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Mark Rothery's Biology Web Site

A level biology resources past paper questions coursework help

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WELCOME: This site is mainly for AQA(B) AS & A2 (A level) Biology, content is reached from the module links below. There are extensive notes, summaries and past paper questions. Other resources can be found in each module's resource centre.

AS & A2

Module1	Biochemistry, cells, cell transport, exchange, enzymes & digestion.
Module2	Genetic code, cell cycle, sexual reproduction & gene technology.
Module3	Transport, breathing & heartbeat control, energy & exercise.
Module4	Photosynthesis, respiration, homeostasis, nervous coordination, muscles, inheritance, variation, and evolution & classification.
Module5	Energy flow through ecosystems, material cycles, studying ecosystems, dynamics of ecosystems and human activities.
Module8	Patterns of behaviour, reproductive behaviour, pregnancy, human growth and development, human populations and health.

PowerPoints

MODULE 1

TOPIC	NOTES	KEY FACTS	QUESTIONS
	Cut down versions		Some are best printed & answered on paper then checked. Newer Q's can be answered online.
BIOCHEM	Notes	Tests Properties Carb. Notes	Q1 Q2 Q3 Q4 Q5 Q6 MCQ
CELLS	Notes	All	Q1 Q2 Q3 Q4 MCQ
EXCHANGE	Notes	All	Q1 Q2 Q3 Q4
ENZYMES	Notes		Q1 Q2 Q3
DIGESTION	Notes	Fungi Human	Q1 Q2 Q3 Q4
TECHNIQUES	Notes M/scope		

exam board specification for module one

- resource centre
- revision quiz on module one
- Email link teacher@mrothery.co.uk

BIOCHEMISTRY

Contents

Water
Carbohydrates
Lipids
Proteins

At least 80% of the mass of living organisms is water, and almost all the chemical reactions of life take place in aqueous solution. The other chemicals that make up living things are mostly organic macromolecules belonging to the 4 groups proteins, nucleic acids, carbohydrates or lipids. These macromolecules are made up from specific monomers as shown in the table below. Between them these four groups make up 93% of the dry mass of living organisms, the remaining 7% comprising small organic molecules (like vitamins) and inorganic ions.

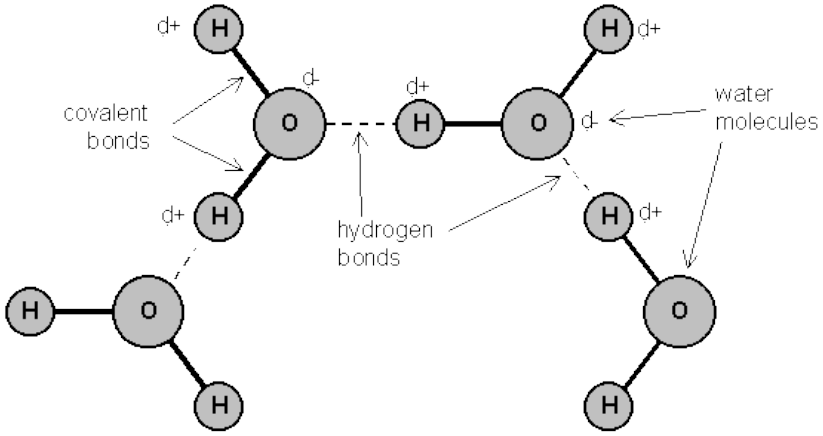
GROUP NAME	MONOMERS	POLYMERS	% DRY MASS
Proteins	amino acids	polypeptides	50
nucleic acids	nucleotides	polynucleotides	18
carbohydrates	monosaccharides	polysaccharides	15

GROUP NAME	COMPONENTS	LARGEST UNIT	% DRY MASS
lipids	fatty acids + glycerol	Triglycerides	10

The first part of this unit is about each of these groups. We'll look at each of these groups in detail, except nucleic acids, which are studied in module 2.

WATER

Water molecules are charged, with the oxygen atom being slightly negative and the hydrogen atoms being slightly positive. These opposite charges attract each other, forming hydrogen bonds. These are weak, long distance bonds that are very common and very important in biology.

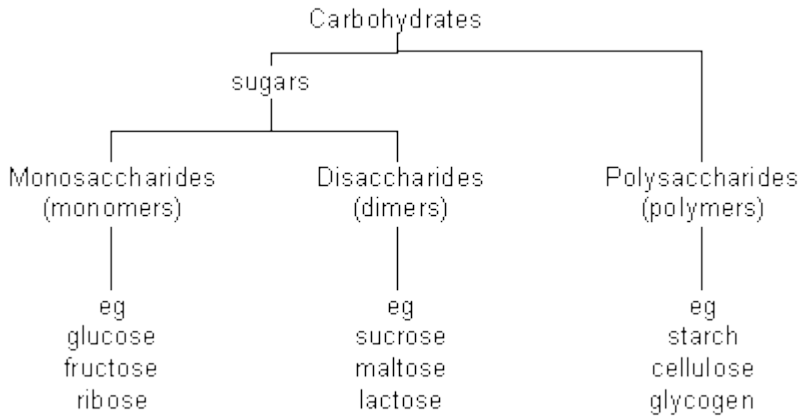


Water has a number of important properties essential for life. Many of the properties below are due to the hydrogen bonds in water.

- **Solvent**. Because it is charged, water is a very good solvent. Charged or polar molecules such as salts, sugars and amino acids dissolve readily in water and so are called hydrophilic ("water loving"). Uncharged or non-polar molecules such as lipids do not dissolve so well in water and are called hydrophobic ("water hating").
- **Specific heat capacity.** Water has a specific heat capacity of 4.2 J g^{-1} $°C^{-1}$, which means that it takes 4.2 joules of energy to heat 1 g of water by 1°C. This is unusually high and it means that water does not change temperature very easily. This minimizes fluctuations in temperature inside cells, and it also means that sea temperature is remarkably constant.
- **Latent heat of evaporation.** Water requires a lot of energy to change state from a liquid into a gas, and this is made use of as a cooling mechanism in animals (sweating and panting) and plants (transpiration). As water evaporates it extracts heat from around it, cooling the organism.
- **Density.** Water is unique in that the solid state (ice) is less dense that the liquid state, so ice floats on water. As the air temperature cools, bodies of water freeze from the surface, forming a layer of ice with liquid water underneath. This allows aquatic ecosystems to exist even in sub-zero temperatures.
- **Cohesion.** Water molecules "stick together" due to their hydrogen bonds, so water has high cohesion. This explains why long columns of water can be sucked up tall trees by transpiration without breaking. It also explains surface tension, which allows small animals to walk on water.
- **Ionization.** When many salts dissolve in water they ionize into discrete positive and negative ions (e.g. NaCl Na^+ + Cl^-). Many important biological molecules are weak acids, which also ionize in solution (e.g. acetic acid $acetate^-$ + H^+). The names of the acid and ionized forms (acetic acid and acetate in this example) are often used loosely and interchangeably, which can cause confusion. You will come across many examples of two names referring to the same substance, e.g.: phosphoric acid and phosphate, lactic acid and lactate, citric acid and citrate, pyruvic acid and pyruvate, aspartic acid and aspartate, etc. The ionized form is the one found in living cells.
- **pH.** Water itself is partly ionized (H_2O H^+ + OH^-), so it is a source of protons (H^+ ions), and indeed many biochemical reactions are sensitive to pH (-log[H^+]). Pure water cannot buffer changes in H^+ concentration, so is not a buffer and can easily be any pH, but the cytoplasms and tissue fluids of living organisms are usually well buffered at about neutral pH (pH 7-8).

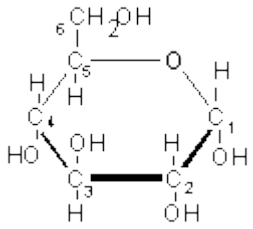
CARBOHYDRATES

Carbohydrates contain only the elements carbon, hydrogen and oxygen. The group includes monomers, dimers and polymers, as shown in this diagram:

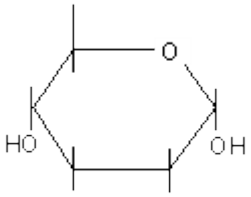


Monosaccharides

All have the formula $(CH_2O)_n$, where n is between 3 and 7. The most common & important monosaccharide is glucose, which is a six-carbon sugar. It's formula is $C_6H_{12}O_6$ and its structure is shown below



or more simply



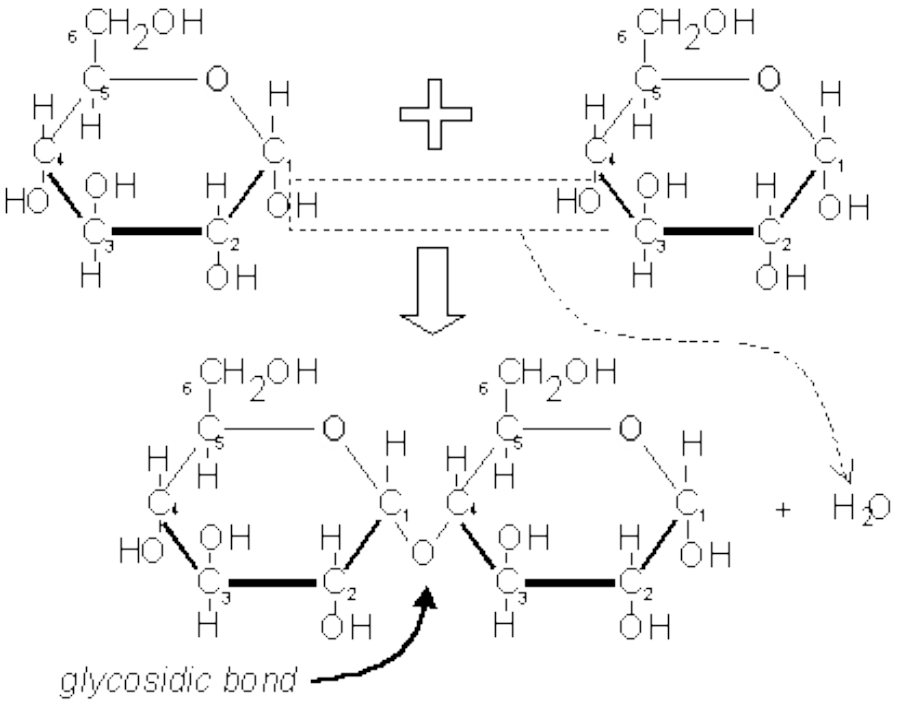
Glucose forms a six-sided ring. The six carbon atoms are numbered as shown, so we can refer to individual carbon atoms in the structure. In animals glucose is the main transport sugar in the blood, and its concentration in the blood is carefully controlled.

There are many monosaccharides, with the same chemical formula ($C_6H_{12}O_6$), but different structural formulae. These include fructose and galactose.

Common five-carbon sugars (where n = 5, $C_5H_{10}O_5$) include ribose and deoxyribose (found in nucleic acids and ATP).

Disaccharides

Disaccharides are formed when two monosaccharides are joined together by a glycosidic bond. The reaction involves the formation of a molecule of water (H_2O):



This shows two glucose molecules joining together to form the disaccharide maltose. Because this bond is between carbon 1 of one molecule and carbon 4 of the other molecule it is called a 1-4 glycosidic bond. This kind of reaction, where water is formed, is called a condensation reaction. The reverse process, when bonds are broken by the addition of water (e.g. in digestion), is called a hydrolysis reaction.

- polymerisation reactions are condensation reactions
- breakdown reactions are hydrolysis reactions

There are three common disaccharides:

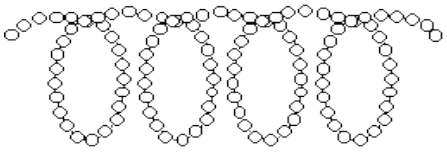
- Maltose (or malt sugar) is glucose & glucose. It is formed on digestion of starch by amylase, because this enzyme breaks starch down into two-glucose units. Brewing beer starts with malt, which is a maltose solution made from germinated barley. Maltose is the structure shown above.
- Sucrose (or cane sugar) is glucose & fructose. It is common in plants because it is less reactive than glucose, and it is their main transport sugar. It's the common table sugar that you put in tea.
- Lactose (or milk sugar) is galactose & glucose. It is found only in mammalian milk, and is the main source of energy for infant mammals.

Polysaccharides

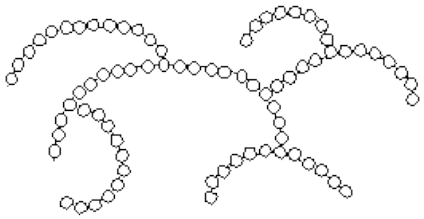
Polysaccharides are long chains of many monosaccharides joined together by glycosidic bonds. There are three important polysaccharides:

Starch is the plant storage polysaccharide. It is insoluble and forms starch granules inside many plant cells. Being insoluble means starch does not change the water potential of cells, so does not cause the cells to take up water by osmosis (more on osmosis later). It is not a pure substance, but is a mixture of amylose and amylopectin.

Amylose is simply poly-(1-4) glucose, so is a straight chain. In fact the chain is floppy, and it tends to coil up into a helix.

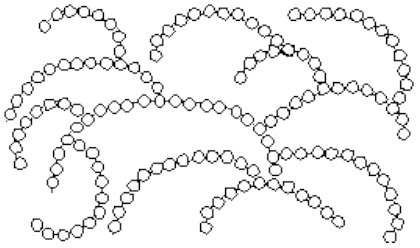


Amylopectin is poly(1-4) glucose with about 4% (1-6) branches. This gives it a more open molecular structure than amylose. Because it has more ends, it can be broken more quickly than amylose by amylase enzymes.

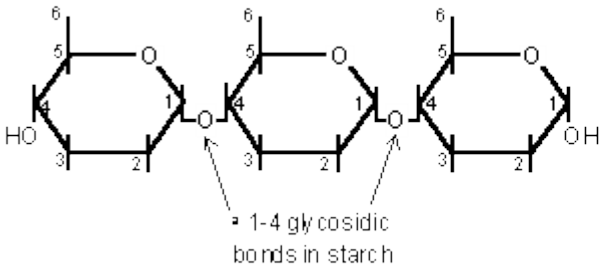


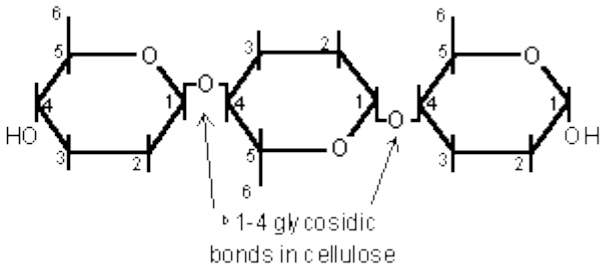
Both amylose and amylopectin are broken down by the enzyme amylase into maltose, though at different rates.

Glycogen is similar in structure to amylopectin. It is poly (1-4) glucose with 9% (1-6) branches. It is made by animals as their storage polysaccharide, and is found mainly in muscle and liver. Because it is so highly branched, it can be mobilised (broken down to glucose for energy) very quickly.

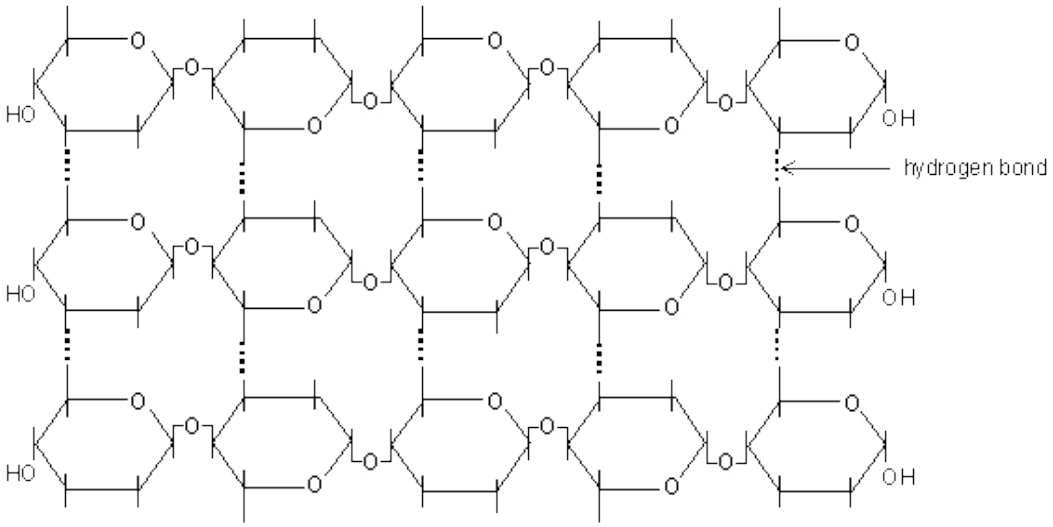


Cellulose is only found in plants, where it is the main component of cell walls. It is poly (1-4) glucose, but with a different isomer of glucose. Cellulose contains beta-glucose, in which the hydroxyl group on carbon 1 sticks up. This means that in a chain alternate glucose molecules are inverted.





This apparently tiny difference makes a huge difference in structure and properties. While the a1-4 glucose polymer in starch coils up to form granules, the beta1-4 glucose polymer in cellulose forms straight chains. Hundreds of these chains are linked together by hydrogen bonds to form cellulose microfibrils. These microfibrils are very strong and rigid, and give strength to plant cells, and therefore to young plants.



