 points to a population increase, therefore birth rate and immigration exceeds death rate and emigration – there are no limiting factors to population growth yet.

Arrow Nº 2 shows the point at which death and emigration exceed birth and immigration (this causes the dip in population).

The dashed black line shows the population greatly exceeding the carrying capacity of the environment – a population crash is likely to follow (shown in red).

Calculating population increase from a graph

Bacterial growth is plotted on a **$\log_{10}$ scale** as the numbers involved are too large for a linear scale. Each increment on the y-axis corresponds to a 10 fold increase in the population number.



Look at the graph above. To calculate the rate of growth (per day) during the exponential phase you need to use the following equation:

$$Growth\ rate = \frac{Number\ bacteria\ day\ 9 - Number\ bacteria\ day\ 4}{Number\ of\ days}\ per\ day$$

The problem is you can't simply read off the number of bacteria, at any given time, from a $\log_{10}$ scale – you must calculate the antilog first! If the log is a whole number this is easy:

- ✓ Antilog 5 = 10^5 = 100 000
- ✓ Antilog 2 = 10^2 = 100

Therefore:

$$Growth\ rate = \frac{100\,000 - 100}{9 - 4} = \frac{99\,900}{5} = 19\,980\ \ per\ day$$

Top Tip – If the $\log_{10}$ number is not a whole number you'll need to use your calculator functions. To find the antilog of 3.5 input the following into your calculator:

- ✓ Press Shift log
- ✓ Type in 3.5
- ✓ Press = to give the answer

Density dependent factors which can limit population size

The effect of **density dependent** factors increases as the density of the population increases, they include:

- ✓ Competition (intraspecific and interspecific)
- ✓ Predation
- ✓ Disease
- ✓ Parasitism

These factors limit the maximum size of the population; they determine the **carrying capacity**.

Density dependent factors weaken individuals and make them less likely to reproduce successfully.

Density independent factors

Density independent factors are abiotic (non-living) and are not linked to population density. Examples include:

- ✓ Earthquakes
- ✓ Tsunami
- ✓ Volcanic eruptions
- ✓ Extreme weather such as flood or drought
- ✓ Wildfires

Measuring abundance

Abundance is the number of individuals, of the same species, in a given area or volume.

Animal abundance can be assessed by:

- ✓ **Capture-mark-recapture** techniques. Individual organisms trapped or caught on day 1 are marked and then released. The same sampling technique is used on day 2. The following equation is used to determine population size:

$$Population\ size = \frac{Number\ on\ day\ 1\ \times\ Number\ on\ day\ 2}{Number\ in\ sample\ 2\ which\ are\ marked}$$

- ✓ **Kick sampling** in a stream and counting freshwater invertebrates.

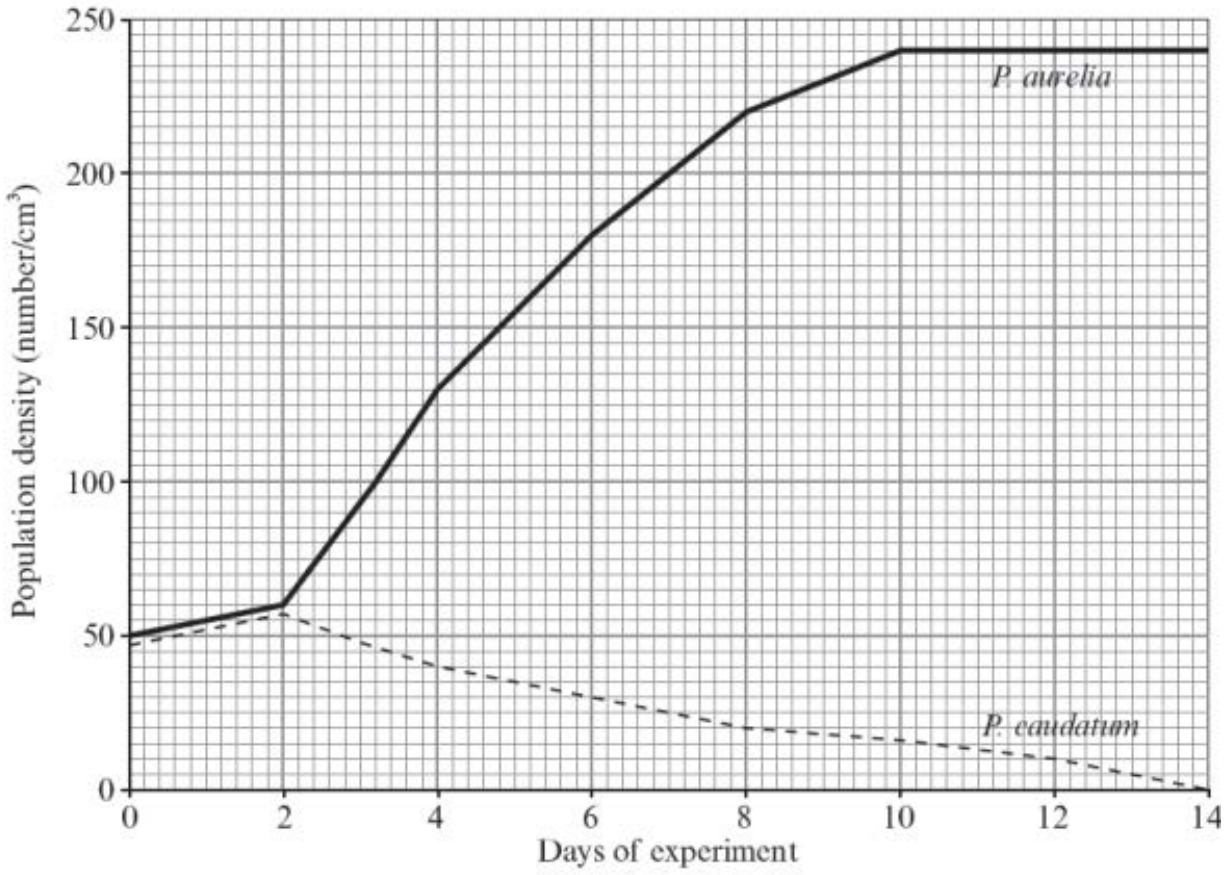
To calculate abundance in sessile organisms, such as plants, lichens or barnacles, the following methods can be used:

- ✓ **Random sampling** using a **quadrat** to find the **density** of organisms in a given area (number of organisms per m^2).
- ✓ **Systematic sampling,** using a **transect**, to determine changes in **percentage cover** of a species due to changes in abiotic factors e.g. salinity or light intensity.

Top Tip – Sampling techniques should have been covered and recorded in your lab-book. You should include this information in your revision notes. Remember 15% of the marks available in the Unit 3 exam will be based on your practical knowledge/skills. Get organised and don't ignore the content in your lab-books.

Competition between paramecium species

Two species of Paramecium, *P. aurelia* and *P. caudatum*, are cultured together with the bacterium *Bacillus pyocyaneus* (which is their food source). Their population densities were measured every two days and the results are plotted on the graph below:



This is an example of **interspecific competition** as two different species are competing for the same resource (food).

Between **Day 0 – 2** both populations increase slightly. At **Day 2** the populations diverge – *P. aurelia* has a competitive advantage as it is smaller and multiplies faster than *P caudatum*; the *P. aurelia* population density increases between **Day 2 and 10** and then levels off (this is the carrying capacity). The *P. caudatum* population density decreases between **Day 2 and 14**, eventually dying out completely.

This illustrates the **competitive exclusion principle**, which stares that when two species occur in the same habitat, one will outcompete the other; two species cannot occupy the same **niche**.

An organism's **niche** is its role or function within an ecosystem. Organisms cannot occupy exactly the same niche due to competition for resources.

Top Tip – If you are asked to **describe** the growth curve of an organism you simply say what you see, but in detail. Use the axes and the axes labels to help you. Split the curve into sections. Look at the example above.

Ecosystems

An **ecosystem** is a characteristic community of interdependent species and their habitat. It is comprised of living (biotic) and non-living (abiotic) elements and is dynamic (changes over time).

Energy flow through ecosystems

Energy flows through ecosystems through food chains. Here are some of the terms you must learn:

- ✓ **Producers** (trophic level 1) – Autotrophic organisms (plants and algae) which absorb light energy to convert simple inorganic compounds into more complex organic compounds such as carbohydrate.
- ✓ **Consumers** – Heterotrophic organisms which cannot fix carbon from inorganic sources like the producers do – they must ingest it or absorb organic carbon from other organisms.
- ✓ **Herbivores** (primary consumers at trophic level 2) – Animals which feed on organic matter produced by the producers.
- ✓ **Carnivores** – Feed on other animals at lower trophic levels.
- ✓ **Trophic level** – An organism's position within a food chain.
- ✓ **Detritivores** (earthworms, woodlice and maggots) feed on dead organic matter e.g. dead plants and animals.
- ✓ **Decomposers**, such as bacteria and fungi, break down organic compounds into simpler inorganic compounds, which are soluble and can be absorbed by plant roots.

Energy lost

Much of the energy which reaches the producers as sunlight is never absorbed and is therefore unavailable for photosynthesis. The mains reasons include:

- ✓ Light is reflected from the leaf surface
- ✓ Wrong wavelength of light which cannot be absorbed by the photosynthetic pigments
- ✓ Light passes through the leaf and does not hit the photosynthetic pigments

Much of the energy absorbed and fixed into carbohydrate by producers is **lost as heat** due to **respiration**. Energy incorporated into plant biomass (tissues) is not all available to the next trophic level. Cellulose in plant cell walls cannot be digested by herbivores as they do not produce the enzyme cellulase; this energy source is **lost as waste** (faeces), but is made available to the decomposers. Ultimately energy leaves an ecosystem as heat.

Energy loss at each trophic level limits the length of food chains to 4 or 5 steps; there is insufficient energy to support more trophic levels.

Photosynthetic efficiency

You may be asked to calculate photosynthetic efficiency (PE).

$$PE = \frac{Quantity\ of\ light\ energy\ incorporated\ into\ biomass}{Quantity\ of\ light\ energy\ hitting\ the\ leaf} \times 100$$

Gross primary productivity (GPP)

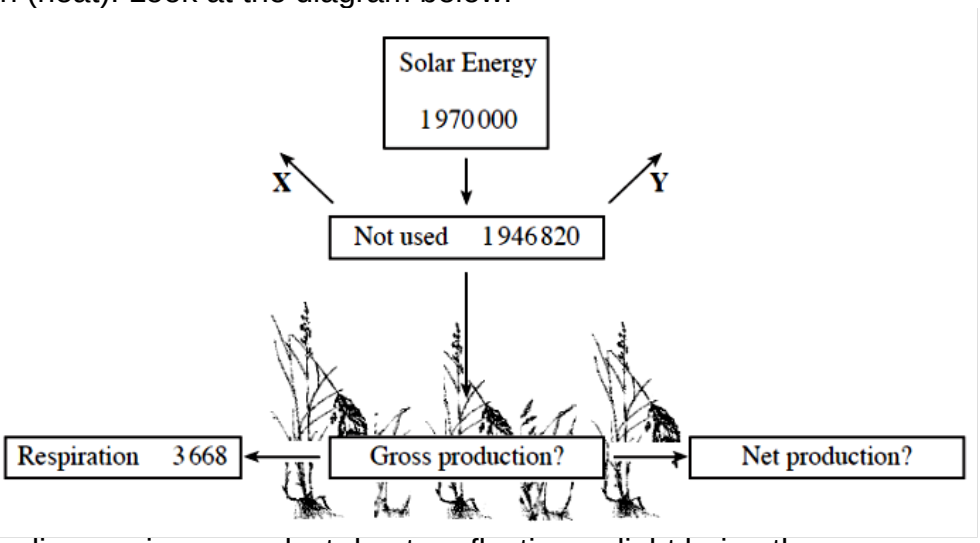
GPP is the **rate of production of chemical energy** in biological molecules **by photosynthesis** per unit area and time (**kJ m^{-2} y^{-1}**). Much of the GPP is used during respiration and some is lost as heat.

Net primary production (NPP)

NPP is the **energy in plant biomass** which **could pass to the primary consumers** at trophic level 2 during feeding. Not all NPP will be available to the herbivores as much of is inaccessible or indigestible – plant roots may not be easily reached and cellulose is indigestible as mammals cannot produce cellulase. NPP is measured in **kJ m^{-2} y^{-1}** too (you need to remember this).

Calculating NPP

You can calculate NPP from a simple flow diagram. You need the GPP and energy lost due to respiration (heat). Look at the diagram below:



X & **Y** on the diagram is energy lost due to reflection or light being the wrong wavelength and not absorbed by the photosynthetic pigments (see page 34). The remaining energy is absorbed and used by photosynthesis to produce carbohydrate and other biological molecules e.g. lipids and proteins – this is the GPP!

$$GPP = Solar\ energy\ hitting\ the\ leaf - Energy\ not\ used$$
$$= 1970\,000 - 1946820$$
$$= 23180\ kJ\ m^{-2}\ y^{-1}$$

Top Tip – Always show your working out, step by step, you may gain credit for your calculation even if your answer is incorrect. Also don't forget to include units!

The NPP is simply the GPP minus respiration.

$$NPP = GPP - Respiration$$
$$= 23180 - 3668$$
$$= 19512\ kJ\ m^{-2}\ y^{-1}$$

Secondary productivity

Secondary productivity is the rate at which **heterotrophs** accumulate energy in the form of new cells and tissues. Heterotrophs cannot fix carbon from inorganic sources like the producers do – they must ingest it or absorb organic carbon from other organisms (feed or consume). Heterotrophs include: animals, fungi, some bacteria and some protoctista.

Herbivores have a lower secondary productivity than carnivores; **carnivores are more efficient at energy conversion** than herbivores. A carnivore's protein-rich diet is more readily and efficiently digested and less energy is lost as waste.

Calculating the efficiency of energy transfer

The efficiency of energy transfer between trophic levels is calculated using the following equation:

$$Efficiency\ of\ energy\ transfer = \frac{Energy\ incorportated\ into\ biomass\ after\ transfer}{Energy\ available\ before\ transfer} \times 100$$

In the exam data is likely to be presented as a **food chain**. In the example below secondary consumers ingested 1603 kJ m^{-2} y^{-1} of energy by feeding on primary consumers; 88 kJ m^{-2} y^{-1} of this is transferred to the tertiary consumers and 192 kJ m^{-2} y^{-1} passes to the decomposers as waste. All you need to do is insert these numbers into the equation above.

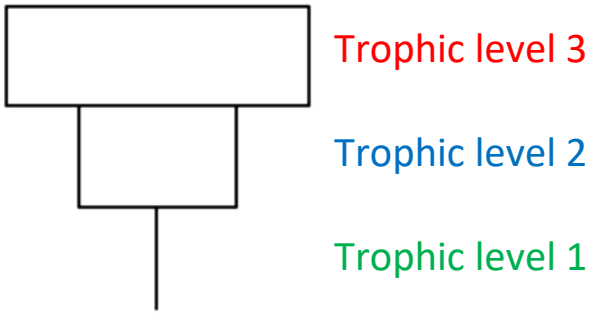
$$Efficiency\ of\ energy\ transfer = \frac{88\ + 192}{1603} \times\ 100$$
$$= 17.5\ \%\ (1dp)$$

Ecological pyramids

Food chains can be represented by **ecological pyramids**. The base of the pyramid will always represent the primary producer at trophic level 1. The consumer at the end of the food chain will always be at the apex of the pyramid.

Pyramids of numbers

Pyramids of numbers are easy to construct, but do not take into account size of the organism and as a result are often inverted (look at the diagram to the right) and are difficult to draw to scale. They provide no meaningful information about the amount of energy present at each trophic level.



Pyramids of biomass

Biomass is the **mass of biological tissue**. Biomass and energy content are related as the biological molecules that make up the tissues, such as carbohydrate, lipid and protein, all contain chemical energy.

Biomass is difficult to measure accurately e.g. roots may be difficult to harvest. Also not all biomass is available to the next trophic level e.g. bones and beaks have mass, but they may not be eaten, and therefore the energy they contain is not transferred.
Pyramids of biomass may be inverted too as no account is taken of reproductive rate or longevity.

Pyramids of energy

A pyramid of energy shows the **quantity of energy transferred** from one trophic level to the next, per unit area or volume, per unit time (kJ m^{-2} y^{-1}).

Pyramids of energy will never be inverted as energy is lost, due to heat from respirations and excretion of waste, at every step; this means that, as you move from the base to the apex, each block will be smaller than the one below (see the example below).

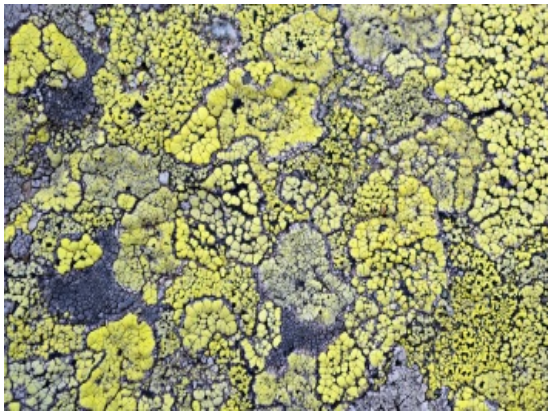
Pyramids of energy allow **comparison of the efficiency of energy transfer** between trophic levels in different communities.

Succession

Succession is a sequence of changes, in the composition of a community, over time. A succession will eventually lead to a stable **climax community**, which has high biodiversity and is highly productive. Each stage of a succession is called a **sere**.

Primary succession

Primary succession begins from bare rock or the site of a recent volcanic eruption. The first organisms to colonise the rock are the **pioneer species**, including lichens, mosses and algae.



Pioneer species, such as the lichens on the right, change the rock surface by penetrating it (forming tiny cracks) and allowing humus (which retains water) to accumulate. This simple soil allows grasses and ferns to colonise the area (see below).

Grasses and ferns further change the rock surface as their roots penetrate further and deeper. Death and decay over several generations allows more soil to accumulate and other higher plant species invade.

As the community of plants becomes more diverse other organisms take advantage of the new habitats and food sources; the diversity of plants and animals increases.
Eventually a **climax community** is established, where species are stable until the environment changes again. An example of a climax community would be woodland.

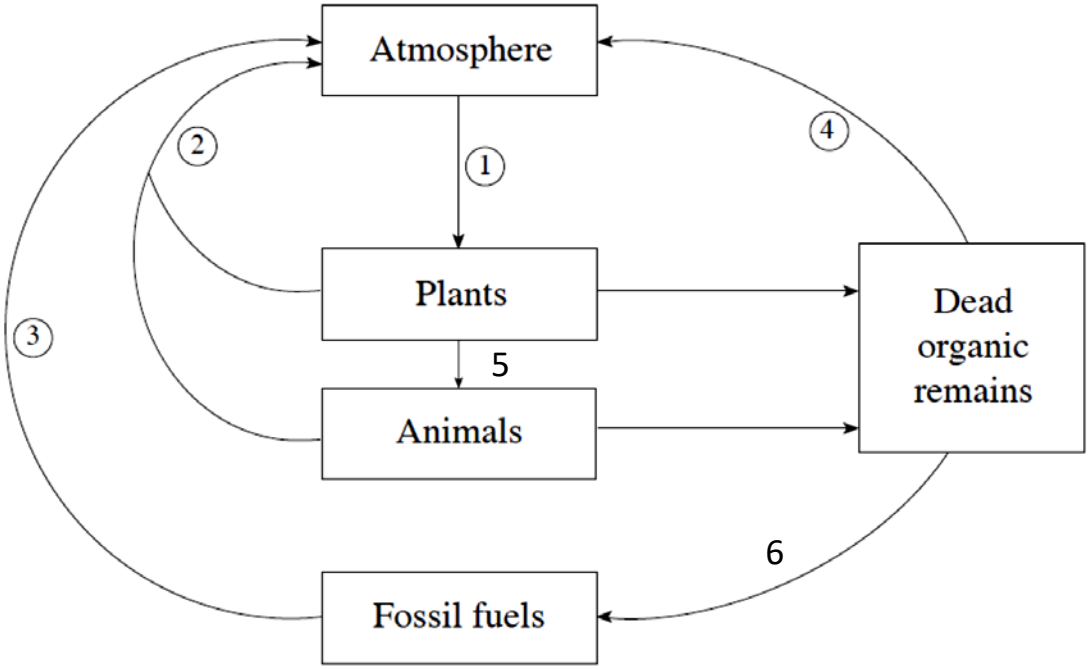
Secondary succession

A **secondary succession** begins from bare soil. A climax community will be achieved much faster as the soil is already present and it may contain viable bulbs, seeds and spores. Bare soil can be exposed after a wildfire.
Human activity may prevent a climax community being achieved. Examples of this include:

- ✓ Grazing sheep.
- ✓ Heather moorland management by controlled burning.
- ✓ Farming of land.
- ✓ Deforestation and soil erosion.

The carbon cycle

Carbon is a key atom as it is an essential component of biological molecules e.g. proteins, carbohydrates, lipids and nucleic acids.



1	Carbon dioxide from the atmosphere is fixed into carbohydrate by the light independent stage of **photosynthesis** (see section 3.2)
2	**Respiration**, in plants and animals, releases carbon dioxide into the atmosphere due to the action of decarboxylase in the link reaction and Krebs cycle (see section 3.3)
3	**Combustion** of fossil fuels releases carbon, in the form of carbon dioxide, into the atmosphere.
4	Microorganisms responsible for **decay** (decomposers) release carbon dioxide into the atmosphere due to respiration
5	Carbon fixed into organic molecules by producers pass from trophic level to trophic level, along **food chains**, during **feeding**
6	**Fossil fuels** are formed, over millions of years, from the remains of dead plants and animals; anaerobic conditions inhibit decay and the carbon rich biological molecules become fossil fuels such as oil, coal and gas over time.

Human activity is disrupting the balance of the carbon cycle due to:

- ✓ Deforestation (less carbon is fixed into carbohydrate by photosynthesis)
- ✓ Burning fossil fuels on a massive scale (more carbon is released due to combustion)
- ✓ An increase in decomposition (more carbon is released due to decomposition e.g. landfill sites)

The enhanced greenhouse effect

Increased atmospheric carbon dioxide leads to an **enhanced greenhouse effect**, commonly referred to as **global warming**. Global warming drives **climate change** which ultimately will affect the distribution of species and increase extinction rate. You should consider the possible global impacts of the following points on **biodiversity** and **agriculture**:

- ✓ Melting polar ice caps and rising sea levels
- ✓ Increased frequency of extreme weather
- ✓ Increased desertification and soil erosion
- ✓ Increased extinction rate
- ✓ Changes in the distribution of disease vectors such as mosquitos

Carbon footprint

This is defined as the **total amount of carbon dioxide** (or carbon equivalents) **produced directly due to the actions of an individual, product or service per year**.
Agriculture has a carbon footprint due to:

- ✓ The production of farm tools
- ✓ The production of insecticides, fungicides and fertilisers
- ✓ Farm machinery, powered by fossil fuels
- ✓ Transport of produce

Changes may need to be made to farming practices to reduce the carbon footprint.

- ✓ Produce less meat – meat production requires more resources (land, chemicals and feed) than crop production and therefore has a larger carbon footprint.
- ✓ Crops should be grown for human consumption, not as animal feed.
- ✓ Rice paddies produce methane (a greenhouse gas 25 times more potent than carbon dioxide), therefore alternatives should be found.
- ✓ Packaging should be reduced to a minimum.
- ✓ Transport distances (food miles) should be reduced and more food produced locally for local people.

The nitrogen cycle

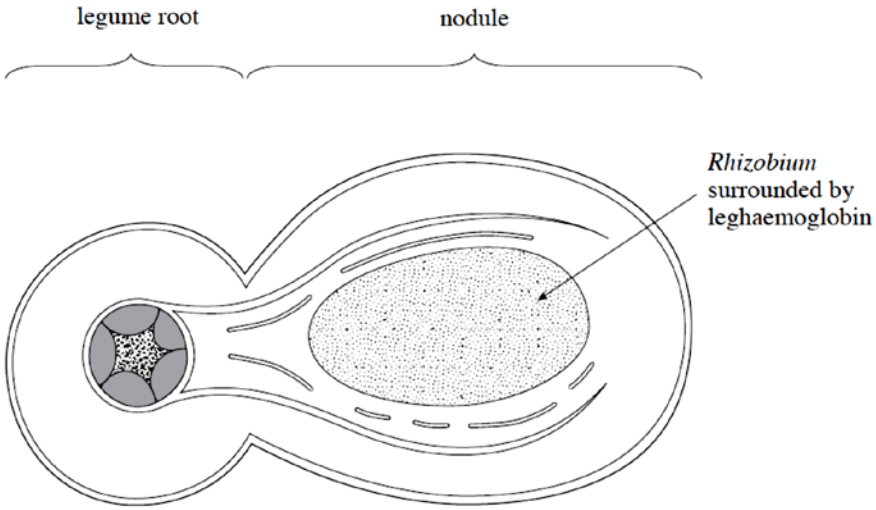
Nitrogen is another essential atom as it is a component part of nucleotides (ATP, DNA & RNA) and amino acids. Nitrogen is also found in chlorophyll!
The nitrogen cycle is the flow of inorganic and organic nitrogen within the abiotic and biotic elements of an ecosystem.
Plant roots can only absorb nitrogen as inorganic ammonium or nitrate ions (by active transport, against a concentration gradient). The nitrogen is then incorporated into the plants biological molecules and ultimately passed along food chains.
Microorganisms play a key role in providing theses inorganic ions:

- ✓ **Nitrogen fixing** – fixing atmospheric nitrogen gas into ammonia and ammonium ions
- ✓ **Nitrification** – converting the products of decay (ammonium ions) into nitrate ions

Nitrogen fixing

Atmospheric nitrogen can be fixed into soluble inorganic compounds by bacteria:

- ✓ **Azotobacter** – free-living bacterium found in soil. It is aerobic and fixes nitrogen gas into ammonium ions.
- ✓ **Rhizobium** is found in the root nodules of legumes (clover, pea & bean plants) and shares a symbiotic relationship with them. Rhizobium uses the enzyme **nitrogenase** to fix nitrogen gas into soluble ammonium. Nitrogenase activity is inhibited by oxygen, therefore the root nodules surround the bacteria with a layer of **leghaemoglobin,** which combines with oxygen, preventing it reaching the anaerobic rhizobium bacteria.



A **mutualistic** or **symbiotic** relationship is one where **both organisms derive benefit**. In this example ammonium, fixed by rhizobium bacteria, is absorbed by the plant roots and in return the legumes share the products of photosynthesis (sucrose & amino acids) with the rhizobium bacteria.