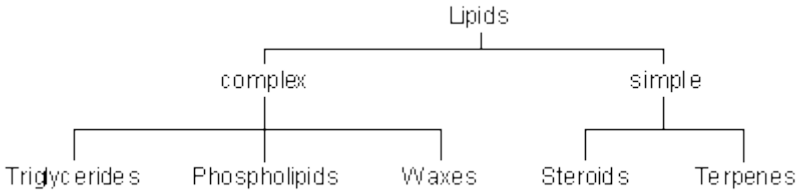
The beta-glycosidic bond cannot be broken by amylase, but requires a specific cellulase enzyme. The only organisms that possess a cellulase enzyme are bacteria, so herbivorous animals, like cows and termites whose diet is mainly cellulose, have mutualistic bacteria in their guts so that they can digest cellulose. Humans cannot digest cellulose, and it is referred to as fibre.

Other polysaccharides that you may come across include:

- Chitin (poly glucose amine), found in fungal cell walls and the exoskeletons of insects.
- Pectin (poly galactose uronate), found in plant cell walls.
- Agar (poly galactose sulphate), found in algae and used to make agar plates.
- Murein (a sugar-peptide polymer), found in bacterial cell walls.
- Lignin (a complex polymer), found in the walls of xylem cells, is the main component of wood.

LIPIDS

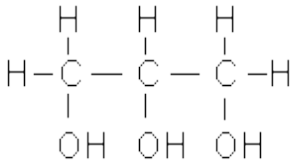
Lipids are a mixed group of hydrophobic compounds composed of the elements carbon, hydrogen and oxygen. They contain fats and oils (fats are solid at room temperature, whereas oils are liquid)



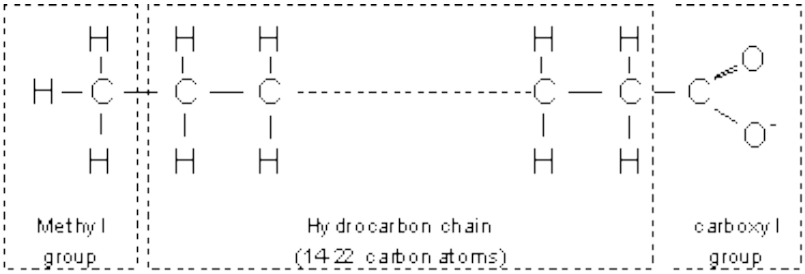
Triglycerides

Triglycerides are commonly called fats or oils. They are made of glycerol and fatty acids.

Glycerol is a small, 3-carbon molecule with three hydroxyl groups.

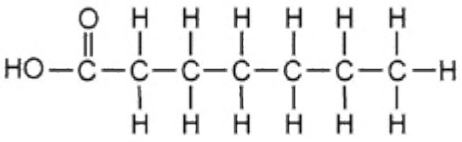


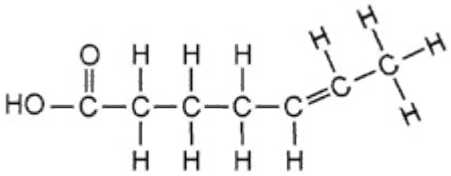
Fatty acids are long molecules with a polar, hydrophilic end and a non-polar, hydrophobic "tail". The hydrocarbon chain can be from 14 to 22 CH_2 units long. The hydrocarbon chain is sometimes called an R group, so the formula of a fatty acid can be written as R-COOH.



- If there are no C=C double bonds in the hydrocarbon chain, then it is a saturated fatty acid (i.e. saturated with hydrogen). These fatty acids form straight chains, and have a high melting point.

- If there are C=C double bonds in the hydrocarbon chain, then it is an unsaturated fatty acid (i.e. unsaturated with hydrogen). These fatty acids form bent chains, and have a low melting point. Fatty acids with more than one double bond are called poly-unsaturated fatty acids (PUFAs).


Saturated fatty acid

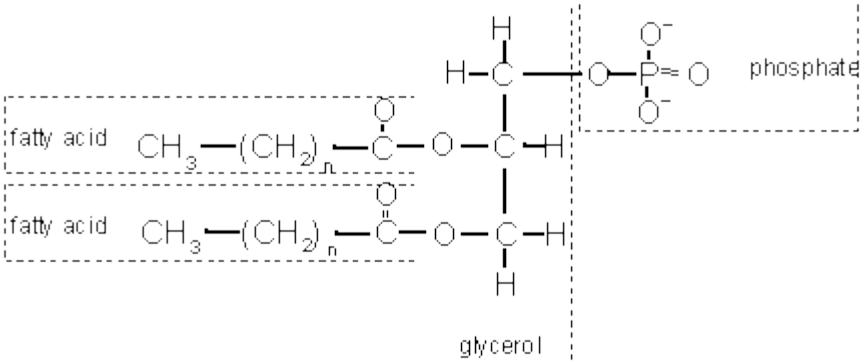

Unsaturated fatty acid

One molecule of glycerol joins togther with three fatty acid molecules to form a triglyceride molecule, in another condensation polymerisation reaction:

Triglycerides are insoluble in water. They are used for storage, insulation and protection in fatty tissue (or adipose tissue) found under the skin (sub-cutaneous) or surrounding organs. They yield more energy per unit mass than other compounds so are good for energy storage. Carbohydrates can be mobilised more quickly, and glycogen is stored in muscles and liver for immediate energy requirements.

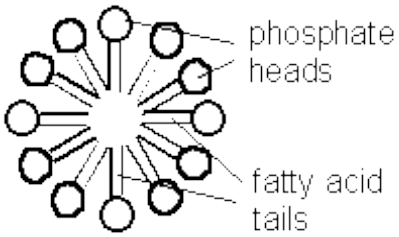
- Triglycerides containing saturated fatty acids have a high melting point and tend to be found in warm-blooded animals. At room temperature they are solids (fats), e.g. butter, lard.
- Triglycerides containing unsaturated fatty acids have a low melting point and tend to be found in cold-blooded animals and plants. At room temperature they are liquids (oils), e.g. fish oil, vegetable oils.

Phospholipids

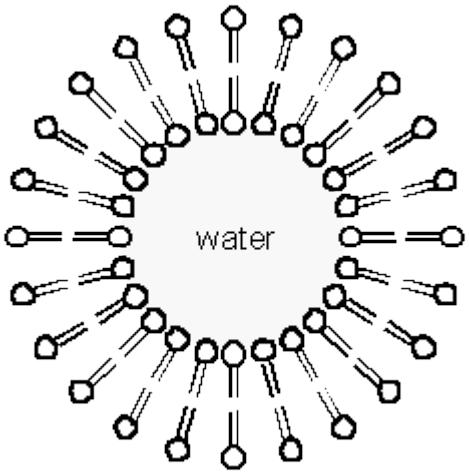


Phospholipids have a similar structure to triglycerides, but with a phosphate group in place of one fatty acid chain. There may also be other groups attached to the phosphate. Phospholipids have a polar hydrophilic "head" (the negatively-charged phosphate group) and two non-polar hydrophobic "tails" (the fatty acid chains). This mixture of properties is fundamental to biology, for phospholipids are the main components of cell membranes.

- When mixed with water, phospholipids form droplet spheres with the hydrophilic heads facing the water and the hydrophobic tails facing each other. This is called a micelle.



- Alternatively, they may form a double-layered phospholipid bilayer. This traps a compartment of water in the middle separated from the external water by the hydrophobic sphere. This naturally-occurring structure is called a liposome, and is similar to a membrane surrounding a cell.



Waxes

Waxes are formed from fatty acids and long-chain alcohols. They are commonly found wherever waterproofing is needed, such as in leaf cuticles, insect exoskeletons, birds' feathers and mammals' fur.

Steroids

Steroids are small hydrophobic molecules found mainly in animals. They include:

- cholesterol, which is found in animals cell membranes to increase stiffness
- bile salts, which help to emulsify dietary fats
- steroid hormones such as testosterone, oestrogen, progesterone and cortisol
- vitamin D, which aids Ca^{2+} uptake by bones.

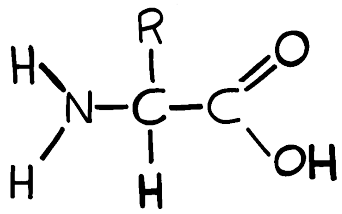
PROTEINS

Proteins are the most complex and most diverse group of biological compounds. They have an astonishing range of different functions, as this list shows.

- structure e.g. collagen (bone, cartilage, tendon), keratin (hair), actin (muscle)
- enzymes e.g. amylase, pepsin, catalase, etc (>10,000 others)
- transport e.g. haemoglobin (oxygen), transferrin (iron)
- pumps e.g. Na^+K^+ pump in cell membranes
- motors e.g. myosin (muscle), kinesin (cilia)
- hormones e.g. insulin, glucagon
- receptors e.g. rhodopsin (light receptor in retina)
- antibodies e.g. immunoglobulins
- storage e.g. albumins in eggs and blood, caesin in milk
- blood clotting e.g. thrombin, fibrin
- lubrication e.g. glycoproteins in synovial fluid
- toxins e.g. diphtheria toxin
- antifreeze e.g. glycoproteins in arctic flea
- and many more!

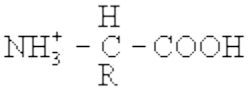
Proteins are made of amino acids. Amino acids are made of the five elements C H O N S. The general structure of an amino acid molecule is shown on the right. There is a central carbon atom (called the "alpha carbon"), with four different chemical groups attached to it:

- a hydrogen atom
- a basic amino group
- an acidic carboxyl group
- a variable "R" group (or side chain)



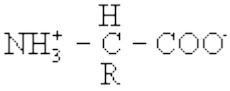
Amino acids are so-called because they have both amino groups and acid groups, which have opposite charges. At neutral pH (found in most living organisms), the groups are ionized as shown above, so there is a positive charge at one end of the molecule and a negative charge at the other end. The overall net charge on the molecule is therefore zero. A molecule like this, with both positive and negative charges is called a zwitterion. The charge on the amino acid changes with pH:

LOW pH (ACID)



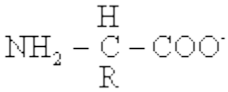
charge = +1

NEUTRAL pH



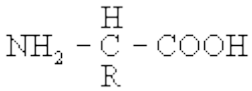
charge = 0

HIGH pH (ALKALI)



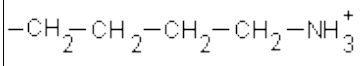
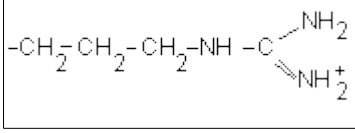
charge = -1

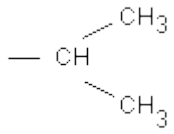
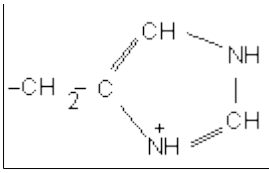
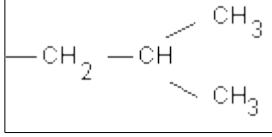
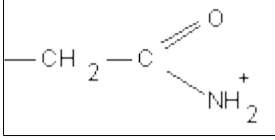
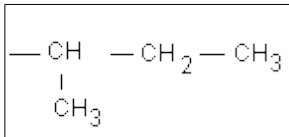
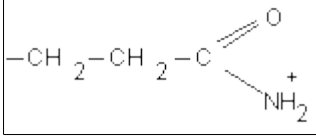
It is these changes in charge with pH that explain the effect of pH on enzymes. A solid, crystallised amino acid has the uncharged structure



however this form never exists in solution, and therefore doesn't exist in living things (although it is the form usually given in textbooks).

There are 20 different R groups, and so 20 different amino acids. Since each R group is slightly different, each amino acid has different properties, and this in turn means that proteins can have a wide range of properties. The following table shows the 20 different R groups, grouped by property, which gives an idea of the range of properties. You do not need to learn these, but it is interesting to see the different structures, and you should be familiar with the amino acid names. You may already have heard of some, such as the food additive monosodium glutamate, which is simply the sodium salt of the amino acid glutamate. Be careful not to confuse the names of amino acids with those of bases in DNA, such as cysteine (amino acid) and cytosine (base), threonine (amino acid) and thymine (base). There are 3-letter and 1-letter abbreviations for each amino acid.

THE TWENTY AMINO ACID R-GROUPS (FOR INTEREST ONLY NO KNOWLEDGE REQUIRED)			
	SIMPLE R GROUPS		**BASIC R GROUPS**
Glycine Gly G	— H	Lysine Lys K	
Alanine Ala A	— CH_3	Arginine Arg R	

Valine Val V		Histidine His H	
Leucine Leu L		Asparagine Asn N	
Isoleucine Ile I		Glutamine Gln Q	
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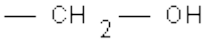
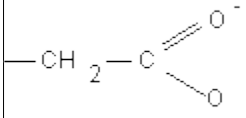
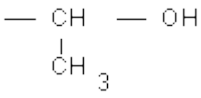
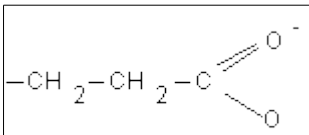
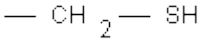
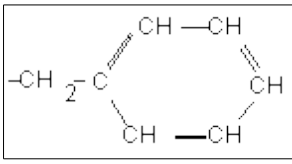
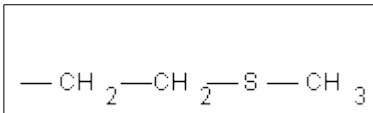
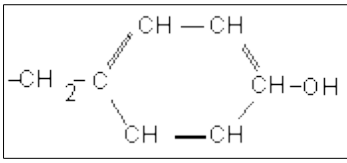
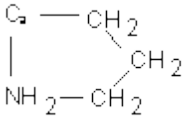
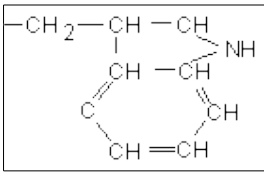
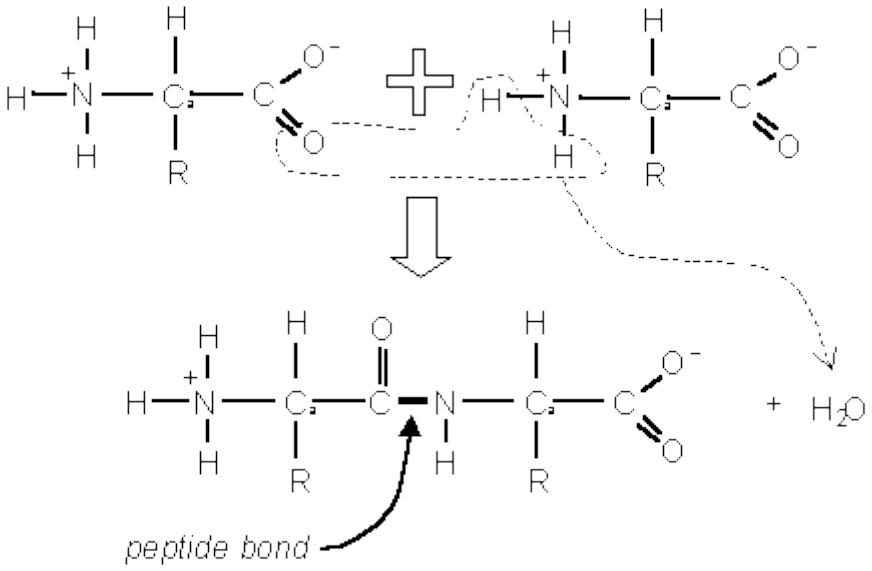
Serine Ser S		Aspartate Asp D	
Threonine Thr T		Glutamate Glu E	
	Sulphur R groups		**Ringed R groups**
Cysteine Cys C		Phenylalanine Phe F	
Methionine Met M		Tyrosine Tyr Y	
	Cyclic R group		
Proline Pro P		Tryptophan Trp W	

Polypeptides

Amino acids are joined together by peptide bonds. The reaction involves the formation of a molecule of water in another condensation polymerisation reaction:



When two amino acids join together a dipeptide is formed. Three amino acids form a tripeptide. Many amino acids form a polypeptide. e.g.:

$^{+}NH_3$-Gly — Pro — His — Leu — Tyr — Ser — Trp — Asp — Lys — Cys-COO^-

In a polypeptide there is always one end with a free amino (NH_2) (NH_3 in solution) group, called the N-terminus, and one end with a free carboxyl (COOH) (COO in solution) group, called the C-terminus.

Protein Structure

Polypeptides are just a string of amino acids, but they fold up to form the complex and well-defined three-dimensional structure of working proteins. To help to understand protein structure, it is broken down into four levels:

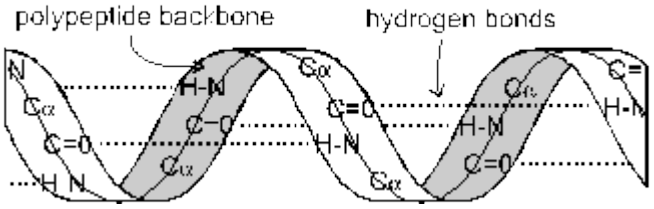
1. Primary Structure

This is just the sequence of amino acids in the polypeptide chain, so is not really a structure at all. However, the primary structure does determine the rest of the protein structure. Finding the primary structure of a protein is called protein sequencing, and the first protein to be sequenced was the protein hormone insulin, by the Cambridge biochemist Fredrick Sanger, for which work he got the Nobel prize in 1958.