Nitrification

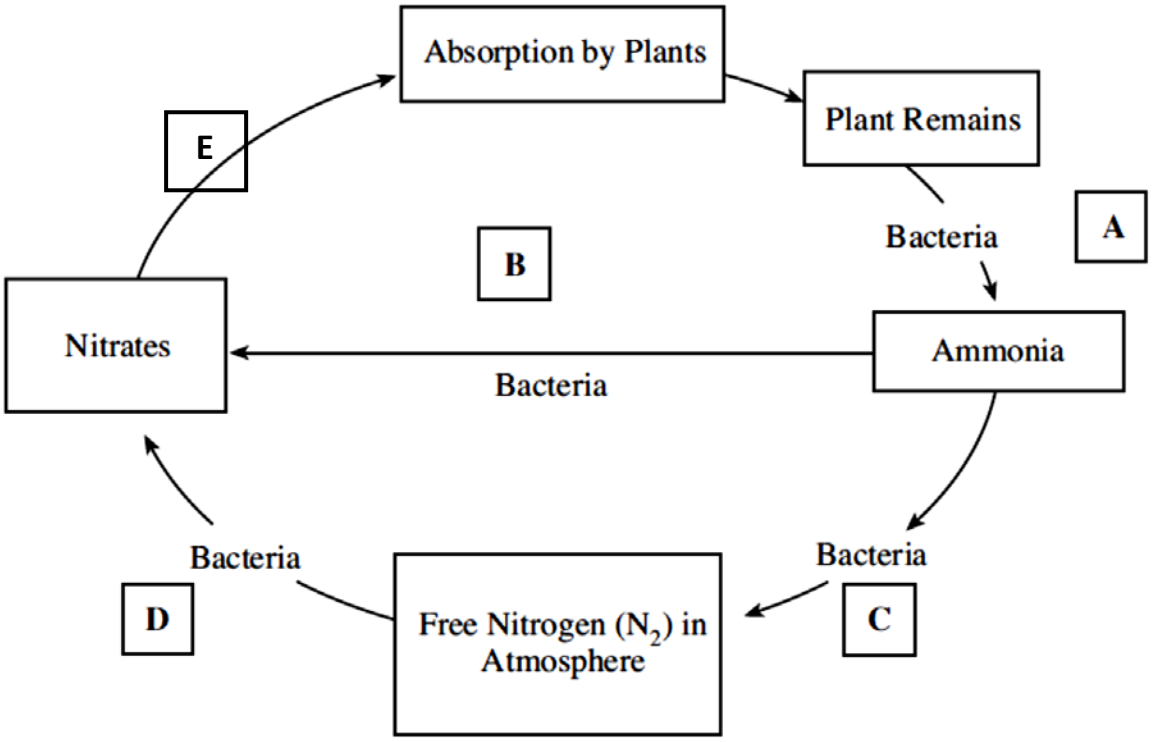
When an organism dies, bacteria and fungi release nitrogenous compounds during decomposition (decay). The products of **decomposition** (sometimes called **putrefaction**) are ammonium ions.

Other microorganisms, namely bacteria, convert ammonium into nitrite and then nitrate in a process called **nitrification**:

- ✓ **Nitrosomonas** (free living aerobic bacteria) convert ammonium into **nitrite**
- ✓ **Nitrobacter** (also free living and aerobic) convert nitrite into **nitrate**. Nitrate is then absorbed into plant root hair cells by active transport.

Denitrification

Denitrification is the loss of soluble nitrate compounds from the soil. Under anaerobic condition nitrate can be converted back into atmospheric nitrogen and lost from the soil – this decreases soil fertility and farmers try to avoid this by ploughing their fields. Ploughing mixes the soil with air; the oxygen from the air inhibits the denitrifying bacteria **pseudomonas** and encourages the growth of nitrosomonas and nitrobacter (nitrifying bacteria) and azotobacter (nitrogen fixing bacteria).



A	Decomposers (bacteria and fungi) break down large organic molecules in the plant remains into inorganic ammonium; this is **putrefaction or decay**
B	Nitrosomonas converts ammonia into nitrite, then nitrobacter converts nitrite into nitrates; this is **nitrification**
C	Inorganic nitrogen sources are converted into nitrogen gas by pseudomonas; this is an anaerobic process called **dentrification**
D	Azotobacter (free living, aerobic bacteria) and rhizobium (in symbiosis with legumes) fix free nitrogen gas into ammonium and nitrates; this process is called **nitrogen fixing**
E	Nitrates are absorbed by root hair cells by **active transport**, against a concentration gradient. Active transport requires ATP from the cell and has a high oxygen demand (for ATP synthesis by aerobic respiration)

Top Tip – The Unit 3 paper will be synoptic in nature. There are strong links here to biological molecules (Unit 1.1), membrane transport (Unit 1.3), enzymes (Unit 1.4), and nucleotide structure & protein synthesis (Unit 1.5). In Unit 2 you could look at biodiversity (Unit 2.1) and transport & circulation (Unit 2.3) and even digestion (Unit 2.4). Be prepared to build on your understanding and knowledge – make connections between AS and A2!

Human activities can affect the nitrogen cycle

Human activity can improve the availability of soluble nitrate, and therefore soil fertility, by:

- ✓ Adding chemical fertilisers (ammonium nitrate)
- ✓ Adding manure (animal waste)
- ✓ Adding treated sewage (human waste)
- ✓ Planting legumes such as clover
- ✓ Ploughing or draining to improve aeration

Human activity can also cause nitrogen pollution and reduce biodiversity:

- ✓ Excess nitrates on grassland leads to increased growth of weeds, such as nettles, this decreases biodiversity due to competition for resources
- ✓ Draining wetlands destroys unique habitats
- ✓ Nitrate pollution in waterways causes eutrophication – ultimately causing a decrease in dissolved oxygen and a decrease in biodiversity

Eutrophication

Chemical fertilisers, manure and slurry can be washed into waterways if used in excess or carelessly. This increases the soluble nitrate content of the water and increases algal and plant growth. An algal bloom may form, which covers the surface of the water, and blocks light at lower depths. Plants die as photosynthesis cannot take place. Aerobic bacteria decompose the dead plants and algae. As the bacteria multiply the dissolved oxygen concentration of the water drops, causing other organism to die due to suffocation. The water becomes anaerobic which encourages denitrifying bacterial growth – nitrate levels fall.

Section 3.6 – Human impact on the environment

Extinction

Extinction is the complete loss of a species. A species may become endangered or extinct for the following reasons:

- ✓ **Natural selection** due to changing selection pressures
- ✓ **Non-contiguous populations** – populations which are too small with insufficient genetic diversity to ensure a healthy and viable increase in number
- ✓ **Loss of habitat** e.g. deforestation, drainage of wetlands and loss of hedgerows
- ✓ **Overhunting by humans** (this includes overfishing)
- ✓ **Competition** from introduced (alien) species (interspecific competition)
- ✓ **Pollution** due to human activity e.g. oil and PCBs

The Sumatran orang-utan population is estimated to be 6 600 individuals, located in the north of the country. This species depends on high-quality primary forest and cannot tolerate habitat disturbance. The population is fragmented into 13 groups, only 6 having more than 250 individuals and therefore regarded as genetically viable in the long term.

Conservation

Conservation is the protection, preservation, management and restoration of natural habitats and their ecological communities. The aim is to maintain species and genetic biodiversity while allowing human activity to continue. Conservation can be achieved by:

- ✓ **Protecting habitats** e.g. SSSIs and Nature Reserves
- ✓ **International co-operation restricting trade** in endangered species and their parts e.g. ivory and the products of whaling (look at the **CITES** web site)
- ✓ **Gene & Sperm banks**
- ✓ **Seed banks** (Kew Gardens)
- ✓ **Rare breed societies**
- ✓ **Species reintroduction** e.g. the red kite in mid-Wales
- ✓ **International organisations** which organise publicity to educate and increase public awareness e.g. WWFN
- ✓ **Legislation** e.g. the EU Habitats Directive
- ✓ **Ecotourism**, which aims to educate, conserve and contribute to local economies

Maintaining biodiversity (both species and genetic) by conservation is essential for protecting potential sources of new crops for agriculture and new pharmaceuticals for medicine. A diversity of alleles will be essential in combating climate change; some alleles may provide a selective advantage to some individuals, thus preventing extinction. Conservation is also an ethical issue – each species, and their combination of genes, is unique and precious.

Agricultural exploitation

Farming practices have changed a great seal since the end of World War 2**. Intensive farming** has seen an increase in the use of chemical fertilisers, pesticides and herbicides. Mechanisation requires larger fields to accommodate large machinery; this has led to a reduction in the number of hedgerows. Hedgerows are an important habitat and their loss reduces biodiversity.

These larger fields are used to grow **monocultures**, in which a single crop, e.g. wheat or barley, is grown on a massive scale.

Monocultures provide only one type of habitat, which reduces biodiversity. Monocultures reduce soil fertility as roots grow to the same length and extract minerals from the same depth – this **increases the need for chemical fertilisers**.

Plants of the same species, grown so close together, are also susceptible to the same pests and diseases, which are able to pass from plant to plant rapidly. To combat this farmer uses **more pesticides**.

Monoculture is the growth of large numbers of genetically identical crop plants in a defined area.
Pesticides include chemicals which kill or inhibit the growth of weeds (herbicides), fungi (fungicides) and insects (insecticides).
Chemical fertilisers include the elements NPK and increase plant growth.

Overgrazing

Overgrazing land can cause soil compaction, reducing air spaces and inhibiting nitrogen fixing and nitrifying bacteria – leading to a loss of soil fertility (look back at Unit 3.5 on pages 39-43). Water is also unable to penetrate compacted soil and grass growth is inhibited.

Farming in the future

The following schemes and legislation aims to reverse the decline in biodiversity and soil fertility:

- ✓ **Organic farming** – reduces the need for chemical fertilisers and pesticides and allows for crop rotation on smaller fields. Different crops provide a variety of habitats and increase biodiversity.
- ✓ **Set-aside schemes** – farmers manage their farms for biodiversity; land is set aside for conservation and wildlife. Government grants compensate the farmer for loss of income.
- ✓ **Legislation e.g. the Environment Act 1995** – loss of hedgerows has been reversed. Hedgerows provide habitats and food sources for insects, birds and small mammals. They provide nesting sites and act as corridors allowing wildlife to move from area to area safely.

Deforestation

Deforestation is the complete loss of trees (due to human activity) in a defined area. The land is then used for agriculture, building or infrastructure. Trees are being cut down faster than forests can regenerate. Consequences of deforestation include:

- ✓ Soil erosion
- ✓ Lowland flooding
- ✓ Desertification
- ✓ Habitat loss
- ✓ Decrease in biodiversity
- ✓ Climate change

Forest management

Woodlands are highly productive and an important recourse for humans. Managed forestry involves sustainable replanting and regeneration. Methods include:

- ✓ **Coppicing** – tree trunks are cut at their base, leaving a stool (stump) a few centimetres above the soil. New shoots grow from the stool which can be harvested at different diameters for different purposes e.g. building, fencing or fire wood. Coppiced woodland at different stages provides a variety of habitats and increases biodiversity.
- ✓ **Selective cutting**
- ✓ **Long rotation time**

Good forest practice includes planting trees an optimum distance apart, controlling the spread of pests and disease, controlled timber cutting and protection of native woodlands.

Overfishing

Overfishing depletes fish stocks. Fish populations may become too low to recover – they are no longer viable. This also impacts food chains and entire ecosystems. Commercial fishing employs the following methods:

- ✓ **Drift netting** – A net, suspended from floats, is stretched between two boats. Thousands of miles of nets are set and non-target species are often caught e.g. dolphins and turtles.
- ✓ **Trawling** – Weighted nets are dragged across the ocean floor. This method catches everything and damages the ocean floor, decimating habitats for many miles.

To **preserve fish stocks** the fishing industry is regulated by:

- ✓ Regulating mesh size
- ✓ Quotas and landing size regulations
- ✓ Exclusion zones
- ✓ Marine stewardship council certification
- ✓ Legislation limiting the size of fishing fleets or controlling the number of days spent at sea
- ✓ Fishing alternative, non-traditional, species
- ✓ Using lines not nets

Fish farming

Fish farming is large-scale, **intensive farming** where fish are bred and mature in enclosed ponds. Food, predation, disease and parasites are controlled. Warm water is used to accelerate growth.

Disadvantages of intensive fish farming include:

- ✓ **Rapid spread of disease and parasites** due to the high density of the farmed population – this is controlled using antibiotics and pesticides and antibiotics which can enter the food chain. **Pesticides bioaccumulate**, causing a reduction in fertility at higher trophic levels.
- ✓ **Nitrogenous waste pollution** leading to eutrophication and dissolved oxygen depletion.
- ✓ The escape of farmed fish may **carry disease and parasites** to wild populations.
- ✓ Farmed fish are larger and **outcompete** wild fish for resources should they escape.
- ✓ Farmed fish, such as salmon, are fed on feed derived from other fish; this is a waste of resources.
- ✓ Farmed fish, such as salmon, contain high levels of toxic chemicals such as **methyl mercury, dioxins, pesticides and PCBs** (polychlorinated biphenyls).

Sustainability and decision-making

Monitoring allows us to determine the quality of the environment and also assess any decline in quality over time. The following factors can be monitored:

- ✓ Air quality
- ✓ Soil quality
- ✓ Water quality – including chemical, biological and microbiological aspects

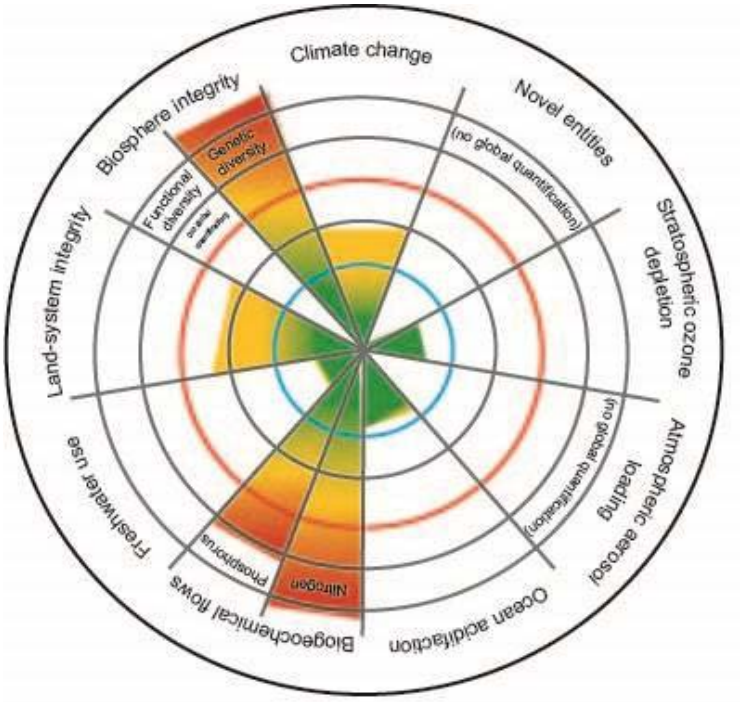
Environmental impact assessment

An environmental impact assessment is a document which uses data to predict the environmental effects of a proposed project. An environmental impact assessment should include:

- ✓ Description of the site and proposed project
- ✓ Description of abiotic and biotic factors
- ✓ **Mitigation** – ways of limiting environmental damage and maintaining biodiversity

Planetary boundaries

Planetary boundaries define the **safe operating space for humanity**. Should we exceed all 9 boundaries planet Earth may no longer be able to support a human population.



Look at the circular graphic to the left. Within the **inner blue circle** is the **safe operating space**. Between this and the **outer red circle** is an area of increasing risk (the **zone of uncertainty**). Beyond the red circle, the values represent **high risk**, the **planetary boundary has been crossed and events are unpredictable**. Our current boundary status is summarised in the table below:

Top Tip – The **WJEC website** has some truly excellent resources. To find the Planetary Boundary resource go to the **WJEC homepage**, scroll down to the bottom, you'll see a dark grey banner. Click on **Educational Resources**, select **Biology KS5** and click on the **Planetary Boundaries** box.

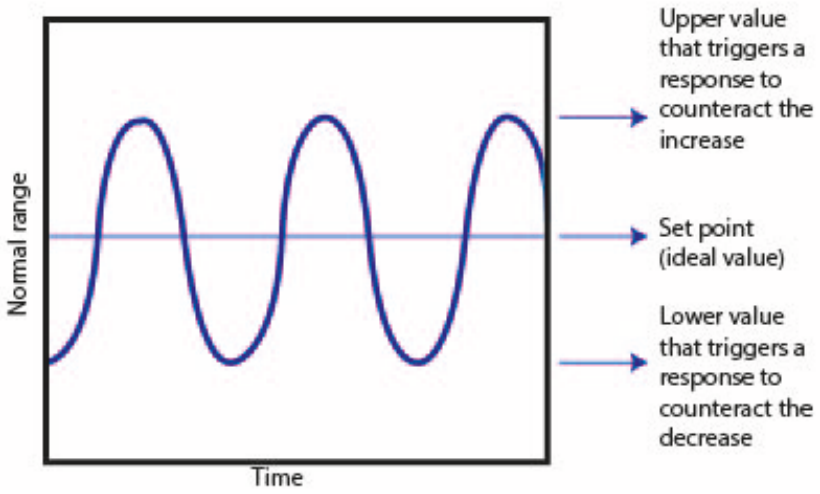
Boundary	Crossed	Avoidable	Not quantified	Avoided
Climate change	✓			
Biosphere integrity	✓			
Land system change	✓			
Biogeochemical flows	✓			
Ozone depletion in the stratosphere				✓
Ocean acidification		✓		
Fresh water use		✓		
Atmospheric aerosols			✓	
Introduction of novel entities			✓	

- ✓ **Climate change boundary** – Disruption of the carbon cycle due to burning fossil fuels, deforestation and increased decomposition. The release of carbon dioxide on a massive scale causes climate change due to enhanced global warming. See pages 39-40.
- ✓ **Biosphere integrity boundary** (biodiversity boundary) – Human activity introduces new selection pressures which decrease biodiversity in relation to natural selection and increases the rate of extinction. See page 44.
- ✓ **The land use boundary** – The balance between protection/conservation of habitats and human needs. Changes in farming practices, monoculture, the use of chemicals such as fertilisers and pesticides. See page 45.
- ✓ **The biogeochemical flows boundary** (nitrogen boundary) – Disruption of the nitrogen cycle due to inhibition of nitrogen fixing and nitrifying bacteria and denitrification in waterlogged or compacted soils. Eutrophication due to nitrogen pollution in waterways. See pages 40-43.
- ✓ **The stratospheric ozone boundary** – The only boundary avoided to date. In the stratosphere ultraviolet light causes CFCs to release chlorine as free radicals; free radicals break down ozone. The use of CFCs in aerosols and refrigeration has been reduced globally, reversing depletion of the ozone layer.
- ✓ **The ocean acidification boundary** – The pH of the ocean is decreasing due to increasing atmospheric carbon dioxide concentrations. Carbon dioxide in the air dissolves in bodies of water as hydrogen carbonate releasing hydrogen ions; this causes acidification and a decrease in pH. Fish gills are damaged by low pH, calcium carbonate also leaches from mollusc shells and arthropod exoskeletons (crabs, shrimps and lobsters) – this causes softening making them more vulnerable to physical and chemical attack.
- ✓ **The fresh water boundary** – All organisms need regular access to fresh water. Water sources include ice sheets, ice caps, glaciers, icebergs, ponds & lakes, rivers & streams and groundwater. Climate change and chemical pollution may limit freshwater sources. Desalination of sea water may be increasingly necessary.
- ✓ **The atmospheric aerosol boundary** – Aerosols are microscopic particles released into the atmosphere by the combustion of fuels and by dust from digging, mining and quarrying. These particles can cause respiratory problems and reduce photosynthesis, as leaves are coated with particles cannot absorb as much light. Sulphates in aerosols reflect sunlight, providing some cooling effect, but other particulates, such as soot absorb sunlight and reradiate it.
- ✓ **The introduction of novel entities boundary** – This was known as the chemical pollution boundary, but new technologies and materials release increasingly novel types of pollution. Organic pollutants, radioactive materials, nano-materials and plastics are included. Some chemicals are so toxic they have already been banned e.g. DDT and PCBs.

Section 3.7 – Homeostasis and the kidney

Homeostasis

Homeostasis is the maintenance of a constant internal environment by **negative feedback**. Homeostasis prevents wild fluctuations, beyond the optimal range, allowing cells and metabolism to function efficiently.
Core body temperature, pH and water potential may change due to changes in our activity or external environment, but they **fluctuate around a set point**. The body is kept in **dynamic equilibrium**; constant changes occur, but corrective mechanisms bring the internal environmental conditions back towards a **set point**.



Negative feedback

A **receptor** detects a deviation from the set point in the internal environment. The receptor sends instructions to a **co-ordinator** or controller. The co-ordinator communicates with one or more **effectors** which make responses which are corrective. The **factor returns to normal** (the set point), this is monitored by the receptor and information is **fed back** to the effectors, which stop making the correction.

Excretion

Excretion is the **removal of wastes** produced by the body **due to metabolism**. The mammalian body excretes compound using four excretory organs:

- ✓ **Lungs** – Carbon dioxide and water in expired air
- ✓ **Kidneys** – Urea, creatinine and uric acid in urine
- ✓ **Skin** – Urea in sweat
- ✓ **Liver** - Bile pigments in faeces

The kidney

The kidney has two main functions:

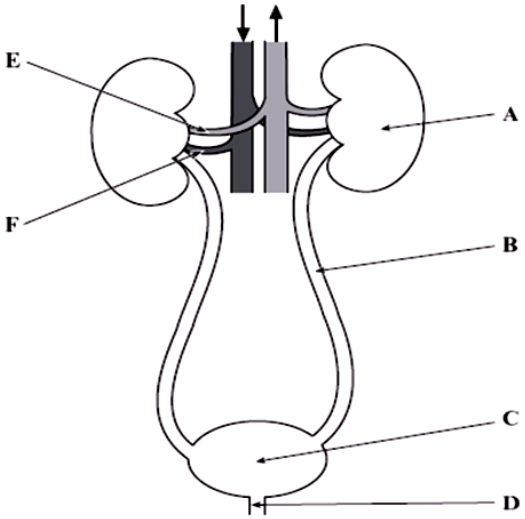
- ✓ **Excretion** – the removal of nitrogenous waste from the body
- ✓ **Osmoregulation** – the control of water potential of the body's fluids

Urea

Excess amino acids are **deaminated** in the liver; the amino group is removed and converted into ammonia (highly toxic) and then to urea (less toxic). Urea is removed by the kidneys.

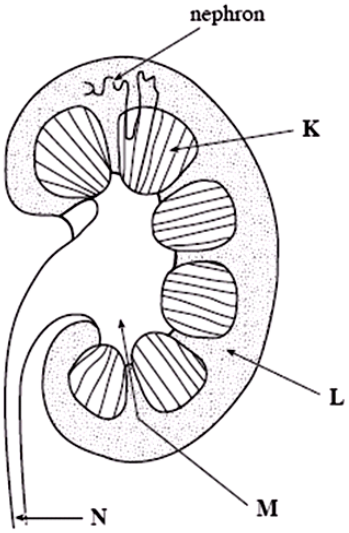
Kidney structure

Humans have two **kidneys**, one either side of the vertebral column. The kidney is enclosed in a tough renal capsule. Blood enter the kidney via the **renal artery** and leaves the kidney via the **renal vein**. You should be able to identify and label the structures shown below.



A – Kidney
B – Ureter (transports urine to the bladder)
C – Bladder (stores urine)
D – Urethra (carries urine out of the body)
E – Renal vein (blood returns to the general circulation)
F – Renal artery (blood enters the kidney)

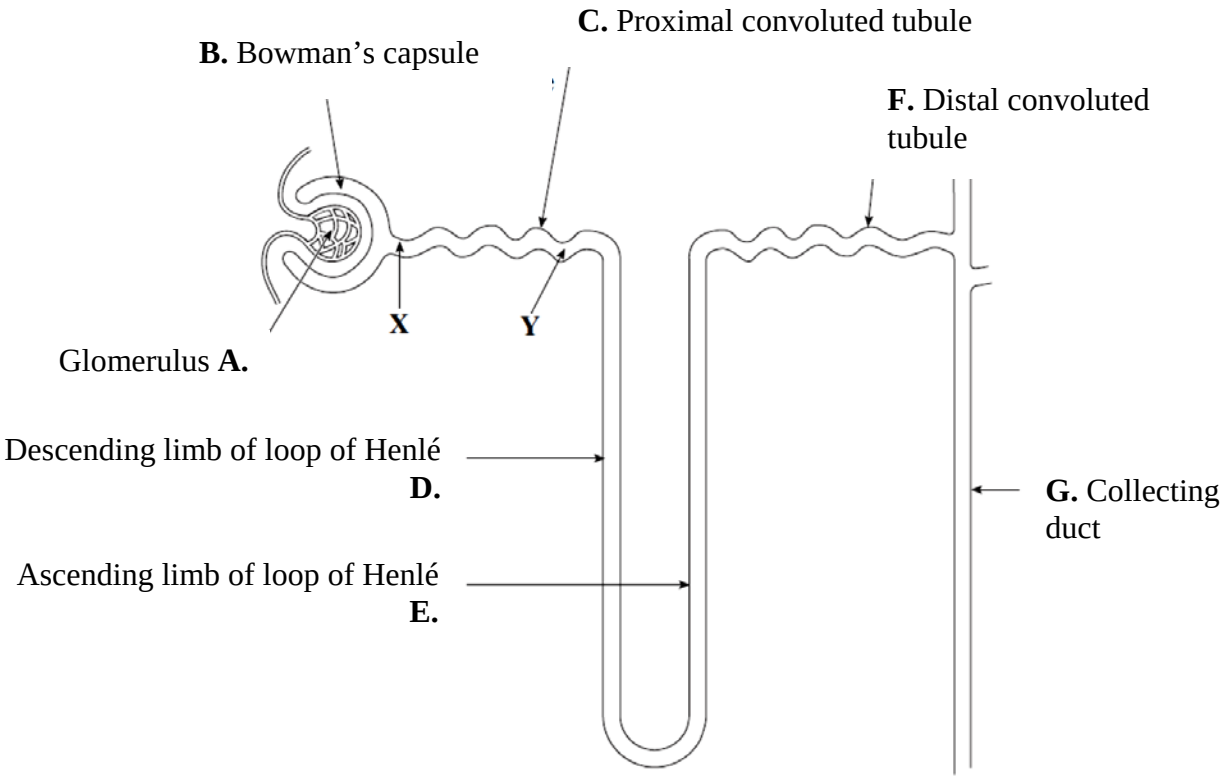
K – Medulla (reabsorption of water occurs here)
L – Cortex (ultrafiltration and selective reabsorption occurs in this region)
M – Pelvis (empties urine into the ureter)
N – Ureter (transports urine to the bladder)



Top Tip – Look at the section through a kidney shown on the right. Notice the position of the **nephron**. The nephron is the functional unit of the kidney and is highly adapted. There are a million nephrons in every kidney! You should practice drawing a nephron onto a kidney to ensure the relative position is correct.

The nephron

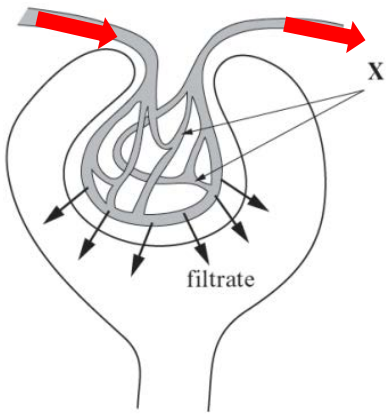
The nephron is the functional unit of the kidney. **Bowman's capsule** and the **proximal and distal convoluted tubules** are present in the cortex. The **loop of Henle** is found in the medulla.



Ultrafiltration

Ultrafiltration is filtration under high pressure. Small molecules and ions are forced into the tubule as filtrate. Large molecules (proteins) and blood cells cannot pass into the tubule as they are too large to be filtered.