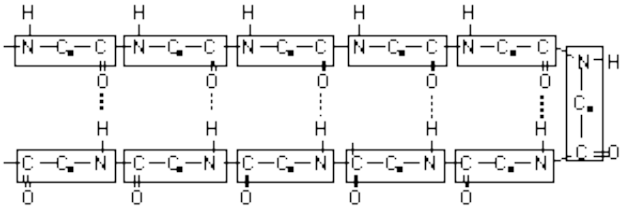
2. Secondary Structure

This is the most basic level of protein folding, and consists of a few basic motifs that are found in all proteins. The secondary structure is held together by hydrogen bonds between the carboxyl groups and the amino groups in the polypeptide backbone. The two secondary structures are the α-helix and the β-sheet.

The α-helix. The polypeptide chain is wound round to form a helix. It is held together by hydrogen bonds running parallel with the long helical axis. There are so many hydrogen bonds that this is a very stable and strong structure. Helices are common structures throughout biology.



The β-sheet. The polypeptide chain zig-zags back and forward forming a sheet. Once again it is held together by hydrogen bonds.



3. Tertiary Structure

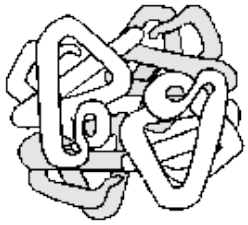
This is the 3 dimensional structure formed by the folding up of a whole polypeptide chain. Every protein has a unique tertiary structure, which is responsible for its properties and function. For example the shape of the active site in an enzyme is due to its tertiary structure. The tertiary structure is held together by bonds between the R groups of the amino acids in the protein, and so depends on what the sequence of amino acids is. There are three kinds of bonds involved:

- hydrogen bonds, which are weak.
- ionic bonds between R-groups with positive or negative charges, which are quite strong.
- sulphur bridges - covalent S-S bonds between two cysteine amino acids, which are strong.

4. Quaternary Structure

This structure is found only in proteins containing more than one polypeptide chain, and simply means how the different polypeptide chains are arranged together. The individual polypeptide chains are usually globular, but can arrange themselves into a variety of quaternary shapes. e.g.:

Haemoglobin, the oxygen-carrying protein in red blood cells, consists of four globular subunits arranged in a tetrahedral (pyramid) structure. Each subunit contains one iron atom and can bind one molecule of oxygen.



These four structures are not real stages in the formation of a protein, but are simply a convenient classification that scientists invented to help them to understand proteins. In fact proteins fold into all these structures at the same time, as they are synthesised.

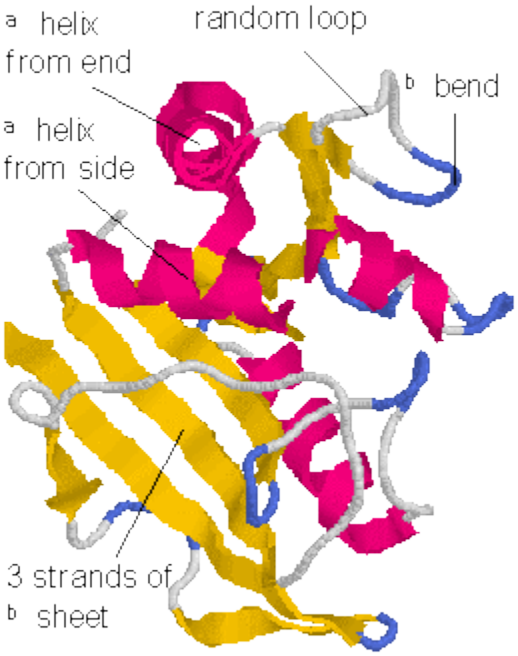
The final three-dimensional shape of a protein can be classified as globular or fibrous.

globular structure

fibrous (or filamentous) structure

The vast majority of proteins are globular, including enzymes, membrane proteins, receptors, storage proteins, etc. Fibrous proteins look like ropes and tend to have structural roles such as collagen (bone), keratin (hair), tubulin (cytoskeleton) and actin (muscle). They are usually composed of many polypeptide chains. A few proteins have both structures: the muscle protein myosin has a long fibrous tail and a globular head, which acts as an enzyme.

This diagram shows a molecule of the enzyme dihydrofolate reductase, which comprises a single polypeptide chain. It has a globular shape



This diagram shows part of a molecule of collagen, which is found in bone and cartilage. It has a unique, very strong triple-helix structure. It is a fibrous protein

Biochemical Tests

Benedicts test for reducing sugars

- grind up sample
- add Benedicts solution
- heat
- colour change from blue to red/brown indicate reducing sugars
- note simple non reducing sugars (mainly disaccharides) can all be hydrolysed to their reducing sugar components by heating with dilute acid (e.g. HCl). If you neutralise after heating you can then perform the Benedicts test
- a positive result indicates the presence of a simple non-reducing sugar

Iodine (I_2) test for starch

- add drops of Iodine to sample
- colour change from brown to blue black indicates presence of starch

Shultz's test for cellulose

- add Shultz's solution
- purple colour indicates presence of cellulose

Biuret test for protein

- grind up sample
- add Biuret solution
- lilac colour indicates protein present

Emulsion test for lipids

- grind up sample
- add ethanol
- decant into water
- cloudy emulsion indicates presence of lipid

Characteristic	Proteins	Carbohydrate	Lipid
Elements present	C H O N	C H O	C H O
Type of bond	Peptide	Glycosidic	Ester
Reagent in Tests	Biuret	Benedict	Ethanol
Simplest form	Amino Acids	Monosaccharide	Glycerol/Fatty acids
How bonds formed	Condensation	Condensation	Condensation
How bonds broken	Hydrolysis	Hydrolysis	Hydrolysis
Formation of long chains	Polypeptides	Polysaccharide	None
Polar	Yes	Yes	No
Type of polarity	Hydrophilic	Hydrophilic	Hydrophobic
Dissolve in water	Yes	Yes	Basically No

CARBOHYDRATES - KEY NOTES

Contain Elements: Carbon, Hydrogen and Oxygen.

Biological Importance…

Energy Source	Carbohydrates are principal respiratory substrates
Structural Compounds	Cellulose (CW of all plant cells) & Lignin
Storage Compounds	Plants, Starch (common plant storage never in animals) Animals. Glycogen (e.g. mammalian liver)

CLASSIFICATION: The basic sugar unit = the saccharide

- 1 sugar unit = Monosaccaride
- 2 sugar units = Disaccharide
- Many sugar units = Polysaccharide

MONOSACCHARIDES

Examples of Monosaccharides: Glucose, Ribose

General There are the building blocks of other important C/H's

Monosaccharides are:

- Sweet tasting
- Soluble in water
- Reducing sugars (see below)

Reducing Sugar Properties (all monosaccharides are reducing sugars). M/S are capable of REDUCING benedicts solution. When this reduction occurs benedicts solution changes from blue to orange/red.

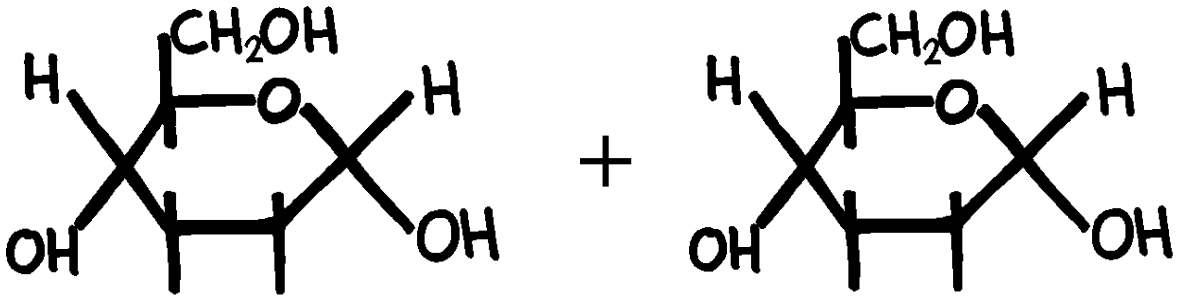
DISACCHARIDES

Examples of D/S: Maltose (Malt sugar), Lactose (milk sugar)

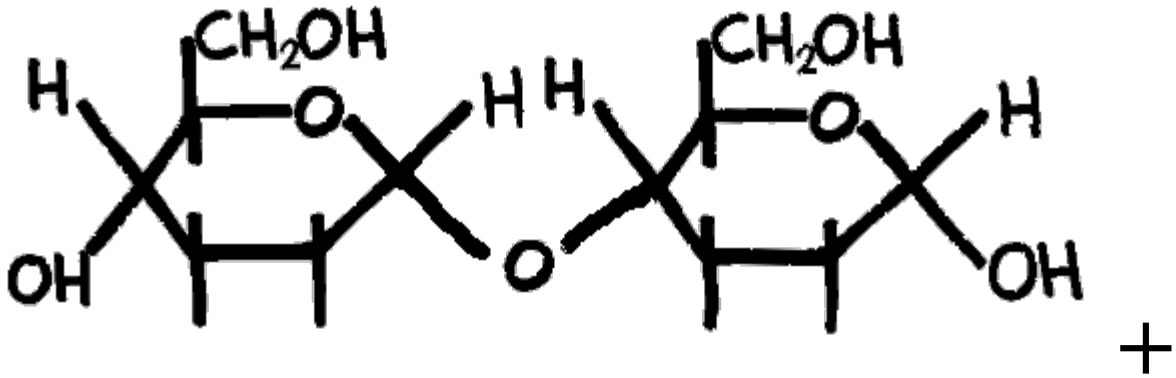
Maltose formed by **CONDENSATION** of 2 units of glucose, the bond is called a glycosidic bond.

Note: In the exam you could be given half of the reaction below and asked to fill in the other half - you wouldn't be asked to come up with it all off the top of your head

2 molecules of glucose



Undergo a condensation reaction to form…



+ H_2O

Maltose and Water

General Summary of disaccharides

- May be non-reducing
- Sweet tasting
- Water soluble

POLYSACCHARIDES

Examples of P/S: Starch, Glycogen, Cellulose

These are an important group of carbohydrates. Two main divisions:

- Structural Polysaccharides e.g. Cellulose, Chitin, Lignin (wood)
- Storage Polysaccharides e.g. Starch and Glycogen

General Properties of P/S

- Non sweet tasting
- Non truly soluble in H_2O

Structural P/S

In these polysaccharides the sugar unit residues present in long chain molecules of the polymer are straight, and cross-linkages between chains occur giving the material its strength.

CELLS

Contents

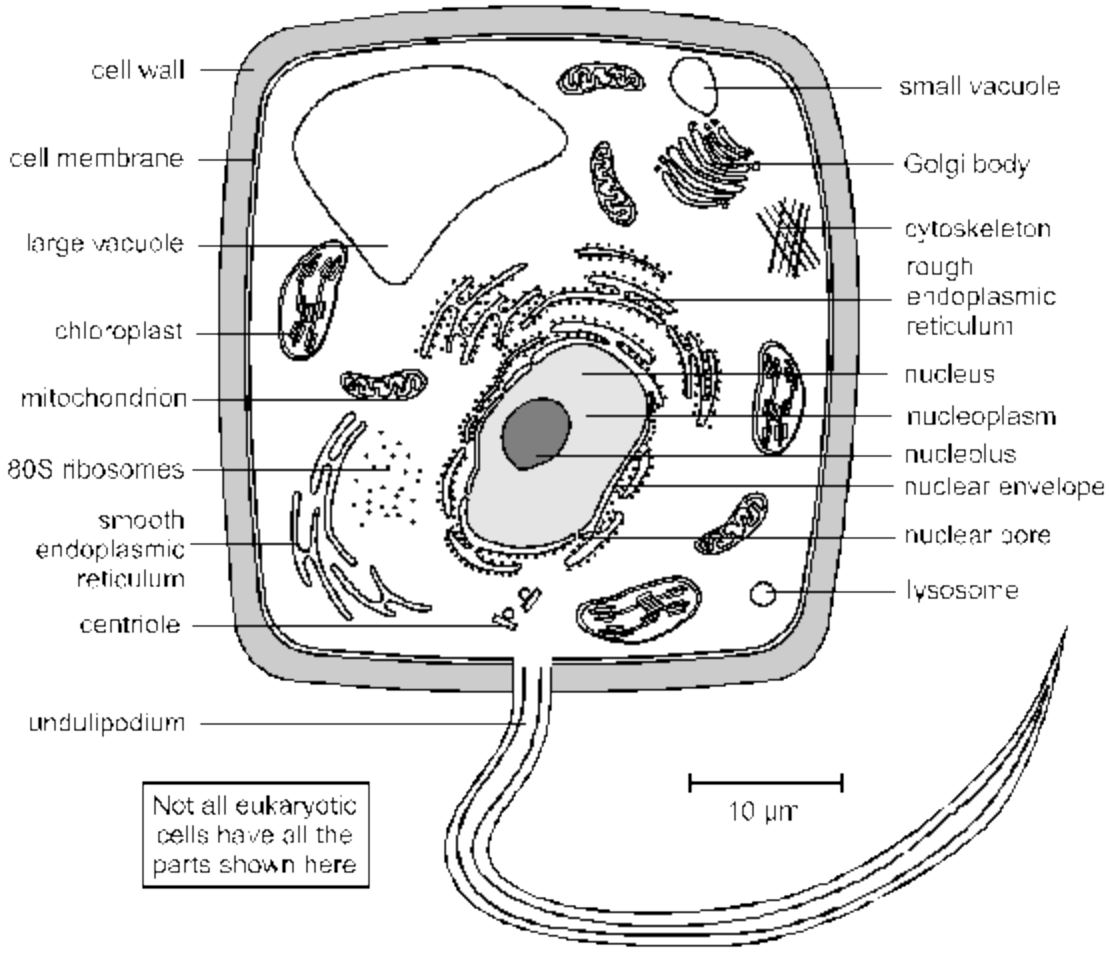
Eukaryotes
Prokaryotes
Cell Membranes
Transport

All living things are made of cells, and cells are the smallest units that can be alive. Life on Earth is classified into five kingdoms, and they each have their own characteristic kind of cell. However the biggest division is between the cells of the prokaryote kingdom (the bacteria) and those of the other four kingdoms (animals, plants, fungi and protoctista), which are all eukaryotic cells. Prokaryotic cells are smaller and simpler than eukaryotic cells, and do not have a nucleus.

- Prokaryote = without a nucleus
- Eukaryote = with a nucleus

We'll examine these two kinds of cell in detail, based on structures seen in electron micrographs (photos taken with an electron microscope). These show the individual organelles inside a cell.

Eukaryotic Cells

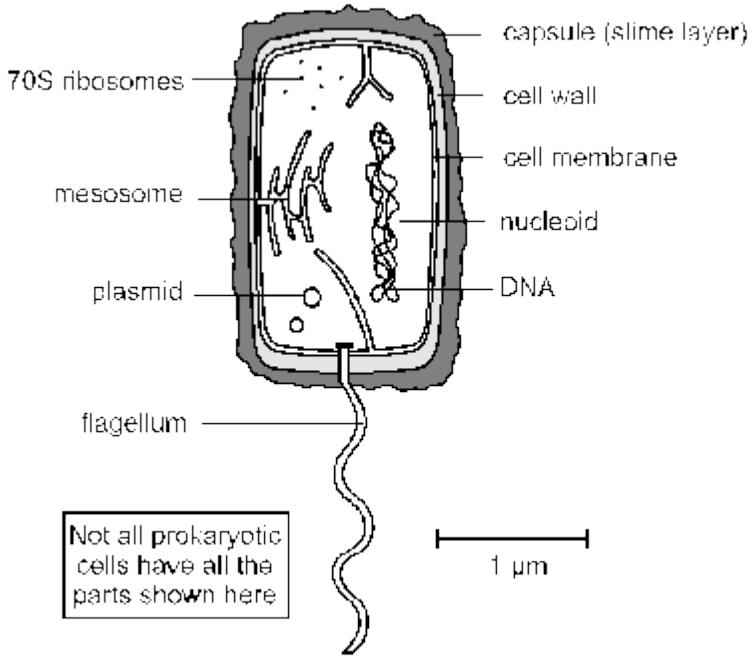


- **Cytoplasm (or Cytosol).** This is the solution within the cell membrane. It contains enzymes for metabolic reactions together with sugars, salts, amino acids, nucleotides and everything else needed for the cell to function.
- **Nucleus**. This is the largest organelle. Surrounded by a nuclear envelope, which is a double membrane with nuclear pores - large holes containing proteins that control the exit of substances such as RNA from the nucleus. The interior is called the nucleoplasm, which is full of chromatin- a DNA/protein complex containing the genes. During cell division the chromatin becomes condensed into discrete observable chromosomes. The nucleolus is a dark region of chromatin, involved in making ribosomes.
- **Mitochondrion (pl. Mitochondria)**. This is a sausage-shaped organelle (8µm long), and is where aerobic respiration takes place in all eukaryotic cells. Mitochondria are surrounded by a double membrane: the outer membrane is simple, while the inner membrane is highly folded into cristae, which give it a large surface area. The space enclosed by the inner membrane is called the matrix, and contains small circular strands of DNA. The inner membrane is studded with stalked particles, which are the site of ATP synthesis.
- **Chloroplast**. Bigger and fatter than mitochondria, chloroplasts are where photosynthesis takes place, so are only found in photosynthetic organisms (plants and algae). Like mitochondria they are enclosed by a double membrane, but chloroplasts also have a third membrane called the thylakoid membrane. The thylakoid membrane is folded into thylakoid disks, which are then stacked into piles called grana. The space between the inner membrane and the thylakoid is called the stroma. The thylakoid membrane contains chlorophyll and stalked particles, and is the site of photosynthesis and ATP synthesis. Chloroplasts also contain starch grains, ribosomes and circular DNA.
- **Ribosomes.** These are the smallest and most numerous of the cell organelles, and are the sites of protein synthesis. They are composed of protein and RNA, and are manufactured in the

nucleolus of the nucleus. Ribosomes are either found free in the cytoplasm, where they make proteins for the cell's own use, or they are found attached to the rough endoplasmic reticulum, where they make proteins for export from the cell. They are often found in groups called polysomes. All eukaryotic ribosomes are of the larger, "80S", type.

- **Smooth Endoplasmic Reticulum (SER).** Series of membrane channels involved in synthesising and transporting materials, mainly lipids, needed by the cell.
- **Rough Endoplasmic Reticulum (RER).** Similar to the SER, but studded with numerous ribosomes, which give it its rough appearance. The ribosomes synthesise proteins, which are processed in the RER (e.g. by enzymatically modifying the polypeptide chain, or adding carbohydrates), before being exported from the cell via the Golgi Body.
- **Golgi Body (or Golgi Apparatus).** Another series of flattened membrane vesicles, formed from the endoplasmic reticulum. Its job is to transport proteins from the RER to the cell membrane for export. Parts of the RER containing proteins fuse with one side of the Golgi body membranes, while at the other side small vesicles bud off and move towards the cell membrane, where they fuse, releasing their contents by exocytosis.
- **Vacuoles.** These are membrane-bound sacs containing water or dilute solutions of salts and other solutes. Most cells can have small vacuoles that are formed as required, but plant cells usually have one very large permanent vacuole that fills most of the cell, so that the cytoplasm (and everything else) forms a thin layer round the outside. Plant cell vacuoles are filled with cell sap, and are very important in keeping the cell rigid, or turgid. Some unicellular protoctists have feeding vacuoles for digesting food, or contractile vacuoles for expelling water.
- **Lysosomes.** These are small membrane-bound vesicles formed from the RER containing a cocktail of digestive enzymes. They are used to break down unwanted chemicals, toxins, organelles or even whole cells, so that the materials may be recycled. They can also fuse with a feeding vacuole to digest its contents.
- **Cytoskeleton.** This is a network of protein fibres extending throughout all eukaryotic cells, used for support, transport and motility. The cytoskeleton is attached to the cell membrane and gives the cell its shape, as well as holding all the organelles in position. There are three types of protein fibres (microfilaments, intermediate filaments and microtubules), and each has a corresponding motor protein that can move along the fibre carrying a cargo such as organelles, chromosomes or other cytoskeleton fibres. These motor proteins are responsible for such actions as: chromosome movement in mitosis, cytoplasm cleavage in cell division, cytoplasmic streaming in plant cells, cilia and flagella movements, cell crawling and even muscle contraction in animals.
- **Centriole**. This is a pair of short microtubules involved in cell division.
- **Cilium and Flagellum**. These are flexible tails present in some cells and used for motility. They are an extension of the cytoplasm, surrounded by the cell membrane, and are full of microtubules and motor proteins so are capable of complex swimming movements. There are two kinds: flagella (pl.) (no relation of the bacterial flagellum) are longer than the cell, and there are usually only one or two of them, while cilia (pl.) are identical in structure, but are much smaller and there are usually very many of them.
- **Microvilli**. These are small finger-like extensions of the cell membrane found in certain cells such as in the epithelial cells of the intestine and kidney, where they increase the surface area for absorption of materials. They are just visible under the light microscope as a brush border.
- **Cell Membrane (or Plasma Membrane).** This is a thin, flexible layer round the outside of all cells made of phospholipids and proteins. It separates the contents of the cell from the outside environment, and controls the entry and exit of materials. The membrane is examined in detail later.
- **Cell Wall.** This is a thick layer outside the cell membrane used to give a cell strength and rigidity. Cell walls consist of a network of fibres, which give strength but are freely permeable to solutes (unlike membranes). Plant cell walls are made mainly of cellulose, but can also contain hemicellulose, pectin, lignin and other polysaccharides. There are often channels through plant cell walls called plasmodesmata, which link the cytoplasms of adjacent cells. Fungal cell walls are made of chitin. Animal cells do not have a cell wall.

Prokaryotic Cells



- **Cytoplasm**. Contains all the enzymes needed for all metabolic reactions, since there are no organelles
- **Ribosomes**. The smaller (70 S) type.
- **Nuclear Zone**. The region of the cytoplasm that contains DNA. It is not surrounded by a nuclear membrane.
- **DNA**. Always circular, and not associated with any proteins to form chromatin.
- **Plasmid**. Small circles of DNA, used to exchange DNA between bacterial cells, and very useful for genetic engineering.
- **Cell membrane**. made of phospholipids and proteins, like eukaryotic membranes.
- **Mesosome**. A tightly-folded region of the cell membrane containing all the membrane-bound proteins required for respiration and photosynthesis.
- **Cell Wall**. Made of murein, which is a glycoprotein (i.e. a protein/carbohydrate complex). There are two kinds of cell wall, which can be distinguished by a Gram stain: Gram positive bacteria have a thick cell wall and stain purple, while Gram negative bacteria have a thin cell wall with an outer lipid layer and stain pink.
- **Capsule** (or **Slime Layer**). A thick polysaccharide layer outside of the cell wall. Used for sticking cells together, as a food reserve, as protection against desiccation and chemicals, and as protection against phagocytosis.
- **Flagellum**. A rigid rotating helical-shaped tail used for propulsion. The motor is embedded in the cell membrane and is driven by a H^+ gradient across the membrane. Clockwise rotation drives the cell forwards, while anticlockwise rotation causes a chaotic spin. This is an example of a rotating motor in nature.

Summary of the Differences Between Prokaryotic and Eukaryotic Cells

Prokaryotic Cells	Eukaryotic cells
small cells (< 5 μm)	larger cells (> 10 μm)
always unicellular	often multicellular
no nucleus or any membrane-bound organelles	always have nucleus and other membrane-bound organelles
DNA is circular, without proteins	DNA is linear and associated with proteins to form chromatin
ribosomes are small (70S)	ribosomes are large (80S)
no cytoskeleton	always has a cytoskeleton
cell division is by binary fission	cell division is by mitosis or meiosis
reproduction is always asexual	reproduction is asexual or sexual

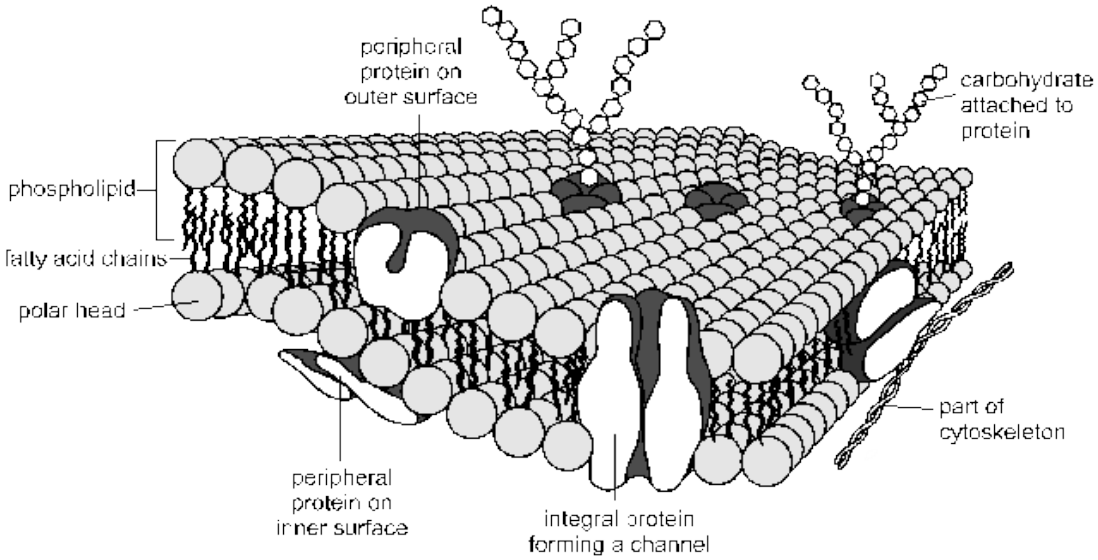
Endosymbiosis

Prokaryotic cells are far older and more diverse than eukaryotic cells. Prokaryotic cells have probably been around for 3.5 billion years - 2.5 billion years longer than eukaryotic cells. It is thought that eukaryotic cell organelles like mitochondria and chloroplasts are derived from prokaryotic cells that became incorporated inside larger prokaryotic cells. This idea is called endosymbiosis, and is supported by these observations:

- organelles contain circular DNA, like bacteria cells.
- organelles contain 70S ribosomes, like bacteria cells.
- organelles have double membranes, as though a single-membrane cell had been engulfed and surrounded by a larger cell.

The Cell Membrane

The cell membrane (or plasma membrane) surrounds all living cells. It controls how substances can move in and out of the cell and is responsible for many other properties of the cell as well. The membranes that surround the nucleus and other organelles are almost identical to the cell membrane. Membranes are composed of phospholipids, proteins and carbohydrates arranged in a fluid mosaic structure, as shown in this diagram.



The phospholipids form a thin, flexible sheet, while the proteins "float" in the phospholipid sheet like icebergs, and the carbohydrates extend out from the proteins.

The phospholipids are arranged in a bilayer, with their polar, hydrophilic phosphate heads facing outwards, and their non-polar, hydrophobic fatty acid tails facing each other in the middle of the bilayer. This hydrophobic layer acts as a barrier to all but the smallest molecules, effectively isolating the two sides of the membrane. Different kinds of membranes can contain phospholipids with different fatty acids, affecting the strength and flexibility of the membrane, and animal cell membranes also contain cholesterol linking the fatty acids together and so stabilising and strengthening the membrane.

The proteins usually span from one side of the phospholipid bilayer to the other (intrinsic proteins), but can also sit on one of the surfaces (extrinsic proteins). They can slide around the membrane very quickly and collide with each other, but can never flip from one side to the other. The proteins have hydrophilic amino acids in contact with the water on the outside of membranes, and hydrophobic amino acids in contact with the fatty chains inside the membrane. Proteins comprise about 50% of the mass of membranes, and are responsible for most of the membrane's properties.

- Proteins that span the membrane are usually involved in transporting substances across the membrane (more details below).
- Proteins on the inside surface of cell membranes are often attached to the cytoskeleton and are involved in maintaining the cell's shape, or in cell motility. They may also be enzymes, catalysing reactions in the cytoplasm.
- Proteins on the outside surface of cell membranes can act as receptors by having a specific binding site where hormones or other chemicals can bind. This binding then triggers other events in the cell. They may also be involved in cell signalling and cell recognition, or they may be enzymes, such as maltase in the small intestine (more in digestion).

The carbohydrates are found on the outer surface of all eukaryotic cell membranes, and are usually attached to the membrane proteins. Proteins with carbohydrates attached are called glycoproteins. The carbohydrates are short polysaccharides composed of a variety of different monosaccharides, and form a cell coat or glycocalyx outside the cell membrane. The glycocalyx is involved in protection and cell recognition, and antigens such as the ABO antigens on blood cells are usually cell-surface glycoproteins.

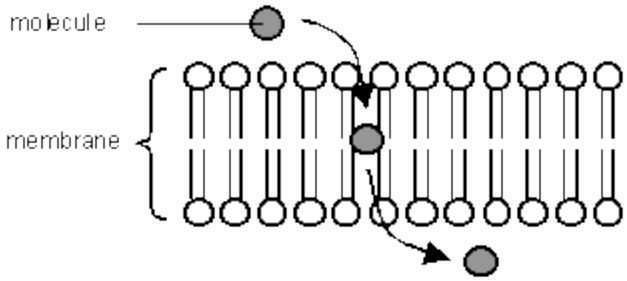
Remember that a membrane is not just a lipid bilayer, but comprises the lipid, protein and carbohydrate parts.

Transport Across The Membrane

Cell membranes are a barrier to most substances, and this property allows materials to be concentrated inside cells, excluded from cells, or simply separated from the outside environment. This is compartmentalization is essential for life, as it enables reactions to take place that would otherwise be impossible. Eukaryotic cells can also compartmentalize materials inside organelles. Obviously materials need to be able to enter and leave cells, and there are five main methods by which substances can move across a cell membrane:

- 1. Simple Diffusion
- 2. Osmosis
- 3. Facilitated Diffusion
- 4. Active Transport
- 5. Vesicles

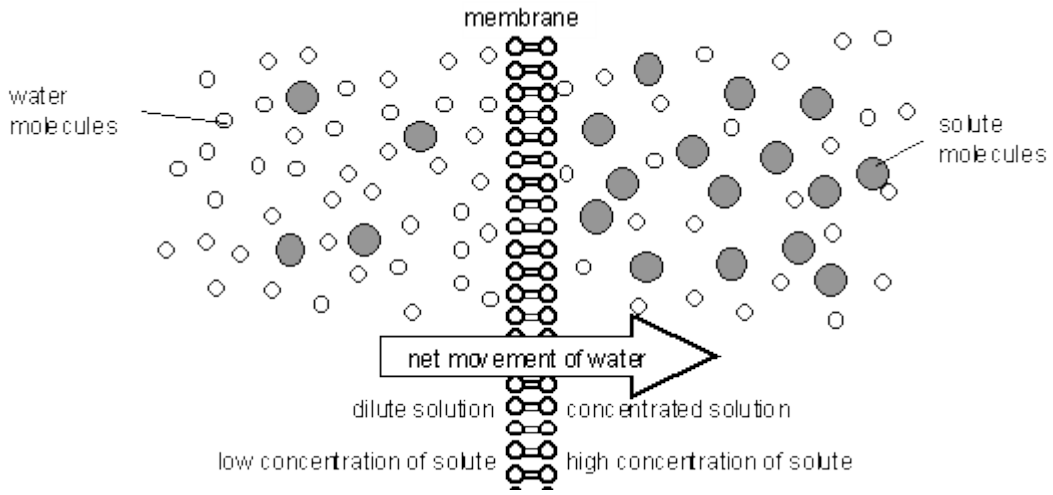
1. Simple Diffusion



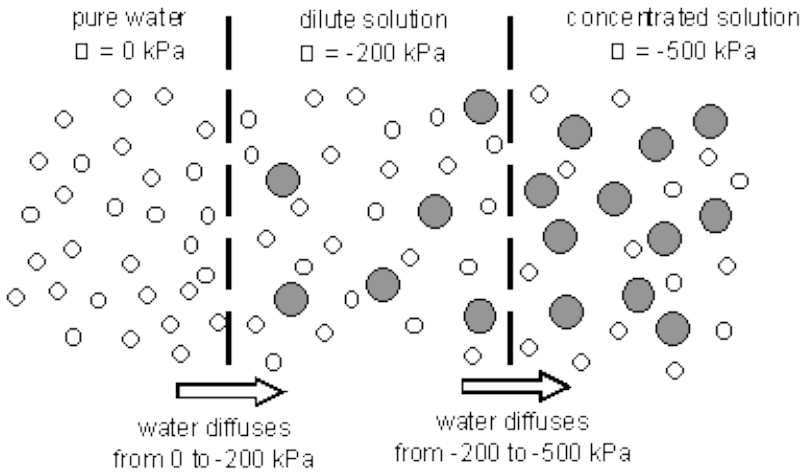
A few substances can diffuse directly through the lipid bilayer part of the membrane. The only substances that can do this are lipid-soluble molecules such as steroids, or very small molecules, such as H_2O, O_2 and CO_2. For these molecules the membrane is no barrier at all. Since lipid diffusion is (obviously) a passive diffusion process, no energy is involved and substances can only move down their concentration gradient. Lipid diffusion cannot be controlled by the cell, in the sense of being switched on or off.

2. Osmosis

Osmosis is the diffusion of water across a membrane. It is in fact just normal lipid diffusion, but since water is so important and so abundant in cells (its concentration is about 50 M), the diffusion of water has its own name - osmosis. The contents of cells are essentially solutions of numerous different solutes, and the more concentrated the solution, the more solute molecules there are in a given volume, so the fewer water molecules there are. Water molecules can diffuse freely across a membrane, but always down their concentration gradient, so water therefore diffuses from a dilute to a concentrated solution.

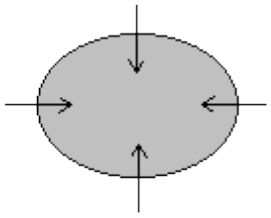
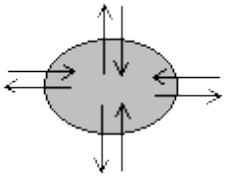
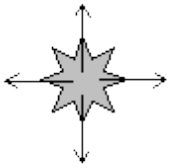
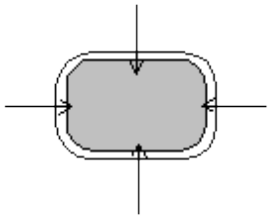
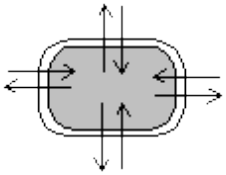
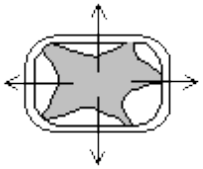


Water Potential. Osmosis can be quantified using water potential, so we can calculate which way water will move, and how fast. Water potential (Ψ, the Greek letter psi, pronounced "sy") is a measure of the water molecule potential for movement in a solution. It is measured in units of pressure (Pa, or usually kPa), and the rule is that water always moves by osmosis from less negative to more negative water potential (in other words it's a bit like gravity potential or electrical potential). 100% pure water has $\Psi = 0$, which is the highest possible water potential, so all solutions have $\Psi < 0$ (i.e. a negative number), and you cannot get $\Psi > 0$.