Bowman's capsule (look at **B** above) and the capillary knot of the glomerulus (**A**) are responsible for ultrafiltration. **High hydrostatic pressure** is generated in the capillary knot as the blood capillaries narrow.



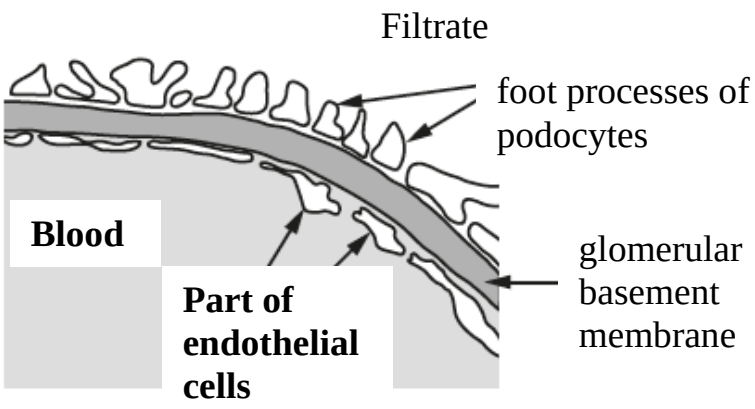
Blood enters the glomerulus (the capillary knot is shown as **X** to the left) via the **afferent arteriole** and leaves via the **efferent arteriole**. The afferent arteriole has a wider diameter than the efferent arteriole; this narrowing generates a high hydrostatic pressure. This provides the driving force for ultrafiltration. Small molecules pass through three filtration layers and enter the Bowman's capsule and tubule as filtrate.

Glomerular filtrate contains:

- Water
- Glucose
- Salts
- Urea
- Amino acids

The fine structure of the glomerulus and Bowman's capsule allows ultrafiltration to take place. The blood entering the glomerulus is separated from the **Bowman's space** by three layers:

- ✓ **Capillary walls** – The wall of the capillaries in the glomerulus is one cell layer thick – this is the **endothelium**. Tiny pores between cells, called **fenestrations** (80nm in diameter), allow solutes to pass to the basement membrane.
- ✓ **Basement membrane** – This is a **selective molecular filter** which only allows small molecules to pass through; blood cells, platelets and large proteins, such as antibodies, are too large.
- ✓ **Squamous epithelial cell layer of the Bowman's capsule (podocytes)** – **Podocytes** have extensions, called **pedicels**, which wrap around a capillary, pulling it closer to the basement membrane. The gaps between the pedicels are called **filtration slits**.



The **high hydrostatic pressure**, generated by narrowing of the capillaries in the capillary knot of the glomerulus, forces small molecules through the **fenestrations** of the endothelial cells, through the selective molecular filter of the **basement membrane** and finally through the **filtration slits** of the pedicels into the Bowman's space – this is glomerular filtrate.

Calculating filtration rate

About 20% of the blood that leaves the heart enters the kidneys. The rate at which fluid passes from the blood in the capillary knot (glomerulus) into the Bowman's space is the **filtration rate**. This is calculated by:

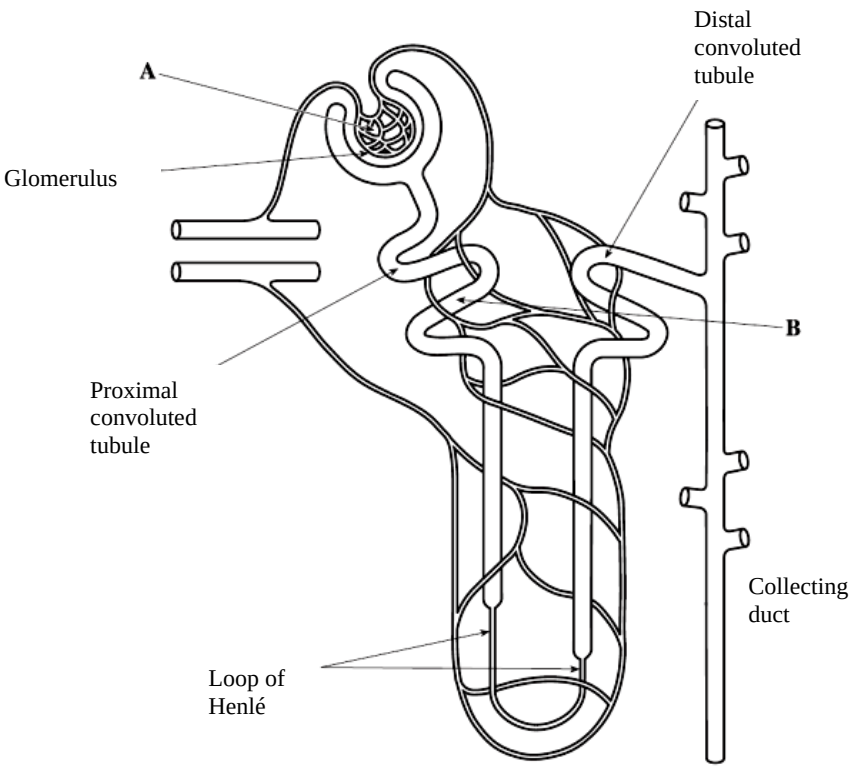
$$\%\ Blood\ filtered = \frac{Volume\ of\ filtrate\ produced\ per\ minute}{Volume\ of\ blood\ entering\ kidneys\ per\ minute} \times 100$$

Top Tip – The kidneys of an adult receive 1.1 $dm^3\ min^{-1}$ of blood and produce 125 $cm^3\ min^{-1}$ of glomerular filtrate. Before you even begin to plug the numbers into the equation above you must make sure the units are consistent (match) e.g. all in $cm^3\ min^{-1}$.

$$\%\ Blood\ filtered = \frac{125}{1100} \times 100$$
$$= 11.4\%\ (1dp)$$

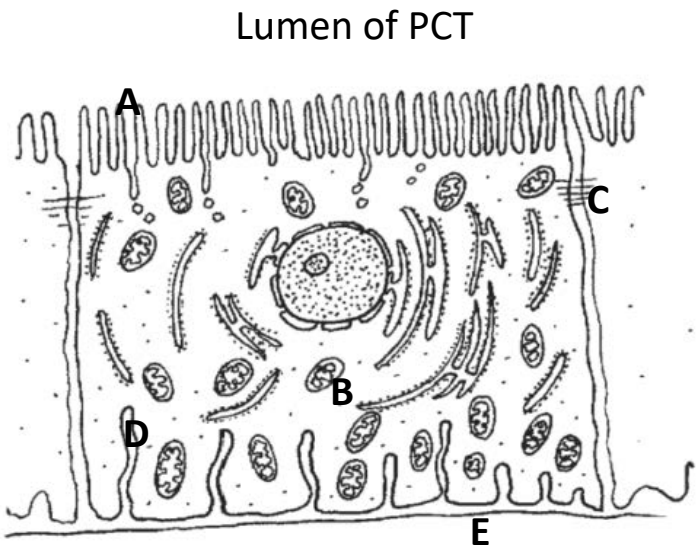
Selective reabsorption by the proximal convoluted tubule

Selective reabsorption is the process by which **useful substances such as glucose, amino acids and salts are reabsorbed back into the blood plasma**. This takes place in the proximal convoluted tubule (in the cortex) by facilitated diffusion and active transport.



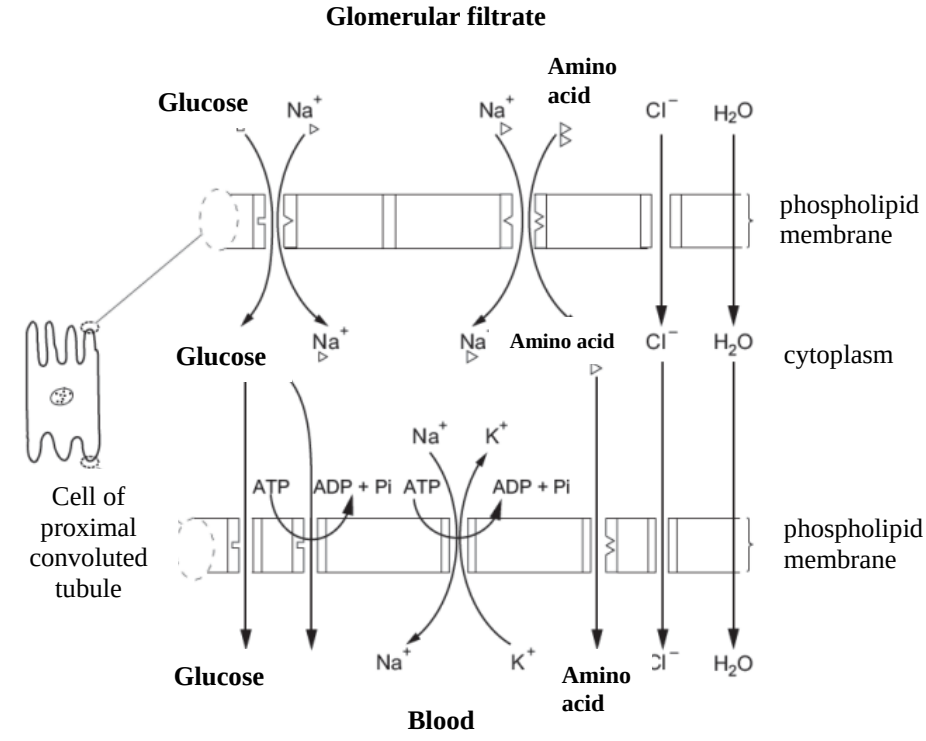
As you can see from the diagram on the left, the nephron is closely associated with blood capillaries called the **vasa recta**. Reabsorbed substances pass from the proximal convoluted tubule into the blood plasma contained in the vasa recta capillaries. The cells lining the wall of the proximal convoluted tubule are **highly specialised cuboidal epithelial cells** (look at the diagram below). The composition of filtrate at the beginning of the proximal convoluted tubule (PCT) will be very different to that at the end of the PCT because useful solutes have been reabsorbed.

The specialised cuboidal epithelium cell to the right has **microvilli** (**A**) protruding into the lumen of the PCT to increase surface area for selective reabsorption. There are **many mitochondria** (**B**) producing ATP for active transport. **Tight junctions between cells** (**C**) to hold neighbouring cells together closely to prevent molecules diffusing between adjacent cells (in either direction). **Basal channels** (**D**) also increase surface area of the cell membrane at the **basement membrane** (**E**). The cells are **closely associated with the blood capillaries** of the vasa recta.



This is how molecules are reabsorbed:

- ✓ **Salts** – Mainly active transport, but some by facilitated diffusion
- ✓ **Glucose & amino acids** – Cotransport with sodium ions into the cell (see the diagram below)
- ✓ **Water** – Osmosis
- ✓ **Urea and small proteins** – Facilitated diffusion



Selective reabsorption is the uptake of specific molecules from the glomerular filtrate into the bloodstream.
Cotransport is the transport of molecules or ions together through the same transport protein (look and glucose & amino acids entering the cell with Na^+ to the left).
Secondary active transport is the coupling of diffusion, down an electrochemical gradient, providing energy for active transport (look at glucose leaving the cell to the left).

Look at the diagram above. This is easier than it looks – just say what you see. Glucose and amino acids enter the cell from the lumen of the PCT by cotransport with sodium ions. Chloride ions enter by facilitated diffusion and water by osmosis. Once inside the cell they diffuse across the cell cytoplasm towards the opposite cell membrane. Glucose leaves the cell by facilitated diffusion and **secondary active transport** via a carrier and pump respectively. Sodium ions leave by active transport via a sodium-potassium pump. Amino acids and chloride ions leave by facilitated diffusion and water by osmosis.

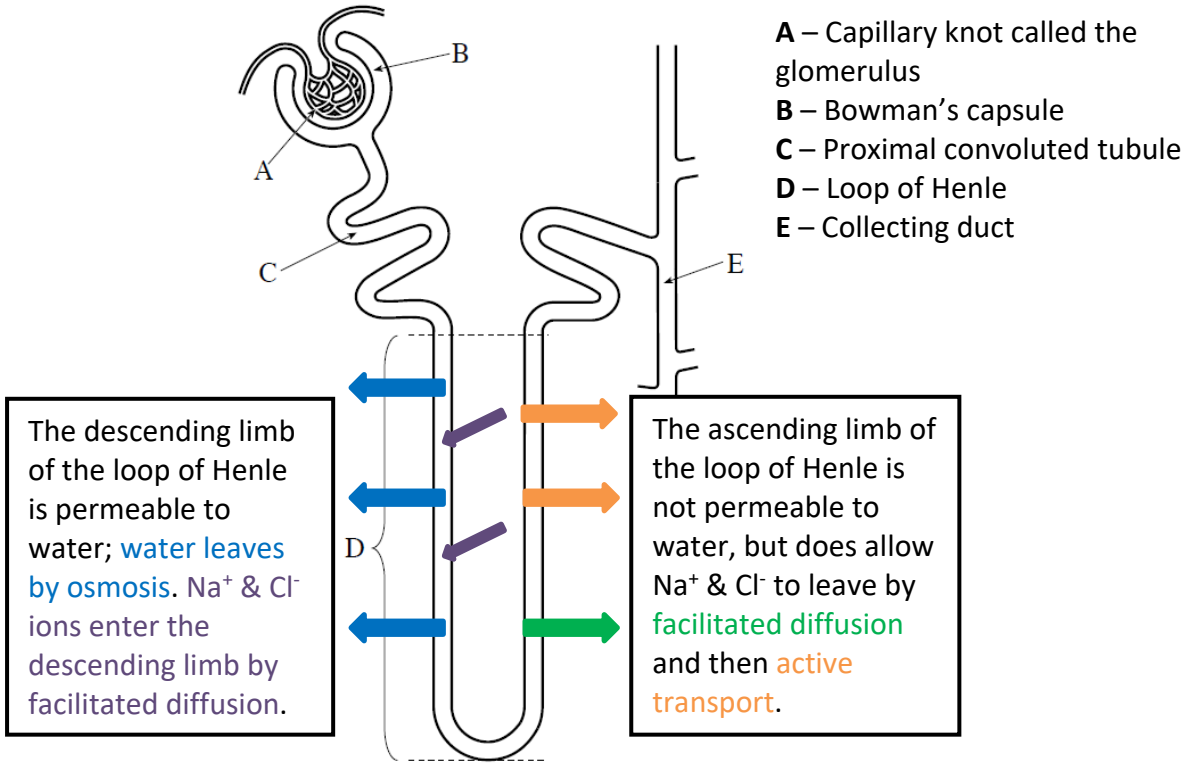
The glucose threshold

Glucose is essential for respiration and is usually **all** reabsorbed by the PCT and re-enters the blood stream via the vasa recta. If the concentration of glucose in the filtrate is too high intrinsic transport proteins may become limiting (there are not enough of them), which means that not all the glucose will be reabsorbed. Glucose will remain in the filtrate and pass out of the body in urine. This may occur if a person secretes too little insulin (diabetes type 1) or liver cells no longer respond to insulin (diabetes type 2 or gestational diabetes in some pregnant women).