Cells and Osmosis. The concentration (or OP) of the solution that surrounds a cell will affect the state of the cell, due to osmosis. There are three possible concentrations of solution to consider:

- Isotonic solution a solution of equal OP (or concentration) to a cell
- Hypertonic solution a solution of higher OP (or concentration) than a cell
- Hypotonic solution a solution of lower OP (or concentration) than a cell
- The effects of these solutions on cells are shown in this diagram:

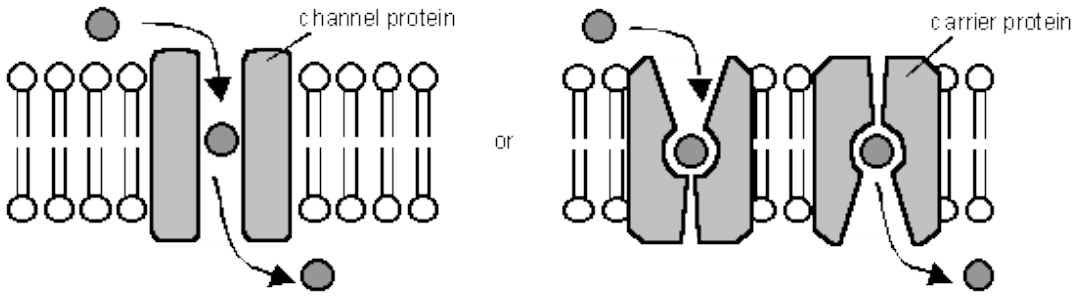
	Hypotonic solution	Isotonic solution	Hypertonic solution
Animal Cell	 water enters, cell swells and may burst (lysis).	 no net movement of water, cell normal size	 water leaves, cell shrinks and crenates
Plant Cell	 water enters, cell swells a bit and becomes turgid.	 no net movement of water, cell normal size	 water leaves, cytoplasm shrinks and plasmolyses

The diagram below shows what happens when 2 fresh raw eggs with their shells removed with acid are placed into sucrose solution (hypertonic) and distilled water (hypotonic). Water enters the egg in water (endosmosis) causing it to swell and water leaves the egg in sucrose causing it to shrink (exosmosis).

These are problems that living cells face all the time. For example:

- Simple animal cells (protozoans) in fresh water habitats are surrounded by a hypotonic solution and constantly need to expel water using contractile vacuoles to prevent swelling and lysis.
- Cells in marine environments are surrounded by a hypertonic solution, and must actively pump ions into their cells to reduce their water potential and so reduce water loss by osmosis.
- Young non-woody plants rely on cell turgor for their support, and without enough water they wilt. Plants take up water through their root hair cells by osmosis, and must actively pump ions into their cells to keep them hypertonic compared to the soil. This is particularly difficult for plants rooted in salt water.

3. Facilitated Diffusion.

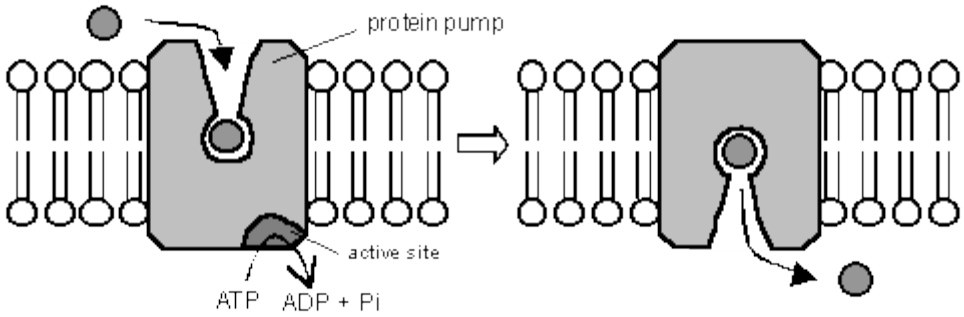


Facilitated diffusion is the transport of substances across a membrane by a trans-membrane protein molecule. The transport proteins tend to be specific for one molecule (a bit like enzymes), so substances can only cross a membrane if it contains the appropriate protein. As the name suggests, this is a passive diffusion process, so no energy is involved and substances can only move down their concentration gradient. There are two kinds of transport protein:

- Channel Proteins form a water-filled pore or channel in the membrane. This allows charged substances (usually ions) to diffuse across membranes. Most channels can be gated (opened or closed), allowing the cell to control the entry and exit of ions.
- Carrier Proteins have a binding site for a specific solute and constantly flip between two states so that the site is alternately open to opposite sides of the membrane. The substance will bind on the side where it at a high concentration and be released where it is at a low concentration.

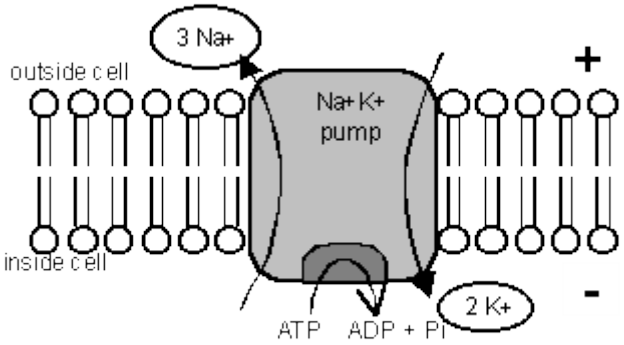
The rate of diffusion of a substance across a membrane increases as its concentration gradient increases, but whereas lipid diffusion shows a linear relationship, facilitated diffusion has a curved relationship with a maximum rate. This is due to the rate being limited by the number of transport proteins.

4. Active Transport (or Pumping).



Active transport is the pumping of substances across a membrane by a trans-membrane protein pump molecule. The protein binds a molecule of the substance to be transported on one side of the membrane, changes shape, and releases it on the other side. The proteins are highly specific, so there is a different protein pump for each molecule to be transported. The protein pumps are also ATPase enzymes, since they catalyse the splitting of ATP into ADP + phosphate (Pi), and use the energy released to change shape and pump the molecule. Pumping is therefore an active process, and is the only transport mechanism that can transport substances up their concentration gradient.

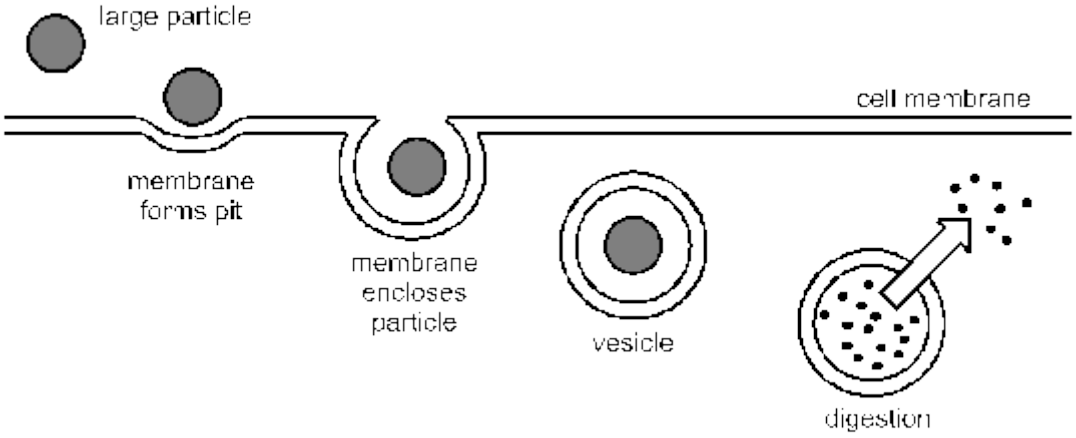
The Na^+K^+ Pump. This transport protein is present in the cell membranes of all animal cells and is the most abundant and important of all membrane pumps. We look at it in more detail in module 4 (A2 course)



5. Vesicles

The processes described so far only apply to small molecules. Large molecules (such as proteins, polysaccharides and nucleotides) and even whole cells are moved in and out of cells by using membrane vesicles.

Endocytosis is the transport of materials into a cell. Materials are enclosed by a fold of the cell membrane, which then pinches shut to form a closed vesicle. Strictly speaking the material has not yet crossed the membrane, so it is usually digested and the small product molecules are absorbed by the methods above. When the materials and the vesicles are small (such as a protein molecule) the process is known as pinocytosis (cell drinking), and if the materials are large (such as a white blood cell ingesting a bacterial cell) the process is known as phagocytosis (cell eating).



Exocytosis is the transport of materials out of a cell. It is the exact reverse of endocytosis. Materials to be exported must first be enclosed in a membrane vesicle, usually from the RER and Golgi Body. Hormones and digestive enzymes are secreted by exocytosis from the secretory cells of the intestine and endocrine glands.

Sometimes materials can pass straight through cells without ever making contact with the cytoplasm by being taken in by endocytosis at one end of a cell and passing out by exocytosis at the other end.

Summary of Membrane Transport

METHOD	USES ENERGY	USES PROTEINS	SPECIFIC	CONTROLLABLE
Simple Diffusion	N	N	N	N
Osmosis	N	N	Y	N
Facilitated Diffusion	N	Y	Y	Y
Active Transport	Y	Y	Y	Y
Vesicles	Y	N	Y	Y

CELLS SUMMARY

Cells

Are the building blocks of organisms.

(Av.size: 20 micrometers)

Small due to:

- Cell membrane considerations
- Nucleus to cytoplasm ratio
- Supply to demand ratio

Electron microscope

Uses a beam of electrons.

ADVANTAGE- HAS A:	DISADVANTAGE- SPECIMEN MUST BE:
• Shorter wavelength • Greater resolution	• Dead • Dehydrated

Organelles

- Are membrane bound structures.
- Have specialised functions to perform.
- Some do not have a membrane surrounding them.
- chloroplast & permanent vacuoles are only found in plant cells.

Nucleus

- Controls all cell activities
- Contains genes
- Contains code for protein synthesis
- Involved in production of Ribosome's & RNA (essential for cell division)

Structure

- Contains- nucleic acids (DNA&RNA)
- **Double membrane =** Nuclear envelope
- Encrusted with ribosome's
- Covered in pores
- Continuous with RER

RER

- Protein isolation & transport

Structure of RER

- Consists of interconnecting flattened tubules (cisternae) stacked together.
- Membrane is encrusted with ribosome's (Polysome configuration).

SER

- Steroid synthesis
- Lipid synthesis
- Lipid & steroid transportation
- Storage of Ca ions

Structure

- No ribosomes
- Rarely form cisternae
- Membrane distinctly more tubular & smooth

ER in general

- Increases in surface area for chemical reactions
- Provides a pathway for transporting materials through the cell
- Collects & stores materials made by the cell.

Ribosomes

2 types

- 70s prokaryotes (+ chloroplasts and mitochondria)

- 80s eukaryotes

structure

Consists of small & large subunit.

Golgi Apparatus

- A stack of flattened cavities
- Forms lysosomes
- Produces enzymes for secretion
- Protein and carbohydrate combine to form glycoprotein

Vesicles

Contain proteins for:

- Secretion
- To become part of plasma membrane
- To become functions of enzymes

Lysosomes

- Contain digestive hydrolytic enzyme
- Fuse with the target, enzymes breakdown the target, products are absorbed by the cell
- Secretes their enzymes outside the cell to breakdown other cells
- Digests stuff taken in from the environment by the cell
- Digests parts of cells e.g.: worn out organelles (autolysis)

Mitochondria

- Synthesis of ATP
- Biosynthesis
- Found in all eukaryotes except mature red blood cells.
- Number depends on activity of cell.
- High metabolically active ones- have large numbers.
- Low ones- have small numbers.

Contains

70s ribosome's, DNA circlet, Matrix- fluid of mitochondria, double membrane & Cristae which is an inner folded membrane containing stalked particles.

Chloroplast

- Site of photosynthesis

Contains

- Lamellae, DNA circlet, double membrane, Stroma- fluid of chloroplast, starch grains, granum, thylakoid & chlorophyll.

Cell wall

- Contains cellulose & hemi cellulose.
- Are fully permeable & strong.

Structure

- X- weave made from interwoven fibres.
- Consists of straight chains of beta-glucose, forms micro fibrils & macro fibrils

Centrioles

- Forms the spindle during cell division

Structure

- 2 cylinders of protein microtubules arranged at 90 degrees
- Not membrane bound

Differences between Prokaryotic & Eukaryotic cells

FEATURE	PROKARYOTE	EUKARYOTE
Size	Small about 0.5 micrometers	Up to 40 micrometers
Genetic material	Circular DNA (in cytoplasm)	DNA in form of linear chromosomes (in nucleus)
Organelles	Few present, none membrane bound	Many organelles: • Double membranes e.g.: nucleus, mitochondria & chloroplasts • Single membrane e.g.: GA, ER & lysosomes
Cell walls	Rigid formed from glycoproteins (mainly murein)	• Fungi: rigid, formed from polysaccharide, chitin. • Plant: rigid, formed from polysaccharides. E.g.: cellulose. • Animals no cell wall
Ribosome's	70s	80s

Bacterial cells also contain flagellum, plasmid and capsule.

- Cells form specialised cells, which form tissues.
- **Tissues**- are cells of one type, which carry out one function. E.g.: muscle, nerves
- **Organ**- is a structure made up of different tissues performing certain tasks.

Epithelial cells of small intestine

- Microvilli increase surface area for absorption.
- Mitochondria synthesises ATP for active transport

Palisade mesophyll cell

- Elongated to absorb light
- Contains many chloroplasts for photosynthesis

Differential centrifugation

Used to obtain a sample of isolated organelles.

- Homogenise sample of cells
  - Conditions
  - Ice cold- to stop biological processes
  - Isotonic solution- to prevent osmotic damage
- Add solution to a centrifuge & spin at a low speed
  - Densest organelles spin down first. E.g.: nucleus
- Place supernatant back into centrifuge & spin at a higher speed
  - Next organelle spins down e.g.: mitochondria
- Repeat & spin & higher speed
  - e.g.: RER, SER, GA.

Cell transport

-

Plasma membranes

- Consists of 40% lipids & 60% protein.
- The polar nature of phospholipids explains membrane assembly.
- Phospholipid heads are hydrophilic.
- Fatty acid tails are hydrophobic.

Fluid mosaic model

- Fluid- phospholipids move around the medium.
- Mosaic- phospholipids are not attached to each other/arranged in sequence.

Movement in + out of cells

1. Diffusion (passive)

- Small, gas mols pass between phospholipid mols in membrane. (H-L)
- Water is a special case (osmosis)

2. Facilitated diffusion (passive)

- Movement of lipid soluble, small & gas mols from a (H-L) conc. through intrinsic membrane proteins
- Fatty acid tails creates a hydrophobic barrier to entry.
- **2 types of f-d proteins**
- **Pore (channel) proteins**
- Can be gated by chemicals / a change in voltage is required to open the protein pore.
- **Carrier proteins**
- Mols. undergo a conformational shape change.
- Small mols. that cannot pass through the phospholipid bilayer, glucose, charged mols., ions. Na, K move by facilitated diffusion.

3. Osmosis (passive)

- Movement of water mols from a less to more (-) WP via a selectively permeable.
- Pure (distilled) water has the highest WP = 0 & has a greater average KE of water mols.
- Water + solute has a (-) WP & has a less average KE of water mols.
- WP is the ability of water mols to move. (Kpa)

Osmosis & plant cells

- WP = OP + PP
- Cell WP = cytoplasm's OP + wall PP
- When plant fully turgid WP=0

Passive transport in general

- Uses KE of mols., or ions, themselves as the motive power to move these materials… so direction of movement depends upon concentration & / electrical (charge) gradients.
- E.g.: diffusion, osmosis, facilitated diffusion

4. Active transport (active)

- Uses energy from ATP to move molecules / ions against unfavourable concentration & /

electrical gradients. (L-H)
- Movement is against conc. gradient
- Requires the hydrolysis of ATP
- E.g.: Na, K pumping by nerve cell membranes.

5. Bulk transport (active)

1. Endocytosis

- Into cells (active process)
- Plasma membrane forms a vesicle around substance & vesicles taken into cell.
- Phagocytosis = cells + solid particles e.g.: macrophages
- Pinocytosis = cells + dissolved molecules

2. Exocytosis

- Out of cell (active process)
- Materials formed by the cell are packaged in secretary vesicles, which fuse with the plasma membrane to release their contents.
- E.g.: secreted proteins (digestive enzymes & hormones)

TRANSPORT	ATP REQUIRED	HIGH TO LOW	PROTEINS INVOLVED
Simple diffusion	N	Y	N
Facilitated diffusion	N	Y	Y
Active Transport	Y	N	Y

EXCHANGE

CONTENTS

Diffusion and size
Gas exchange
Gas exchange in plants
Gas exchange in fish
Gas exchange in humans

DIFFUSION AND THE PROBLEM OF SIZE

All organisms need to exchange substances such as food, waste, gases and heat with their surroundings. These substances must diffuse between the organism and the surroundings. The rate at which a substance can diffuse is given by Fick's law:

$$\text{Rate of Diffusion} \propto \frac{\text{surface area} \times \text{concentration difference}}{\text{distance}}$$

So rate of exchange of substances depends on the organism's surface area that's in contact with the surroundings. Requirements for materials depends on the volume of the organism, So the ability to meet the requirements depends on the surface area : volume ratio. As organisms get bigger their volume and surface area both get bigger, but volume increases much more than surface area. This can be seen with some simple calculations for different-sized organisms. Although it's innacurate lets assume the organisms are cube shaped to simplify the maths - the overall picture is still the same. The surface area of a cube with length of side L is LxLx6, while the volume is LxLxL.

Organism	Length	SA (m^2)	Vol. (m^3)	S/A:Vol
bacterium	1 µm	6×10^{-12}	10^{-18}	6,000,000:1
amoeba	100 µm	6×10^{-8}	10^{-12}	60,000:1
fly	10 mm	6×10^{-4}	10^{-6}	600:1
dog	1 m	6×10^{0}	10^{0}	6:1
whale	100 m	6×10^{4}	10^{6}	0.06:1

So as organisms get bigger their surface area/volume ratio gets smaller. Bacteria are all surface with not much inside, while whales are all insides without much surface. So as organisms become bigger it is more difficult for them to exchange materials with their surroundings.

Organisms also need to exchange heat with their surroundings, and here large animals have an advantage in having a small surface area/volume ratio: they lose less heat than small animals. Large mammals keep warm quite easily and don't need much insulation or heat generation. Small mammals and birds lose their heat very readily, so need a high metabolic rate in order to keep generating heat, as well as thick insulation. So large mammals can feed once every few days while small mammals must feed continuously. Human babies also loose heat more quickly than adults, which is why they need woolly hats.

Systems that increase the rate of exchange

Fick's law showed that for a fast rate of diffusion you must have a large surface area, a small distance between the source & the destination, and maintain a high concentration gradient. All large organisms have developed systems that are well-adapted to achieving these goals, as this table shows. For

comparison, a tennis court has an area of about 260 m² and a football pitch has an area of about 5000 m².

SYSTEM	LARGE SURFACE AREA	SMALL DISTANCE	CONCENTRATION GRADIENT
Human lungs	600 million alveoli with a total area of 100m²	each alveolus = 1 cell thick	constant ventilation replaces the air
Fish gills	feathery filaments with secondary lamellae	lamellae = 2 cells thick	water pumped over gills in countercurrent to blood
Leaves	For a tree - SA of leaves =200m²; - SA of spongy cells inside leaves = 6000m².	gases diffuse straight into leaf cells	wind replaces air round leaves

GAS EXCHANGE

Gas exchange takes place at a respiratory surface - a boundary between the external environment and the interior of the body. For unicellular organisms the respiratory surface is simply the cell membrane, but for large organisms it is part of specialised organs like lungs, gills or leaves. This name can cause problems - in biology the word "respiration" means cellular respiration (ATP generation inside cells), however sometimes (such as here) it can also refer to breathing, which is what most non-biologists mean by it anyway.

Gases cross the respiratory surface by diffusion, so from Fick's law we can predict that respiratory surfaces must have:

- a large surface area
- a thin permeable surface
- a moist exchange surface

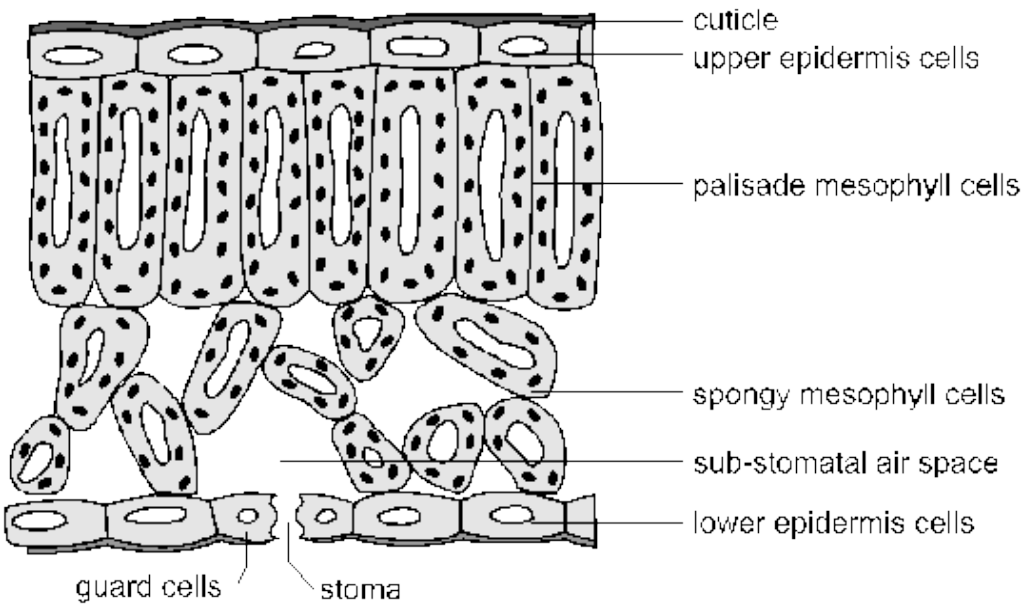
Many also have

- a mechanism to maximise the diffusion gradient by replenishing the source and/or sink.

We shall examine how these requirements are met in the gas exchange systems of humans, fish and plants.

GAS EXCHANGE IN PLANTS

All plant cells respire all the time, and when illuminated plant cells containing chloroplasts also photosynthesise, so plants also need to exchange gases. The main gas exchange surfaces in plants are the spongy mesophyll cells in the leaves. Leaves of course have a huge surface area, and the irregular-shaped, loosely-packed spongy cells increase the area for gas exchange still further. You are expected to know leaf structure in the detail shown in the diagram

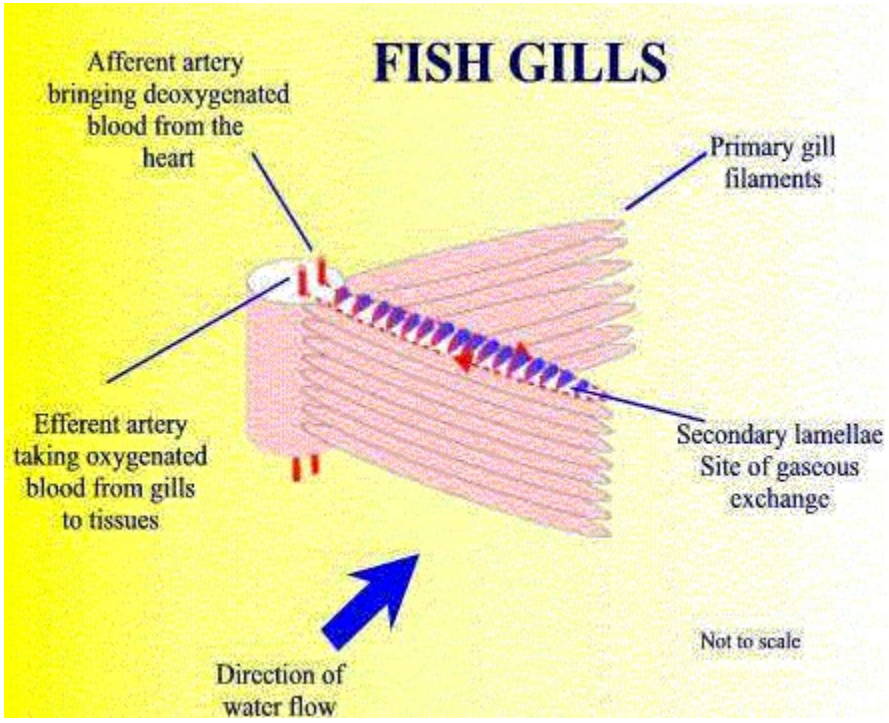


Gases enter the leaf through stomata -usually in the lower surface of the leaf. Stomata are enclosed by guard cells that can swell up and close the stomata to reduce water loss. The gases then diffuse through the air spaces inside the leaf, which are in direct contact with the spongy and palisade mesophyll cells. **Plants do not need a ventilation mechanism** because their leaves are exposed, so the air surrounding them is constantly being replaced in all but the stillest days. In addition, during the hours of daylight photosynthesis increases the oxygen concentration in the sub-stomatal air space, and decreases the carbon dioxide concentration. This increases the concentration gradients for these gases, increasing diffusion rate.

The palisade mesophyll cells are adapted for photosynthesis. They have a thin cytoplasm densely packed with chloroplasts, which can move around the cell on the cytoskeleton to regions of greatest light intensity. The palisade cells are closely packed together in rows to maximise light collection, and in plants adapted to low light intensity there may be two rows of palisade cells.

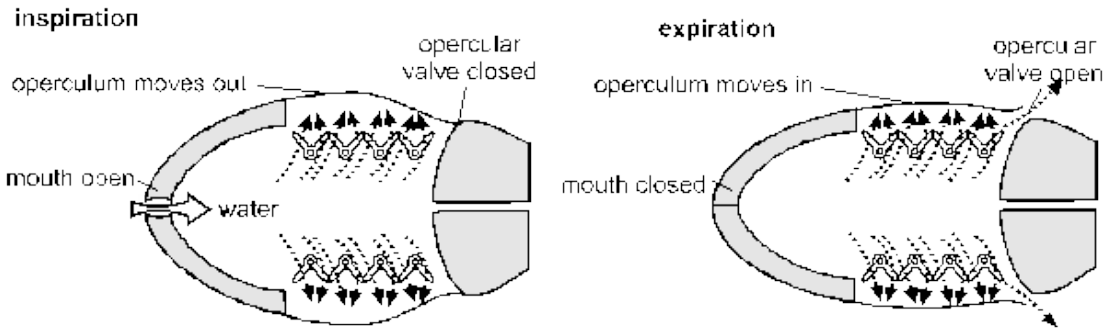
The spongy mesophyll cells are adapted for gas exchange. They are loosely-packed with unusually large intercellular air spaces where gases can collect and mix. They have fewer chloroplasts than palisade cells, so do less photosynthesis.

GAS EXCHANGE IN FISH



Gas exchange is more difficult for fish than for mammals because the concentration of dissolved oxygen in water is less than 1%, compared to 20% in air. (By the way, all animals need molecular oxygen for respiration and cannot break down water molecules to obtain oxygen.) Fish have developed specialised gas-exchange organs called gills, which are composed of thousands of filaments. The filaments in turn are covered in feathery lamellae which are only a few cells thick and contain blood capillaries. This structure gives a large surface area and a short distance for gas exchange. Water flows over the filaments and lamellae, and oxygen can diffuse down its concentration gradient the short distance between water and blood. Carbon dioxide diffuses the opposite way down its concentration gradient. The gills are covered by muscular flaps called opercula on the side of a fish's head. The gills are so thin that they cannot support themselves without water, so if a fish is taken out of water after a while the gills will collapse and the fish suffocates.

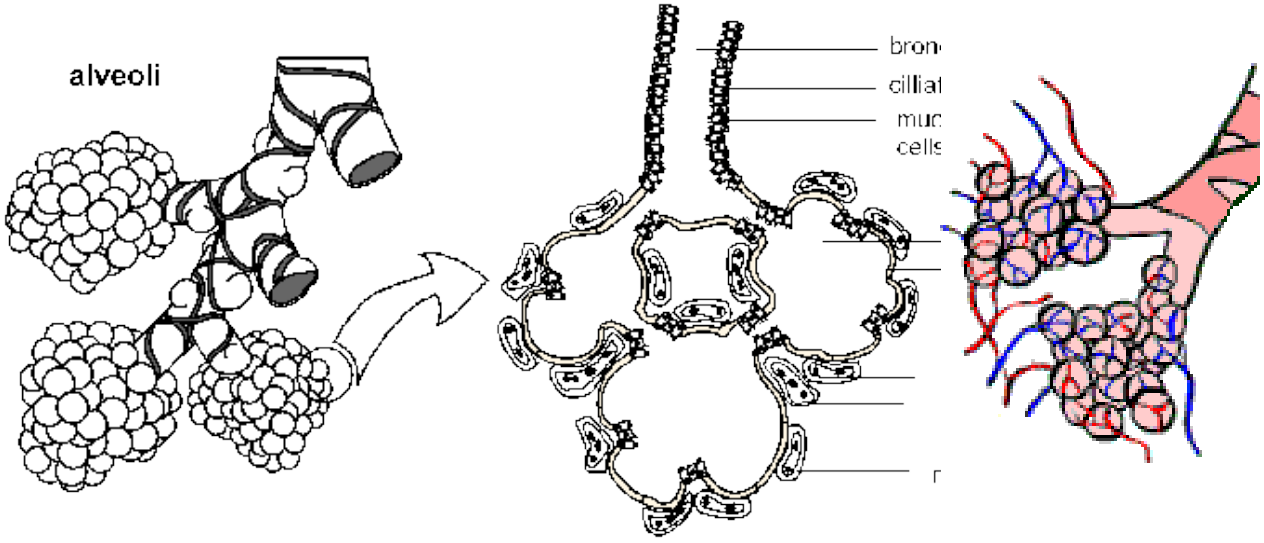
Fish ventilate their gills to maintain the gas concentration gradient. They continuously pump their jaws and opercula to draw water in through the mouth and then force it over the gills and out through the opercular valve behind the gills. This one-way ventilation is necessary because water is denser and more viscous than air, so it cannot be contained in delicate sac-like lungs found in air-breathing animals. In the gill lamellae the blood flows towards the front of the fish while the water flows towards the back. This countercurrent system increases the concentration gradient and increases the efficiency of gas exchange. About 80% of the dissolved oxygen is extracted from the water.



GAS EXCHANGE IN HUMANS

In humans the gas exchange organ system is the respiratory or breathing system. The main features are shown in this diagram.

The actual respiratory surface is on the alveoli inside the lungs. An average adult has about 600 million alveoli, giving a total surface area of about 100m², so the area is huge. The walls of the alveoli are composed of a single layer of flattened epithelial cells, as are the walls of the capillaries, so gases need to diffuse through just two thin cells. Water diffuses from the alveoli cells into the alveoli so that they are constantly moist. Oxygen dissolves in this water before diffusing through the cells into the blood, where it is taken up by haemoglobin in the red blood cells. The water also contains a soapy surfactant which reduces its surface tension and stops the alveoli collapsing. The alveoli also contain phagocyte cells to kill any bacteria that have not been trapped by the mucus.



The steep concentration gradient across the respiratory surface is maintained in two ways: by blood flow on one side and by air flow on the other side. This means oxygen can always diffuse down its concentration gradient from the air to the blood, while at the same time carbon dioxide can diffuse down its concentration gradient from the blood to the air. The flow of air in and out of the alveoli is called ventilation and has two stages: inspiration (or inhalation) and expiration (or exhalation). Lungs are not muscular and cannot ventilate themselves, but instead the whole thorax moves and changes size, due to the action of two sets of muscles: the intercostal muscles and the diaphragm.

Inspiration

- The diaphragm contracts and flattens downwards
- The external intercostal muscles contract, pulling the ribs up and out
- this increases the volume of the thorax
- this increases the lung and alveoli volume
- this decreases the pressure of air in the alveoli below atmospheric (Boyle's law)
- air flows in to equalise the pressure

Normal expiration

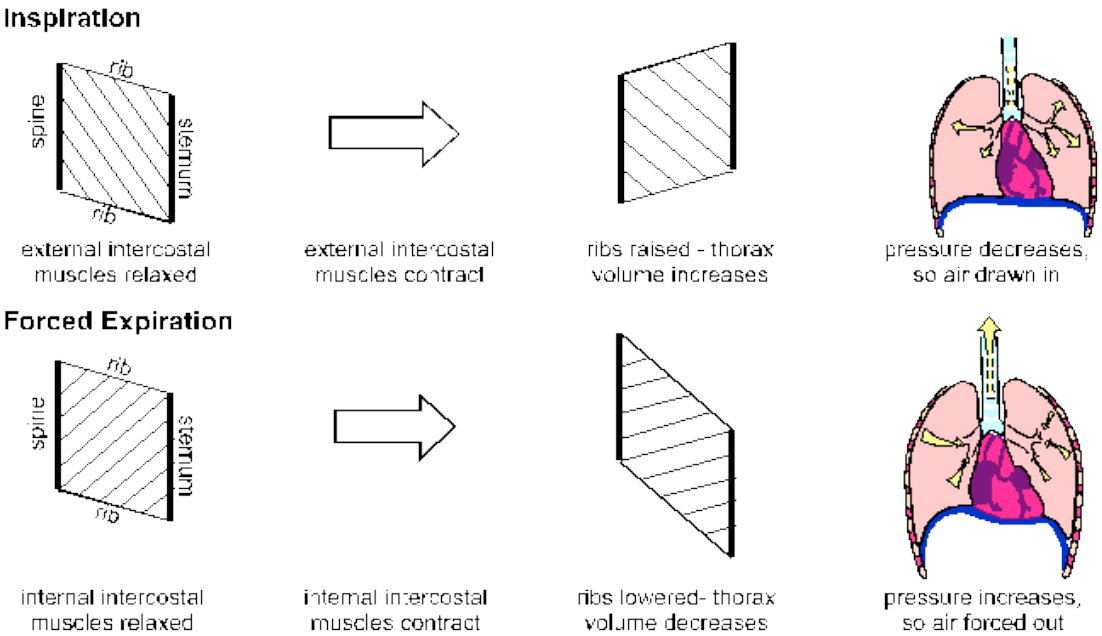
- The diaphragm relaxes and curves upwards

- The external intercostal muscles relax, allowing the ribs to fall
- this decreases the volume of the thorax
- this decreases the lung and alveoli volume
- this increases the pressure of air in the alveoli above atmospheric (Boyle's law)
- air flows out to equalise the pressure

Forced expiration

- The abdominal muscles contract, pushing the diaphragm upwards
- The internal intercostal muscles contract, pulling the ribs downward
- This gives a larger and faster expiration, used in exercise

These movements are transmitted to the lungs via the pleural sac surrounding each lung. The outer membrane is attached to the thorax and the inner membrane is attached to the lungs. Between the membranes is the pleural fluid, which is incompressible, so if the thorax moves, the lungs move too. The alveoli are elastic and collapse if not held stretched by the thorax (as happens in stab wounds).