Reabsorption of water by the loop of Henle

Filtrate leaves the PCT and enters the **descending limb of the loop of Henle** (look at **D** below). The descending limb is permeable to water and water leaves the filtrate and enters the blood by osmosis, down a water potential gradient. At the same time Na^+ & Cl^- ions diffuse into the descending limb from the medulla.

The medulla has a low water potential which is maintained by the **ascending limb of the loop of Henle** expelling Na^+ & Cl^- by facilitated diffusion and then active transport. As water leaves the descending limb by osmosis the filtrate becomes more concentrated, reaching maximum concentration at the apex of the loop. The ascending limb is impermeable to water, but is permeable to Na^+ & Cl^-. Initially Na^+ & Cl^- leaves the ascending limb by facilitated diffusion, but later as the concentration of solutes decreases (as Na^+ & Cl^- leave the filtrate), active transport takes over the expulsion of Na^+ & Cl^- into the tissue fluid of the medulla.



A – Capillary knot called the glomerulus
B – Bowman's capsule
C – Proximal convoluted tubule
D – Loop of Henle
E – Collecting duct

The loop of Henle is called a **counter current multiplier** because the filtrate flows in opposite directions (a counter current) and the concentration of solutes in the filtrate increases towards the apex (is multiplied); the longer the loop of Henle the higher the concentration of solutes at the apex.

Variation in loop length

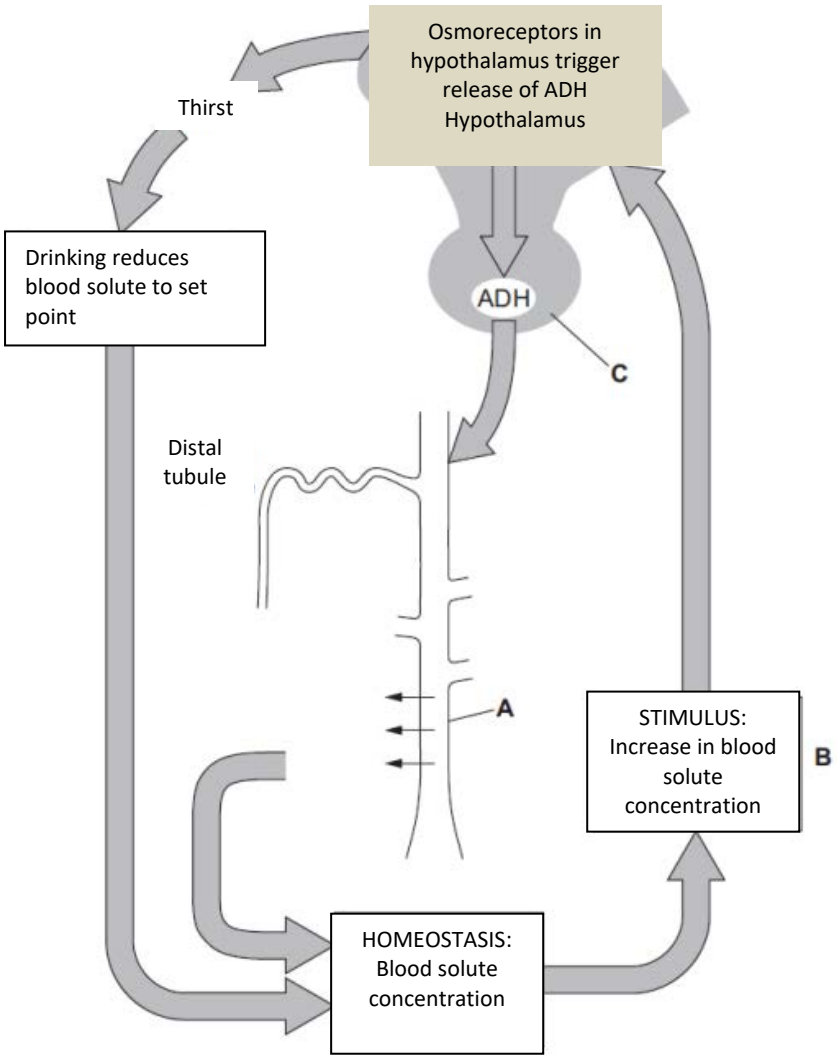
Animals with long loops of Henle are adapted to dry environments, such as the desert e.g. camels. Animals with short loops live in fresh water environments e.g. otters. The longer the loop the more ions can be pumped into the medulla. This lowers the water potential of the medulla further allowing more water to be reabsorbed into the bloodstream by osmosis.

Osmoregulation

Osmoregulation Is the control of body fluid water potential by negative feedback – this is a type of homeostasis. Osmoregulation is under hormonal control. **Osmoreceptors** (detectors) in the **hypothalamus** detect a decrease in blood plasma water potential. A signal is sent to the **posterior lobe of the pituitary gland** (the co-ordinator – look at **C** below) which releases the hormone ADH into the bloodstream. ADH is carried to the kidneys and binds to receptor proteins on the wall of the collecting duct (**A**) and distal convoluted tubule (these are the effectors). **Aquaporins** are added to the cell membranes of the effectors allowing more water to be reabsorbed by osmosis. This increases the water potential of the blood back towards the set point. This information is fed back to the hypothalamus and less ADH (or no ADH) is produced.

ADH increases the permeability of the collecting duct and distal convoluted tubule allowing more water to be reabsorbed; urine produced will be more concentrated and a lower volume.

If insufficient water can be reabsorbed due to the action of ADH to restore the water potential of the plasma, thirst (triggered by the hypothalamus) will encourage the individual to drink more fluids too (look at the diagram below).

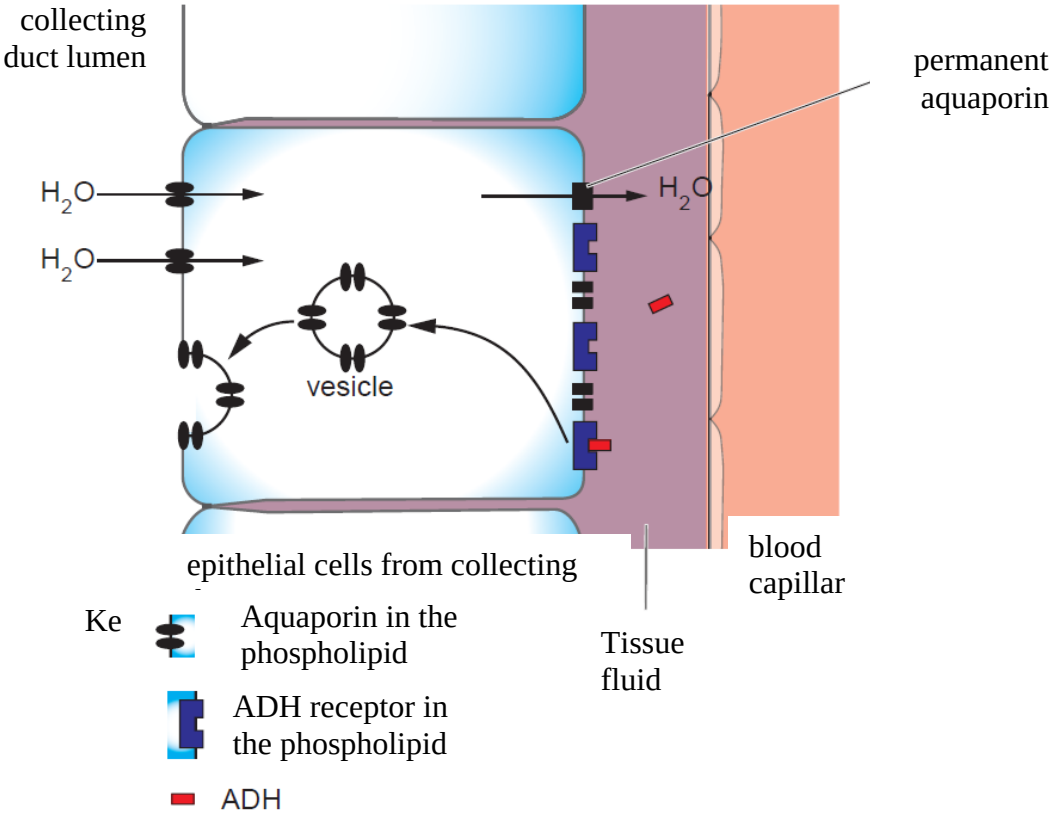


Antidiuretic hormone (ADH) is produced in the hypothalamus and secreted by the posterior lobe of the pituitary gland. ADH increases the permeability of cells of the collecting duct and distal convoluted tubule to water, allowing more water to be reabsorbed by osmosis. **Aquaporins** are intrinsic protein channels which transport water across the phospholipid bilayer during osmosis.

Increasing the permeability of cell membranes using aquaporins

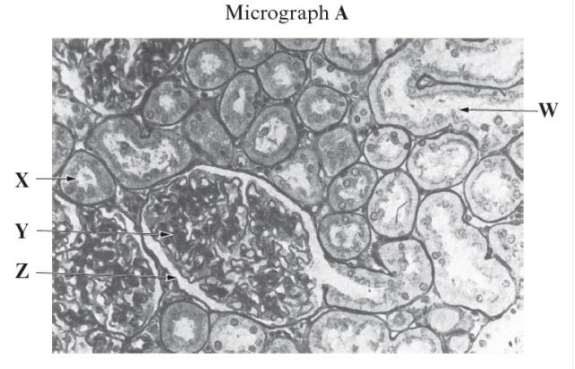
ADH binds to **ADH receptor proteins** in the phospholipid bilayer of the cell membrane. Vesicles containing aquaporins fuse with the cell membrane – increasing the number of available aquaporins for osmosis. This increases the permeability of the cell membrane to water, allowing more water to be reabsorbed into the blood plamsa of the vasa recta capillaries.

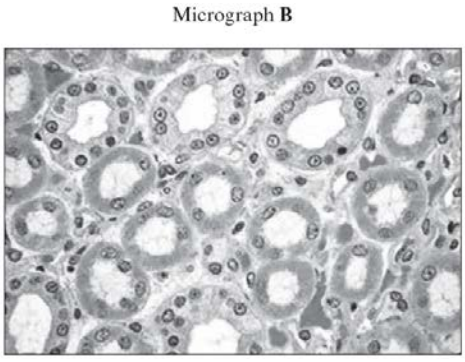
This is reversible, when ADH is released from the receptors the cell membrane folds, forming **aquaporin vesicles**, thus reducing the number of aquaporins and reducing permeability.



Identifying regions of the kidney using a microscope

You must be able to identify micrographs of different regions of the kidney and justify your decision. **Micrograph A** shows a Bowman's capsule (**Z**) and capillary knot (**Y**) and therefore must be a section through the cortex. **W** & **Z** are convoluted tubules. In **micrograph B** there are only tubules, which must be loops of Henle and collecting ducts – this is the medulla.



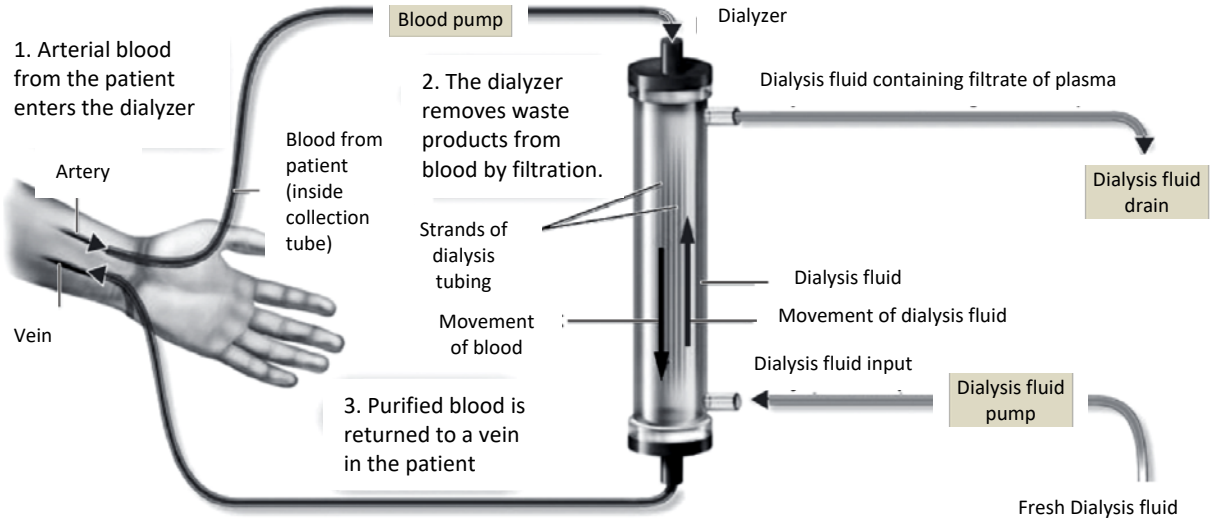


Kidney failure and treatment

If both kidneys fail treatment will be needed to balance fluids in the blood and remove waste. Medication can be taken to control blood potassium and calcium levels. A low protein diet will reduce the need for deamination in the liver and less urea will be produced. Drugs can be used to reduce blood pressure. **Dialysis** and a **kidney transplant** may also be needed.