Exchange

Surface Area to Volume Ratio

Surface area: volume ratio crops up in many exam questions. They can be questions relating to trees, plants, fish or mammals. The question will be about the size/shape of the particular organism or how its size/shape is adapted to its usually adverse surroundings.

Exchange In Organisms

A small organism, like an amoeba, has a large surface area: volume ratio and so it can take all the oxygen it needs by diffusion across the body surface. However, a large organism, like a mammal, has a much smaller surface area: volume ratio, so it cannot get all the oxygen it needs in this way. (A large surface area: volume ratio is preferable for carrying out exchange of substances). Such large organisms need special respiratory organs such as lungs for taking in oxygen.

Examples

- Alveoli in the lungs have a large surface area: volume ratio meaning gas exchange in humans occurs at a fast rate.
- The filaments used in gas exchange for fish also have a large surface area: volume ratio as its surfaces are covered in lamellae. This larger ratio means it is suitable for diffusion.
- The leaves of plants have a large ratio meaning again exchange is carried out more effectively.

Heat and water loss

Heat/water loss is affected by surface area: volume. In large organisms heat/water loss is less than in small organisms. This is because the organism has longer pathways and longer distances, probably more insulation so it is harder for the heat to escape. Conversely, in smaller organisms heat/water loss is greater than in large organisms. The organism has much shorter pathways; all its internal organs are closer to the surface and have less insulation.

Calculating the ratio

- Look at surface area and volume
- Check they are in the same units
- Divide the larger one by the smaller one= ANSWER
- The answer: 1is the ratio, where the answer is the figure for the larger volume

Large Mammals have difficulties regulating body temperature in hot climates due to:

- Small Surface Area to Volume Ratio
- Less heat is lost to the environment
- Homeotherms – Generate heat by metabolic processes

Blood vessels near the surface of the skin help to regulate body temperature by:

- Cooling the body from the core of the body
- More heat is lost due to Radiation
- More heat is lost due to Convection
- More heat is lost due to Conduction
- More heat is lost due to sweating
- Air flow over surface can be increased

The importance of a larger body size and mass to mammals in colder climates are:

- They have a small surface area to volume ratio
- They are homiothermic
- Lose less heat to the environment
- They have Fat for Insulation
- Lose less heat by Radiation/Conduction/Convection

Fish Gas Exchange

Structure of Respiratory Surfaces

- Gills provide a large **Surface Area**, mainly given by the filaments and secondary lamellae.
- The gills are highly capillarised which gives a **good blood supply**.
- Gills have a **short diffusion distance**; this is provided by flattened cells in capillaries and epithelium (surface of gill plates). This enables 0_2 to get into the bloodstream faster.

- In the respiratory system of a fish there is a **countercurrent,** this increases the **efficiency** of gas exchange. The blood flows in the opposite direction to water, this helps to maintain a **diffusion gradient** right along the gill. A result of this more 0_2 can diffuse from the water to the blood.

Fish Ventilation

- Fish ventilate using **unidirectional respiration** – this is due to the density of water being too great for the fish to breathe tidally as humans.
- The fish firstly **expands** its **Buccal Cavity** creating a **large surface area** for the intake of water.
- **Pressure decreases** in the buccal cavity lower than that of the external atmospheric pressure and water enters **down a pressure gradient.**
- As the fish closes it's mouth it **raises** the floor of the buccal cavity, **decreasing volume, increasing pressure.**
- **Water is forced over the gills.**
- At the same time the **Opperculum cavity bulges out, decreasing the pressure** within the cavity – **water is drained over the gills.**
- Removal of carbon dioxide occurs as the blood containing high concentrations of the waste gas goes to the gills and diffuses out into the water down a diffusion gradient (external water has lower concentrations of carbon dioxide than levels in the blood –sets up a diffusion gradient.)

Ventilation in Mammals

Very small organisms such as those consisting of a single cell, have no special tissues, organs or systems for gaseous exchange. Mammals are large, multi cellular organisms and they have a complex system for gaseous exchange. Mammals needs such a system single celled organism does not.

Single celled organisms	Mammals
• Large surface area to volume (ratio) for diffusion; • short diffusion pathway (to all parts of organism) • oxygen/ carbon dioxide diffuse in and out.	• Small surface area to volume • long diffusion pathway • waterproof/ gastight skinneed internal gas exchange surface which is moist with a large s/a

Breathing In:

- Diaphragm contracts and flattens.

- Intercostal muscles contract, therefore ribs move up and out.
- The volume of the thorax increases, decreasing pressure below atmospheric pressure.
- Oxygen flows into large air passages i.e Trachea => Bronchi => largest Bronchioles
- Final pathway – oxygen diffuses into alveoli along the concentration gradient. In the alveoli, oxygen dissolves into a film of liquid, which then diffuses the short distance into the blood capillaries.

Enzymes

Enzymes are biological catalysts. There are about 40,000 different enzymes in human cells, each controlling a different chemical reaction. They increase the rate of reactions by a factor of between 10^6 to 10^{12} times, allowing the chemical reactions that make life possible to take place at normal temperatures. They were discovered in fermenting yeast in 1900 by Buchner, and the name enzyme means "in yeast". As well as catalysing all the metabolic reactions of cells (such as respiration, photosynthesis and digestion), they also act as motors, membrane pumps and receptors.

Enzyme Structure

Enzymes are proteins, and their function is determined by their complex structure. The reaction takes place in a small part of the enzyme called the active site, while the rest of the protein acts as "scaffolding". This is shown in this diagram of a molecule of the enzyme amylase, with a short length of starch being digested in its active site. The amino acids around the active site attach to the substrate molecule and hold it in position while the reaction takes place. This makes the enzyme specific for one reaction only, as other molecules won't fit into the active site.

Many enzymes need cofactors (or coenzymes) to work properly. These can be metal ions (such as Fe^{2+}, Mg^{2+}, Cu^{2+}) or organic molecules (such as haem, biotin, FAD, NAD or coenzyme A). Many of these are derived from dietary vitamins, which is why they are so important. The complete active enzyme with its cofactor is called a holoenzyme, while just the protein part without its cofactor is called the apoenzyme.

How do enzymes work?

There are three ways of thinking about enzyme catalysis. They all describe the same process, though in different ways, and you should know about each of them.

1. Reaction Mechanism

In any chemical reaction, a substrate (S) is converted into a product (P):

S P

(There may be more than one substrate and more than one product, but that doesn't matter here.) In an enzyme-catalysed reaction, the substrate first binds to the active site of the enzyme to form an enzyme-substrate (ES) complex, then the substrate is converted into product *while attached to the enzyme*, and finally the product is released. This mechanism can be shown as:

E + S ES EP E + P

The enzyme is then free to start again. The end result is the same (S P), but a different route is taken, so that the S P reaction as such never takes place. In by-passing this step, the reaction can be made to happen much more quickly.

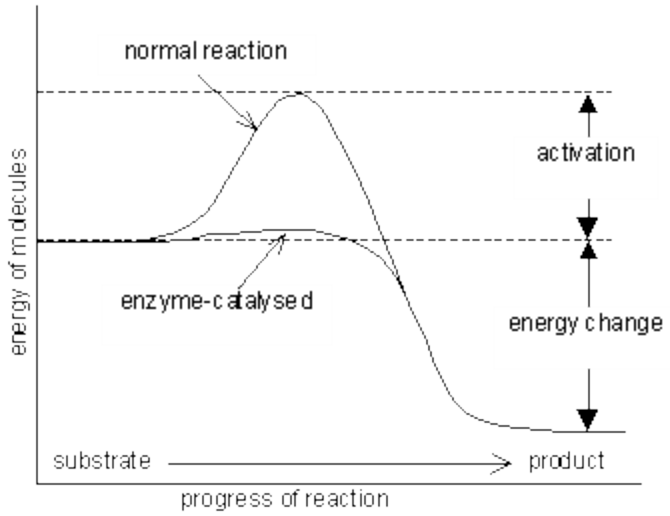
2. Molecule Geometry

The substrate molecule fits into the active site of the enzyme molecule like a key fitting into a lock (in fact it is sometimes called a lock and key mechanism). Once there, the enzyme changes shape slightly, distorting the molecule in the active site, and making it more likely to change into the product. For example if a bond in the substrate is to be broken, that bond might be stretched by the enzyme, making it more likely to break. Alternatively the enzyme can make the local conditions inside the active site quite different from those outside (such as pH, water concentration, charge), so that the reaction is more likely to happen.

It's a bit more complicated than that though. Although enzymes can change the speed of a chemical reaction, they cannot change its direction, otherwise they could make "impossible" reactions happen and break the laws of thermodynamics. So an enzyme can just as easily turn a product into a substrate as turn a substrate into a product, depending on which way the reaction would go anyway. In fact the active site doesn't really fit the substrate (or the product) at all, but instead fits a sort of half-way house, called the transition state. When a substrate (or product) molecule binds, the active site changes shape and fits itself around the molecule, distorting it into forming the transition state, and so speeding up the reaction. This is sometimes called the induced fit mechanism.

3. Energy Changes

The way enzymes work can also be shown by considering the energy changes that take place during a chemical reaction. We shall consider a reaction where the product has a lower energy than the substrate, so the substrate naturally turns into product (in other words the equilibrium lies in the direction of the product). Before it can change into product, the substrate must



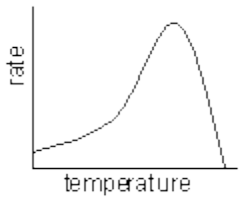
overcome an "energy barrier" called the activation energy (E_A). The larger the activation energy, the slower the reaction will be because only a few substrate molecules will by chance have sufficient energy to overcome the activation energy barrier. Imagine pushing boulders over a hump before they can roll down hill, and you have the idea. Most physiological reactions have large activation energies, so they simply don't happen on a useful time scale. Enzymes dramatically reduce the activation energy of a reaction, so that most molecules can easily get over the activation energy barrier and quickly turn into product.

For example for the catalase reaction ($2H_2O_2$ $2H_2O + O_2$) the activation energy is 86 kJ mol^{-1} with no catalyst, 62 kJ mol^{-1} with an inorganic catalyst of iron filings, and just 1 kJ mol^{-1} in the presence of the enzyme catalase.

The activation energy is actually the energy required to form the transition state, so enzymes lower the activation energy by stabilising the transition state, and they do this by changing the conditions within the active site of the enzyme. So the three ideas above are really three ways of describing the same process.

Factors that Affect the Rate of Enzyme Reactions

1. Temperature

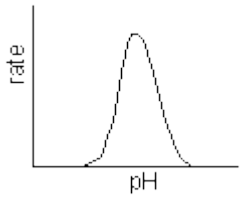


Enzymes have an optimum temperature at which they work fastest. For mammalian enzymes this is about 40°C, but there are enzymes that work best at very different temperatures, e.g. enzymes from the arctic snow flea work at -10°C, and enzymes from thermophilic bacteria work at 90°C.

Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the enzyme and substrate molecules both have more kinetic energy so collide more often, and also because more molecules have sufficient energy to overcome the (greatly reduced) activation energy. The increase in rate with $\left(Q_{10} = \frac{\text{rate at temp } (t+10)°C}{\text{rate at temp } t°C}\right)$ temperature can be quantified as a Q_{10}, which is the relative increase for a 10°C rise in temperature. Q_{10} is usually 2-3 for enzyme-catalysed reactions (i.e. the rate doubles every 10°C) and usually less than 2 for non-enzyme reactions.

The rate is not zero at 0°C, so enzymes still work in the fridge (and food still goes off), but they work slowly. Enzymes can even work in ice, though the rate is extremely slow due to the very slow diffusion of enzyme and substrate molecules through the ice lattice.

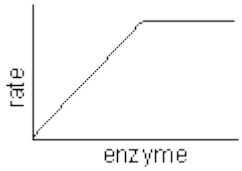
Above the optimum temperature the rate decreases as more and more of the enzyme molecules denature. The thermal energy breaks the hydrogen bonds holding the secondary and tertiary structure of the enzyme together, so the enzyme (and especially the active site) loses its shape to become a random coil. The substrate can no longer bind, and the reaction is no longer catalysed. At very high temperatures this is irreversible. Remember that only the weak hydrogen bonds are broken at these mild temperatures; to break strong covalent bonds you need to boil in concentrated acid for many hours.



2. pH

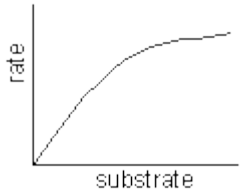
Enzymes have an optimum pH at which they work fastest. For most enzymes this is about pH 7-8 (physiological pH of most cells), but a few enzymes can work at extreme pH, such as protease enzymes in animal stomachs, which have an optimum of pH 1. The pH affects the charge of the amino acids at the active site, so the properties of the active site change and the substrate can no longer bind. For example a carboxyl acid R groups will be uncharged a low pH (COOH), but charged at high pH (COO^-).

3. Enzyme concentration



As the enzyme concentration increases the rate of the reaction increases linearly, because there are more enzyme molecules available to catalyse the reaction. At very high enzyme concentration the substrate concentration may become rate-limiting, so the rate stops increasing. Normally enzymes are present in cells in rather low concentrations.

4. Substrate concentration



The rate of an enzyme-catalysed reaction shows a curved dependence on substrate concentration. As the substrate concentration increases, the rate increases because more substrate molecules can collide with enzyme molecules, so more reactions will take place. At higher concentrations the enzyme molecules become saturated with substrate, so there are few free enzyme molecules, so adding more substrate doesn't make much difference (though it will increase the rate of E-S collisions).

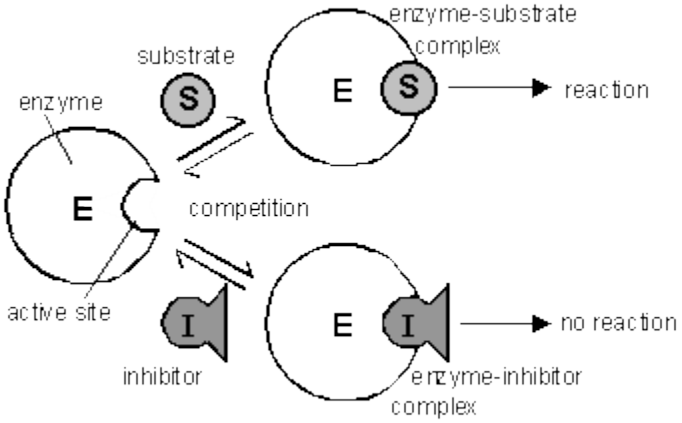
The maximum rate at infinite substrate concentration is called v_{max}, and the substrate concentration that give a rate of half v_{max} is called K_M. These quantities are useful for characterising an enzyme. A good enzyme has a high v_{max} and a low K_M.

5. Covalent modification

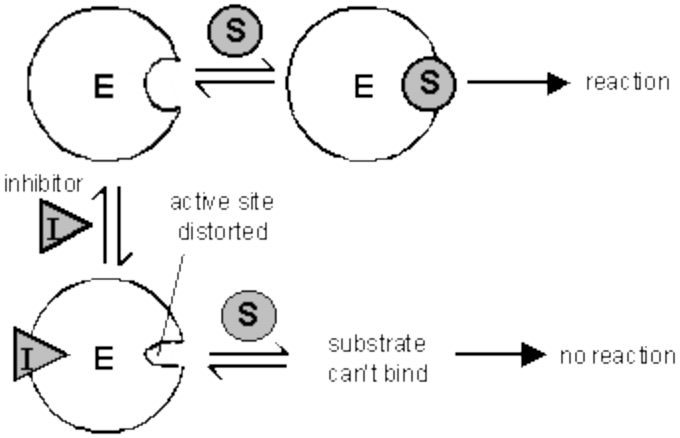
The activity of some enzymes is controlled by other enzymes, which modify the protein chain by cutting it, or adding a phosphate or methyl group. This modification can turn an inactive enzyme into an active enzyme (or vice versa), and this is used to control many metabolic enzymes and to switch on enzymes in the gut (see later) e.g. hydrochloric acid in stomach® activates pepsin® activates rennin.

6. Inhibitors

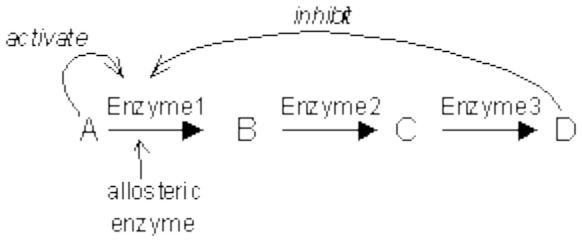
Inhibitors inhibit the activity of enzymes, reducing the rate of their reactions. They are found naturally, but are also used artificially as drugs, pesticides and research tools. There are two kinds of inhibitors.