Dialysis

A dialysis machine removes waste products and excess salts from the blood; this type of dialysis is called **haemodialysis**. Blood is taken from an artery in the arm (**1**) and is passed through thousands of long narrow strands of selectively permeable dialysis tubing. The fibres are surrounded by dialysis fluid. Waste products pass out of the blood plasma, through the pores in the dialysis tubing, into the dialysis fluid (**2**). The fluid flows in the opposite direction to the blood (counter-current flow) and is continuously replaced to maintain steep concentration gradients. Clean blood is returned to the patient through a vein (**3**). This type of dialysis is carried out three days a week, with each session lasting four hours.



Molecule/Substance	Relative concentration		Process and direction of movement
	Blood plasma	Dialysis fluid	
Urea (waste)	High	Low	**Out of the blood** into the dialysis fluid by **diffusion**
Salts	High	Low	**Out of the blood** into the dialysis fluid by **diffusion**
Glucose (needed by the body)	Equal	Equal	**No net movement** of glucose
Amino acids (needed by the body)	Equal	Equal	**No net movement** of amino acids

Top Tip – You may be asked to compare the dialysis machine to the nephron:

- ✓ The selectively permeable dialysis membrane corresponds to the capillary knot and Bowman's capsule – endothelium fenestrations, basement membrane and podocyte filtration slits (see page 53).
- ✓ The tube taking blood to the dialysis machine corresponds to the renal artery.
- ✓ The tube returning blood to the patient's body is the renal vein.
- ✓ The dialysis fluid is the filtrate in the nephron tubule.

Kidney transplant

A **donor** (living or dead) donates a kidney to the patient. The donor and patient must have **compatible tissue types and blood groups**. To reduce the risk of rejection post-transplant the patient must take **immunosuppressant drugs** for the rest of their lives.

Nitrogenous waste disposal in other organisms

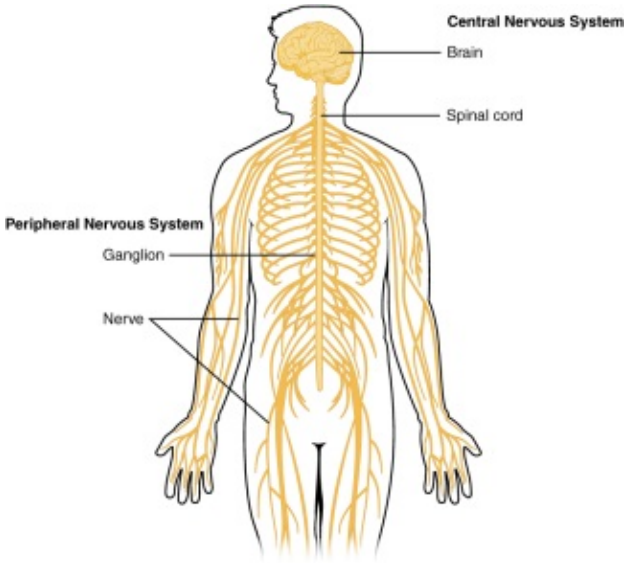
Other organisms deal with the excretion of their nitrogenous waste in different ways:

- ✓ **Fish** excrete ammonia into the surrounding water by diffusion across their gills. Ammonia is highly toxic, but also highly soluble and is flushed away quickly.
- ✓ **Birds and insects** produce uric acid. It is non-toxic and very little water is needed for its excretion – this reduces water loss.
- ✓ **Mammals** produce urea, which requires ATP, but is less toxic than ammonia.

Section 3.8 – The nervous system

The nervous system

The nervous system allows us to respond to changes in our environment. A **stimulus** is any detectable change in the internal or external environment of the organism. Specialised **receptor cells** act as transducers as they detect energy in one form and convert it to electrical energy. This electrical energy travels along **neurones** (nerve cells) as a **nerve impulse**. Nerve impulses initiate a **response** in an **effector**, which is always a muscle or gland.



The **central nervous system** (CNS) is composed the brain and spinal cord. The CNS processes information provided by a stimulus and co-ordinates a response.

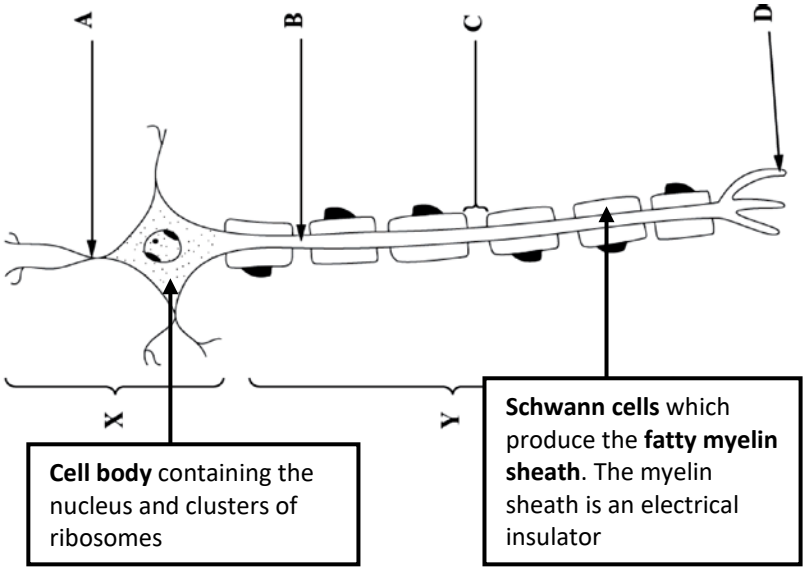
The **peripheral nervous system** (PNS) is made up of neurones (nerve cells). It has two parts:

- ✓ The **somatic nervous** system is made up of pairs of nerves, branching from the brain and spinal cord. These neurones carry impulses from receptor cells to the CNS and then from the CNS to the effectors.
- ✓ The **autonomic nervous system** provides unconscious control of the internal organs e.g. heartbeat and breathing.

Neurones

Neurones are highly specialised cells which carry nerve impulses in one direction. There are three main types in a vertebrate:

- ✓ **Sensory neurone** – Carries nerve impulses from the receptor cells in the sense organ to the CNS
- ✓ **Relay neurone** – Found in the CNS and connects the sensory and motor neurones
- ✓ **Motor neurone** – Transports the nerve impulse from the CNS to the effectors (muscles and glands)

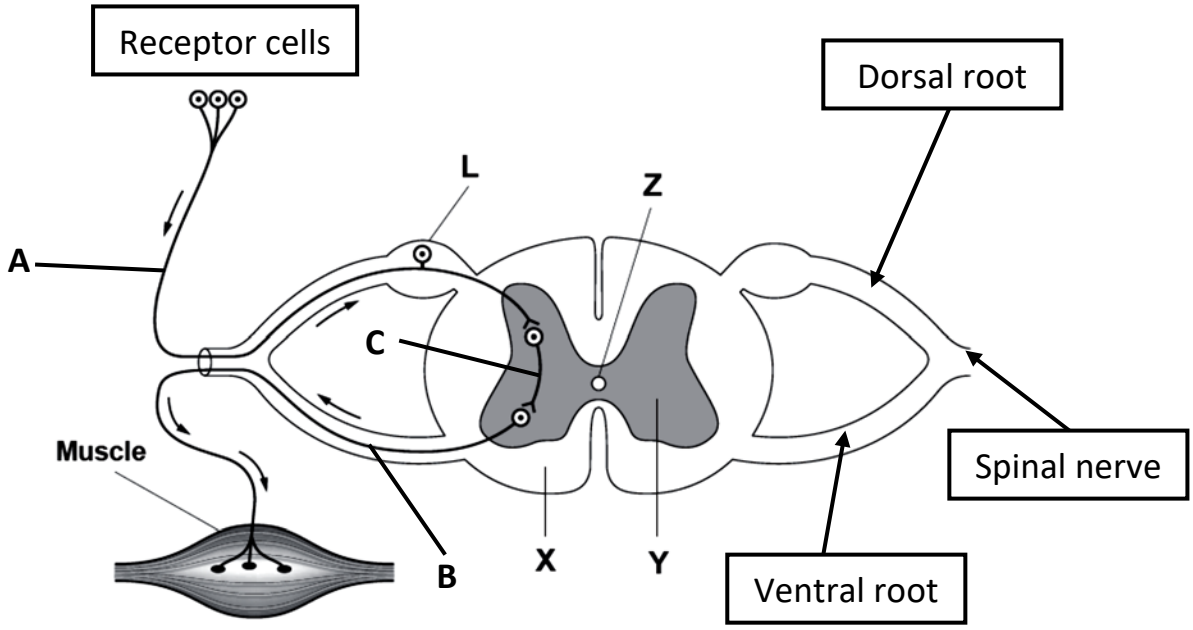


The diagram to the right is **a motor neurone**. **A** is a dendrite, which carries impulses towards the cell body. **B** is the axon; this long fibre carries impulses away from the cell body. **C** is a Node of Ranvier, a region of exposed cell membrane which can be depolarised – these nodes speed up nerve transmission. **D** is the synapse; synapses produce neurotransmitters. Region **X** is found in the grey matter of the spinal cord and **Y** in the white matter.

The spinal cord

The **spinal cord** is a flattened cylinder of nervous tissue, running from the base of the brain to the lumbar region. The spinal cord is protected by vertebrae. The diagram below shows a section through the spinal cord. The grey central region (**Y**) is the grey matter, which contains cell bodies and synapses. The outer, white region (**X**) is called white matter and contains axons coated in fatty myelin – it's the myelin which gives this region its distinctive colour.

Pairs of roots allow neurones to enter and exit the spinal cord. The dorsal root allows the sensory neurone (**A**) to enter; it has a swelling called the **dorsal root ganglion** (**L**) which houses the cell bodies. The ventral root allows the motor neurone (**B**) to exit. Nerve cell **C** is the relay neurone within the grey matter.



The reflex arc

A **reflex** is **fast automatic and protective**. The stimulus may be heat from a flame. Receptor cells in the skin convert heat energy into electrical energy. The nerve impulse travels along the sensory neurone to the spinal cord (via the dorsal root). The impulse passes to the relay neurone and then to the motor neurone. The nerve impulse exits the spinal cord along the motor neurone (via the ventral root) to the muscle (which is an effector). When the impulse reaches the muscle it contacts to pull the arm/hand away from the source of heat – this is a **withdrawal reflex**.

Examples of other reflexes include:

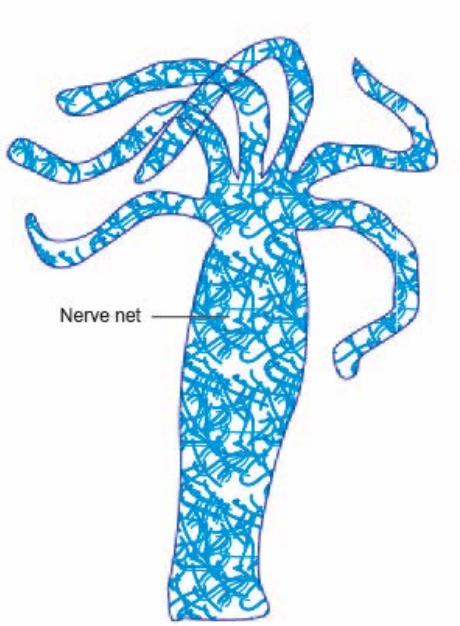
- ✓ Blinking to protect the eye
- ✓ Contraction of the iris to reduce the amount of light hitting the retina

Top Tip – You may be asked to draw the neurones onto an outline of the spinal cord, make sure you can draw the cell bodies and synapses in the correct positions. The elements of a reflex must include: **STIMULUS → RECEPTOR → SENSORY NEURONE → RELAY NEURON → MOTOR NEURONE → EFFECTOR → RESPONSE**

Nerve nets

Invertebrates such as jellyfish and hydra have simple **nerve nets**. You must be able to compare a nerve net with the mammalian neurone system. Hydra has no recognisable CNS and fewer types of receptor cells and therefore responds to a limited number of stimuli. Hydra cannot detect the direction of a stimulus. The greater the intensity of the stimulus the more nerve cells are triggered initiating a greater response.

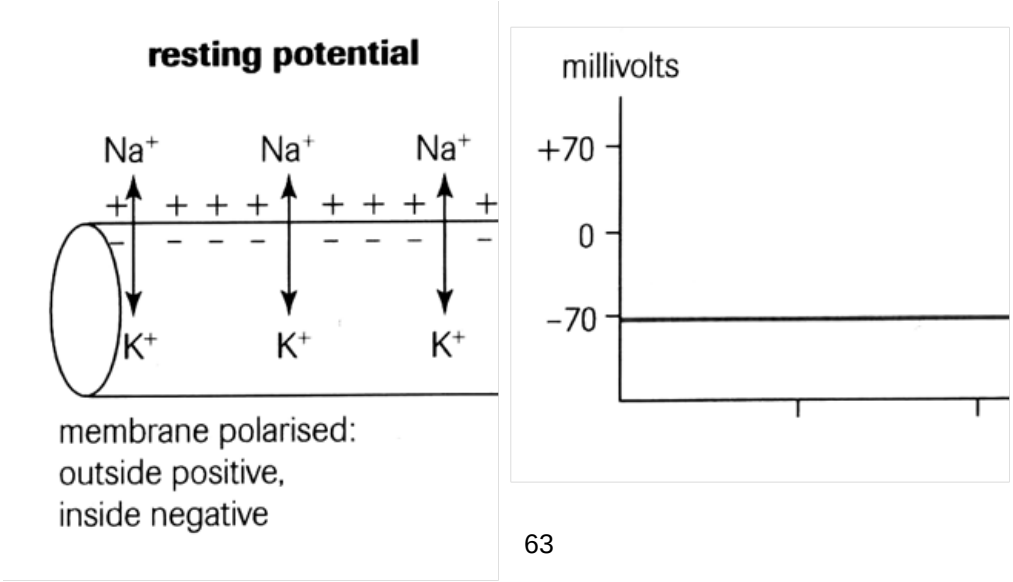
The nerve net has **shorter neurones**, which branch in all directions (vertebrates have longer neurones, which branch in one direction – the axon). Impulses travel slower and in **all directions** (in vertebrates impulses travel quicker and in one direction). Nerve nets have only **one type of neurone**, in mammals there are three.



Top Tip – You must compare like for like! Use terms such as slower instead of slow, shorter instead of short.

Resting potential

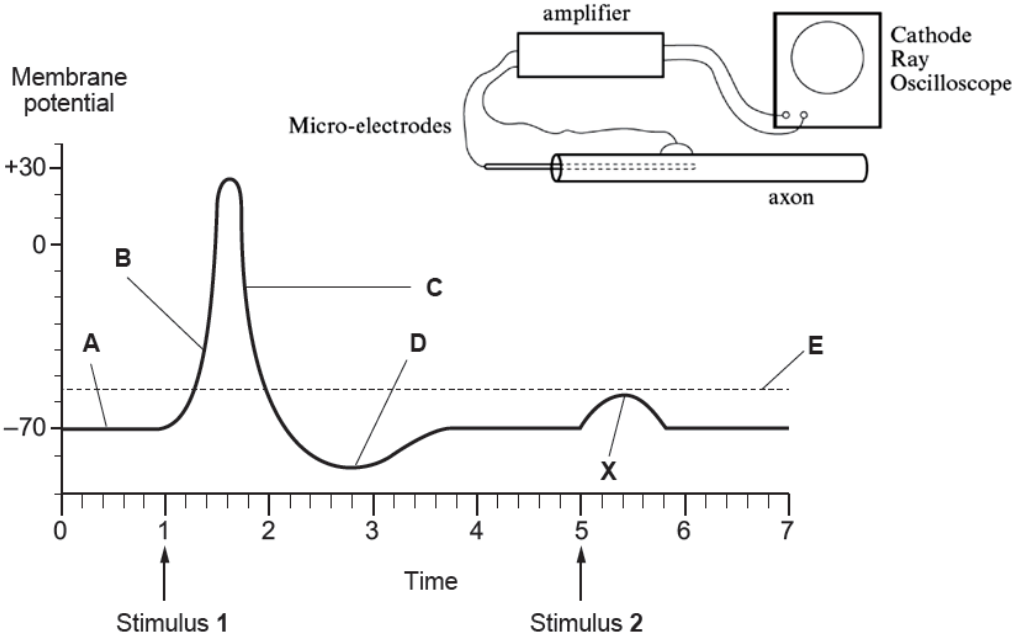
When no nerve impulse is being transported along the axon a **resting potential** is maintained across the axon membrane. The potential difference, across the axon membrane, during a resting potential is -70mV.This is achieved by active transport; the sodium-potassium pump pumps 3 Na^+ out of the axoplasm and only 2 K^+ in. Also the axon membrane is highly permeable to K^+ and they leak out by facilitated diffusion thorough open channels. This outward movement of positive ions means the outside of the axon membrane is positive relative to the inside. The membrane is **polarised**. The ATP needed to maintain a resting potential is produced by the numerous mitochondria present in the axoplasm of the axon.



The action potential

A **stimulus opens voltage gated Na+ channels**. If the stimulus is of sufficient intensity, enough Na^+ channel will open to cause **depolarisation** of the axon. Na^+ ions flood into the axon, down their concentration gradient, until the potential difference across the membrane becomes +40mV – this is an **action potential**. The voltage gated Na^+ channels now close.

An action potential can be measured using a **Cathode Ray Oscilloscope** (look at the diagram below). You will need to be able to interpret an oscilloscope trace.



Point **A** shows a **polarised** axon during the resting potential. After **stimulus 1**, voltage gated Na^+ channels open and **depolarisation** begins (**B)** as Na^+ ions flood into the axon. At the **apex of the peak** the axon is **depolarised** and the voltage gated Na^+ channels close and K^+ channels open. K^+ ions leave the axon, down their concentration gradient, causing **repolarisation** of the axon (**C**). **Hyper-polarisation** occurs to -80mV due to the high permeability of the membrane to K^+ (too many K^+ ions leave the axoplasm). The sodium-potassium pump restores the resting potential and the axon becomes polarised again.

Stimulus 2 is not strong enough to generate a full action potential. Too few voltage gated Na^+ channels are opened and not enough sodium ions pass into the axon to cause full depolarisation – the action potential is not generated (look at the blip **X** on the graph above). To generate a full action potential depolarisation must cross the threshold (**E**).

Refractory period

No further action potentials can be propagated along the axon until polarisation has been restored – this is called the **refractory period**. Depolarisation and repolarisation cause an absolute refractory period where no new action potential can be generated. During the hyperpolarisation phase an action potential can be generated providing the stimulus is stronger than usual.

The all or nothing law

If the intensity of the stimulus is below a certain **threshold**, no action potential will be generated. If the stimulus exceeds the threshold a full action potential is generated and a nerve impulse will be propagated along the axon. Further increases in stimulus intensity will not generate a larger action potential – they are always the same size. This property of a nerve impulse acts as a **filter**, preventing minor stimuli overloading the brain.

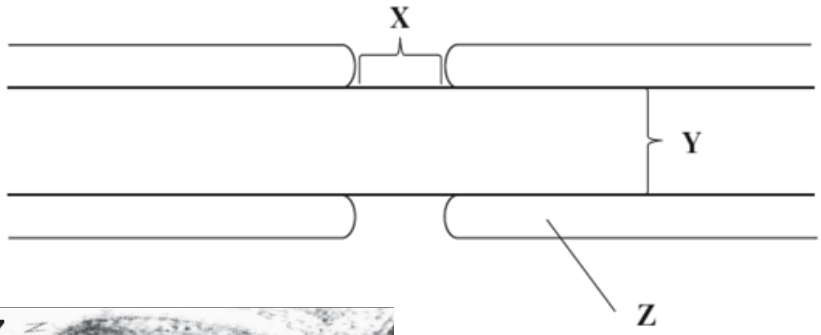
Propagation of the nerve impulse

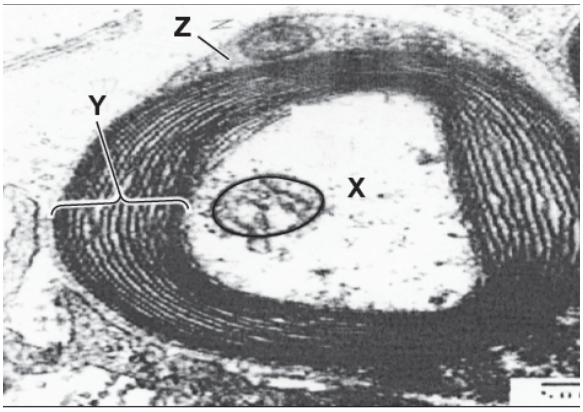
Once a stimulus generates a full action potential the impulse is propagated due to the juxtaposition of positive and negative charges of the polarised and depolarised regions of the axon cell membrane. This generates localised electrical currents which open voltage gates Na+ channels, causing depolarisation of the axon section of the axon cell membrane.

Factors affecting the speed of transmission

The following factors affect the speed of transmission:

- ✓ **Temperature** – Increases in temperature increase kinetic energy and therefore speeds up the transport of ions; this speeds up nerve transmission.
- ✓ **Diameter of the axon** – The greater the diameter of the axon the lower the resistance to the movement of ions. Giant squids have axons up to 1mm in diameter allowing them to react quickly in low temperatures.
- ✓ **Myelination** – **Schwann cells** wrap around the axon (**Y** on the diagram below) and secrete a **fatty myelin sheath** (**Z**) which is an electrical insulator. Only the **Nodes of Ranvier** (**X**), which are gaps in the myelin sheath exposing the cell membrane, can become depolarised (only these regions have voltage gated Na^+ channels). The action potential jumps from node to node and speeds up the rate of transmission; this is called **saltatory transmission**. The greater the distance between the nodes the greater the rate of transmission. Myelination is only found in vertebrates.



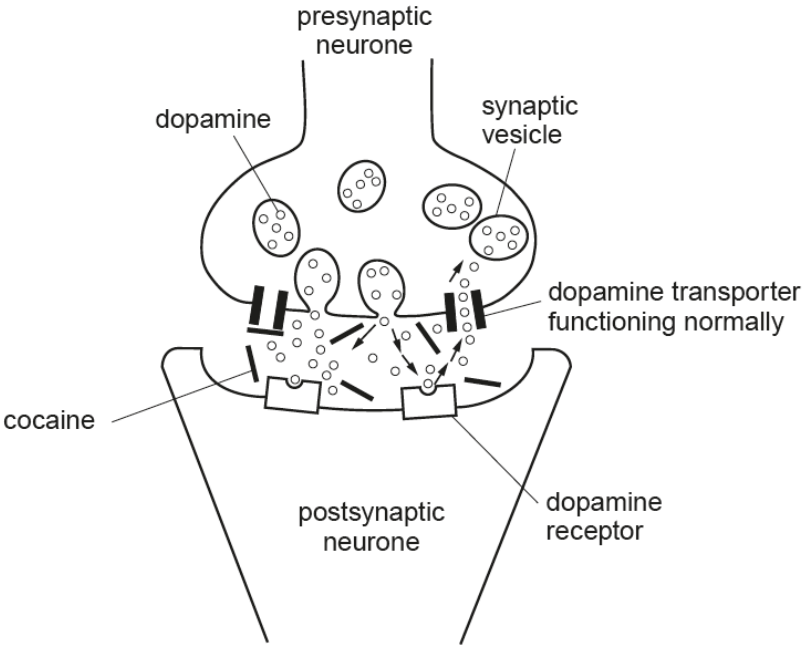


A section through an axon and fatty myelin sheath is shown to the left. **X** is the axon and axoplasm. **Y** is the fatty myelin sheath between layers of the coiled Schwann cell membrane. **Z** is the Schwann cell with a large nucleus.

Synapses

Synapses produce neurotransmitters and pass nerve impulses from neurone to neurone. You need to be able to draw a synapse and label it fully!

The diagram to the right shows some of the main features of a synapse. The synaptic knob also has many **mitochondria** for ATP production (these are not shown). In this example the **drug cocaine** is blocking the dopamine transporter preventing its return from the cleft, across the presynaptic membrane, to the synaptic knob. The dopamine remains in the synaptic cleft too long and remains bound to the receptors on the post synaptic membrane. This intensifies/prolongs stimulation of the post synaptic neurone.



Synaptic transmission

When an action potential reaches the presynaptic membrane **Ca^{2+} channel open** allowing Ca^{2+} to enter the synaptic knob. **Synaptic vessels**, containing neurotransmitter, migrate towards the presynaptic membrane and fuse with it. The neurotransmitter is released by exocytosis. The neurotransmitter diffuses across the synaptic cleft and binds to **receptor proteins** on the post synaptic membrane. This opens Na^+ channel allowing Na+ to flood into the post synaptic neurone. If sufficient Na^+ enters the post synaptic neuron an action potential will be generated in that nerve cell.

Acetylcholine

Acetylcholine is an example of a neurotransmitter. Acetylcholine cannot be allowed to remain in the synaptic cleft, as it would constantly initiate new impulses. It is hydrolysed (broken down) by the enzyme **cholinesterase** into choline and ethanoic acid. These products diffuse into the synaptic knob and are regenerated into acetylcholine and repackaged into synaptic vesicles – this requires ATP, which is why the synaptic knob has many mitochondria.

Properties of the synapse

The synapse has the following functions:

- ✓ Transmit information form neurone to neurone
- ✓ Pass impulses in one direction
- ✓ Act as junctions
- ✓ Prevents overstimulation
- ✓ Filter out low level stimuli

The effect of chemicals on the synapse

Many drugs act at the synapse and can either **amplify or inhibit** synaptic transmission. **Psychoactive drugs** act on the CNS by affecting neurotransmitters or their receptors. The following table summarises how the synapse could be affected by different drugs.

Amplification	Inhibition
Pre-synaptic: Accelerating neurotransmitter production in the synaptic knob (cocaine). Opening calcium channels in the pre-synaptic membrane. Accelerating the release of neurotransmitter from the synaptic knob by exocytosis. Blocking the removal or recycling of neurotransmitter substance from the synaptic cleft back into the synaptic knob (cocaine).	**Pre-synaptic:** Inhibiting neurotransmitter production in the synaptic knob. Closing calcium channels in the pre-synaptic membrane. Inhibiting the release of neurotransmitter from the synaptic knob by exocytosis.
Post-synaptic: Making the post-synaptic receptors more sensitive to the neurotransmitter. Opening the sodium channels on the post synaptic membrane. Inhibiting cholinesterase activity in the synaptic cleft. Mimicking the neurotransmitter substance (cannabis).	**Post-synaptic:** Making the post-synaptic receptors less sensitive to the neurotransmitter. Closing sodium channels on the post-synaptic membrane. Increasing cholinesterase activity in the synaptic cleft. Masking the effect of the neurotransmitter substance. Blocking receptors on the post-synaptic membrane.

Top Tip – Always look at diagrams carefully. Start by describing what you see then apply your knowledge and understanding.

Acknowledgements

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