(a) A competitive inhibitor molecule has a similar structure to the normal substrate molecule, and it can fit into the active site of the enzyme. It therefore competes with the substrate for the active site, so the reaction is slower. Competitive inhibitors increase K_M for the enzyme, but have no effect on v_{max}, so the rate can approach a normal rate if the substrate concentration is increased high enough. The sulphonamide anti-bacterial drugs are competitive inhibitors.



(b) A non-competitive inhibitor molecule is quite different in structure from the substrate molecule and does not fit into the active site. It binds to another part of the enzyme molecule, changing the shape of the whole enzyme, including the active site, so that it can no longer bind substrate molecules. Non-competitive inhibitors therefore simply reduce the amount of active enzyme (just like decreasing the enzyme concentration), so they decrease v_{max}, but have no effect on K_M. Inhibitors that bind fairly weakly and can be washed out are sometimes called reversible inhibitors, while those that bind tightly and cannot be washed out are called irreversible inhibitors. Poisons like cyanide, heavy metal ions and some insecticides are all non-competitive inhibitors.



7. Allosteric Effectors

The activity of some enzymes is controlled by certain molecules binding to a specific regulatory (or allosteric) site on the enzyme, distinct from the active site. Different molecules can inhibit or activate the enzyme, allowing sophisticated control of the rate. Only a few enzymes can do this, and they are often at the start of a long biochemical pathway. They are generally activated by the substrate of the pathway and inhibited by the product of the pathway, thus only turning the pathway on when it is needed.

DIGESTION

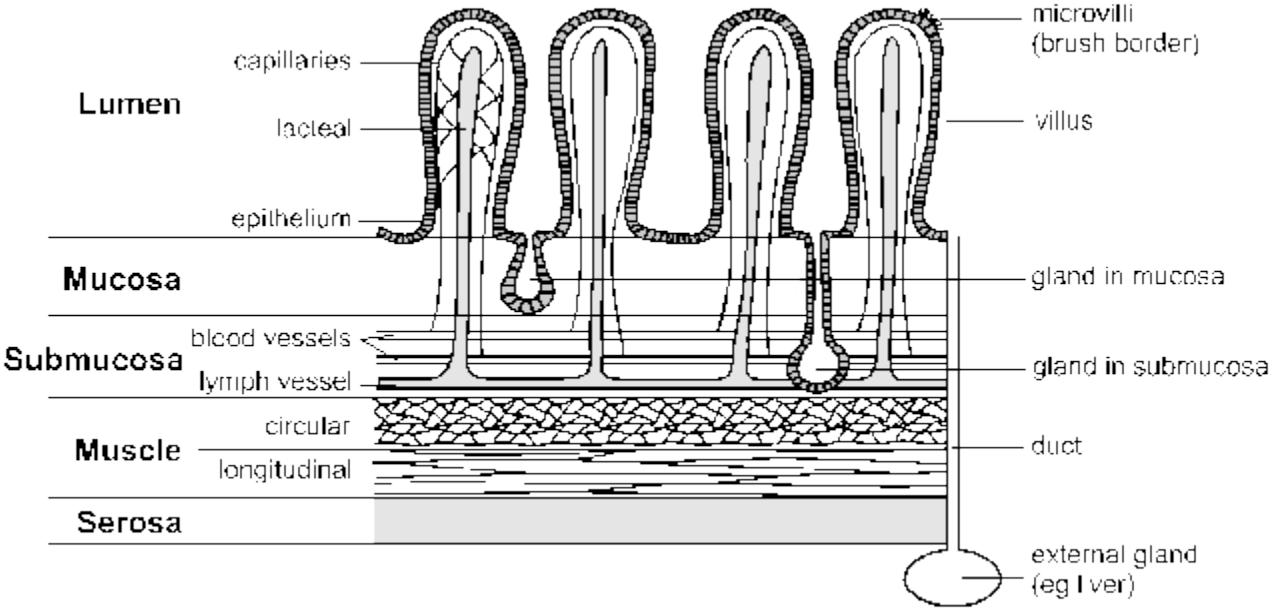
Humans, like all animals, use holozoic nutrition, which consists of these stages:

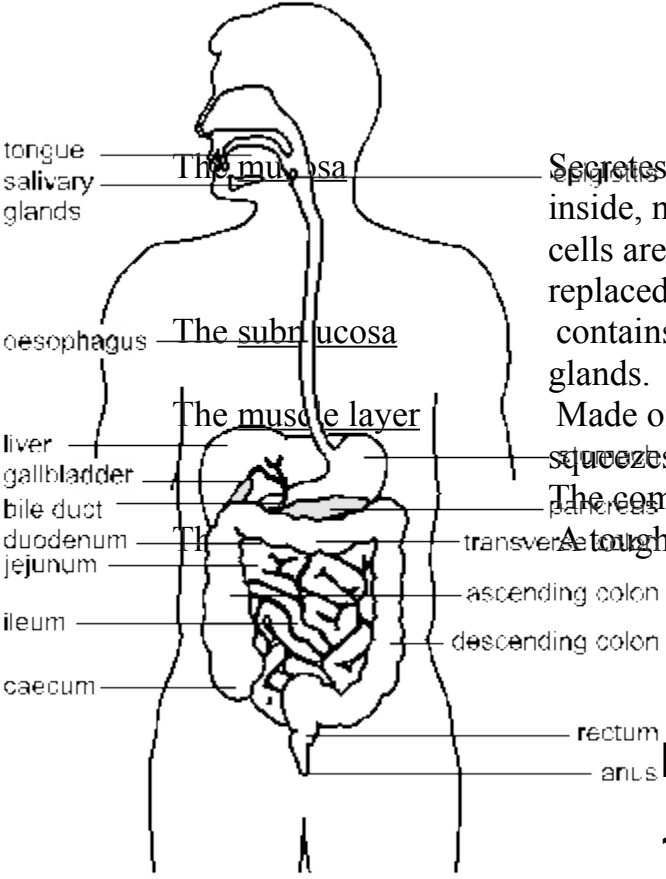
ingestion	- taking large pieces of food into the body
digestion	- breaking down the food by mechanical and chemical means
absorption	- taking up the soluble digestion products into the body's cells
assimilation	- using the absorbed materials
egestion	- eliminating the undigested material

Note

Egestion is elimination of material from the body caviry
Excretion is elimination of substances from within body cells

The human digestive system is well adapted to all of these functions. It comprises a long tube, the alimentary canal (digestive tract or simply gut) that runs from the mouth to the anus, together with a number of associated glands. The digestive systems' made up of different tissues doing different jobs. The lining wall of the alimentary canal appears different in different parts of the gut, reflecting their different roles, but always has the same basic layers:





The mucosa

Secretes digestive juices and absorbs digested food. It is often folded to inc
inside, next to the lumen (the space inside the gut) is a thin layer of cells ca
cells are constantly worn away by the friction of food moving through the g
replaced.

The submucosa

contains blood vessels, lymph vessels and nerves to control the muscles. It
glands.

The muscle layer

Made of smooth muscle, under involuntary control. It can be subdivided in
squeezes the gut when it contracts) and longitudinal muscle (which shorten
The combination of these two muscles allows a variety of different moveme

The

A tough layer of connective tissue that holds the gut together, and attaches

Parts of the Alimentary Canal

1. Mouth (Buccal cavity)

The teeth, tongue and chewing action break up the food physically which increases surface area, and they form it into a ball or bolus. The salivary glands secrete saliva, which contains water to dissolve soluble substances, mucus for lubrication, lysozymes to kill bacteria and amylase to digest starch. The food bolus is swallowed by an involuntary reflex action through the pharynx (the back of the mouth). During swallowing the trachea is blocked off by the epiglottis to stop food entering the lungs.

2. Oesophagus (gullet)

This is a simple tube through the thorax, which connects the mouth to the rest of the gut. No digestion takes place. There is a thin epithelium, no villi, a few glands secreting mucus, and a thick muscle layer, which propels the food by peristalsis. This is a wave of circular muscle contraction, which passes down the oesophagus and is completely involuntary. The oesophagus is a soft tube that can be closed, unlike the trachea, which is a hard tube, held open by rings of cartilage.

3. Stomach

This is an expandable bag where the food is stored for up to a few hours. There are three layers of muscle to churn the food into a liquid called chyme. This is gradually released in to the small intestine by a sphincter, a region of thick circular muscle that acts as a valve. The mucosa of the stomach wall has no villi, but numerous gastric pits (10^4 cm^{-2}) leading to gastric glands in the mucosa layer. These secrete gastric juice, which contains: hydrochloric acid (pH 1) to kill bacteria (the acid does not help digestion, in fact it hinders it by denaturing most enzymes); mucus to lubricate the food and to line the epithelium to protect it from the acid; and the enzymes pepsin and rennin to digest proteins.

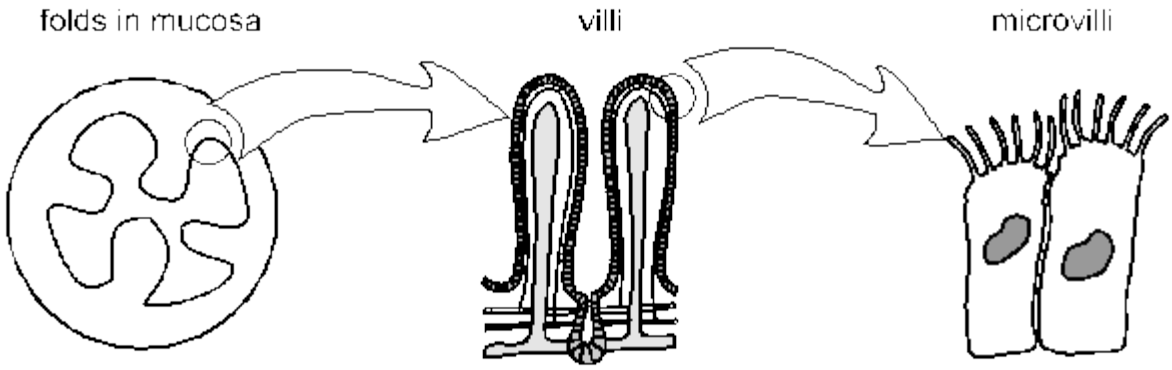
4. Small Intestine

This is about 6.5 m long, and can be divided into three sections:

The duodenum (30 cm long). Although this is short, almost all the digestion takes place here, due to two secretions: Pancreatic juice, secreted by the pancreas through the pancreatic duct. This contains numerous carbohydrase, protease and lipase enzymes. Bile, secreted by the liver, stored in the gall bladder, and released through the bile duct into the duodenum. Bile contains bile salts to aid lipid digestion, and the alkali sodium hydrogen carbonate to neutralise the stomach acid. Without this, the pancreatic enzymes would not work. The bile duct and the pancreatic duct join just before they enter the duodenum. The mucosa of the duodenum has few villi, since there is no absorption, but the submucosa contains glands secreting mucus and sodium hydrogen carbonate.

The jejunum (2 m long) and the ileum (4 m long). These two are similar in humans, and are the site of final digestion and all absorption. There are numerous glands in the mucosa and submucosa secreting enzymes, mucus and sodium hydrogen carbonate.

The internal surface area is increased enormously by three levels of folding: large folds of the mucosa, villi, and microvilli. Don't confuse these: villi are large structures composed of many cells that can clearly be seen with a light microscope, while microvilli are small sub-cellular structures formed by the folding of the plasma membrane of individual cells. Microvilli can only be seen clearly with an electron microscope, and appear as a fuzzy brush border under the light microscope.



Circular and longitudinal muscles move the liquid food by peristalsis.

5. Large Intestine

This comprises the caecum, appendix, colon and rectum. Food can spend 36 hours in the large intestine (mind you that's if your pretty constipated!), while water is absorbed to form semi-solid faeces. The mucosa contains villi but no microvilli, and there are numerous glands secreting mucus. Faeces is made up of cellulose, cholesterol, bile, mucus, mucosa cells (250g of cells are lost each day), bacteria and water, and is released by the anal sphincter. This is a rare example of an involuntary muscle that we can learn to control (during potty training).

Chemistry of Digestion

1. Digestion of Carbohydrates

The most abundant carbohydrate in the human diet is starch (in bread, potatoes, cereal, rice, pasta, biscuits, cake, etc), but there may also be a lot of sugar (mainly sucrose) and some glycogen (in meat).

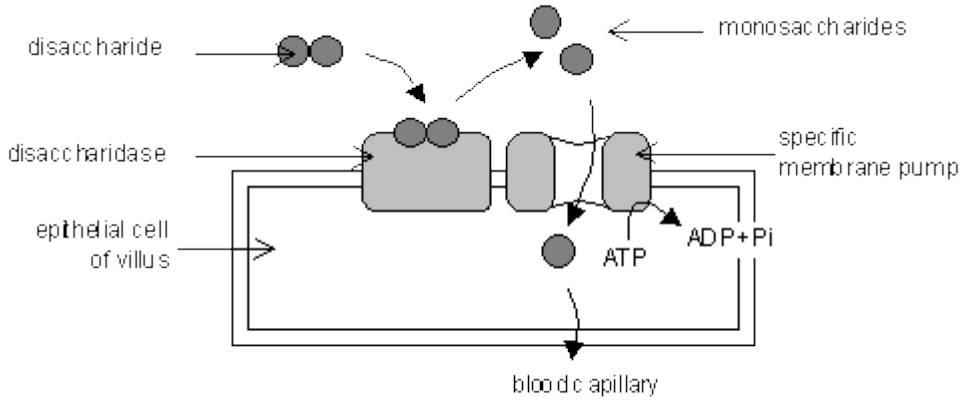
- Salivary amylase starts the digestion of starch. Very little digestion actually takes place, since amylase is quickly denatured in the stomach, but is does help to clean the mouth and reduce bacterial infection.
- Pancreatic amylase digests all the remaining starch in the duodenum. Amylase digests starch molecules from the ends of the chains in two-glucose units, forming the disaccharide maltose. Glycogen is also digested here.
- Disaccharidases in the membrane of the ileum enzymes attached to the epithelial cells complete the digestion of disaccharides to monosaccharides. This includes maltose from starch digestion as well as any sucrose and lactose in the diet. There are three important disaccharidase enzymes:

$$\text{maltose} \xrightarrow{\text{maltase}} \text{glucose}$$

$$\text{sucrose} \xrightarrow{\text{sucrase}} \text{glucose} + \text{fuctose}$$

$$\text{lactose} \xrightarrow{\text{lactase}} \text{glucose} + \text{galactose}$$

- The monsaccharides (glucose, fructose and galactose) are absorbed by active transport into the epithelial cells of the ileum, whence they diffuse into the blood capillaries of the villi. Active transport requires energy in the form of ATP, but it allows very rapid absorption, even against a concentration gradient. The membrane-bound disaccharidases and the monosaccharide pumps are often closely associated:



- The carbohydrates that make up plant fibres (cellulose, hemicellulose, lignin, etc) cannot be digested, so pass through the digestive system as fibre.

2. Digestion of Proteins

- Rennin (in gastric juice) converts the soluble milk protein caesin into its insoluble calcium salt. This keeps in the stomach longer so that pepsin can digest it. Rennin is normally only produced by infant mammals. It is used commercially to make cheese.
- Pepsin (in gastric juice) digests proteins to peptides, 6-12 amino acids long. Pepsin is an endopeptidase, which means it hydrolyses peptide bonds in the middle of a polypeptide chain. It is unusual in that it has an optimum pH of about 2 and stops working at neutral pH.
- Pancreatic endopeptidases continue to digest proteins and peptides to short peptides in the duodenum. Different endopeptidase enzymes cut at different places on a peptide chain because they have different target amino acid sequences, so this is an efficient way to cut a long chain up into many short fragments, and it provides many free ends for the next enzymes to work on.

- Exopeptidases in the membrane of the ileum epithelial cells complete the digestion of the short peptides to individual amino acids. Exopeptidases remove amino acids one by one from the ends of peptide chains. Carboxypeptidases work from the C-terminal end, aminopeptidases work from the N-terminal end, and dipeptidases cut dipeptides in half.
- The amino acids are absorbed by active transport into the epithelial cells of the ileum, whence they diffuse into the blood capillaries of the villi. Again, the membrane-bound peptidases and the amino acid transporters are closely associated.

Protease enzymes are potentially dangerous because they can break down other enzymes (including themselves!) and other proteins in cells. To prevent this they are synthesised in the RER of their secretory cells as inactive forms, called zymogens. These are quite safe inside cells, and the enzymes are only activated in the lumen of the intestine when they are required.

- Pepsin is synthesised as inactive pepsinogen, and activated by the acid in the stomach
- Rennin is synthesised as inactive prorennin, and activated by pepsin in the stomach
- The pancreatic exopeptidases are activated by specific enzymes in the duodenum
- The membrane-bound peptidase enzymes do not have this problem since they are fixed, so cannot come into contact with cell proteins.

The lining of mucus between the stomach wall and the food also protects the cells from the protease enzymes once they are activated.

3. Digestion of Triglycerides

- Fats are emulsified by bile salts to form small oil droplets called micelles, which have a large surface area.
- Pancreatic lipase enzymes digest triglycerides to fatty acids and glycerol in the duodenum.
- Fatty acids and glycerol are lipid soluble and diffuse across the membrane (by lipid diffusion) into the epithelial cells of the villi in the ileum.
- In the epithelial cells of the ileum triglycerides are re-synthesised (!) and combine with proteins to form tiny lipoprotein particles called chylomicrons.
- The chylomicrons diffuse into the lacteal - the lymph vessel inside each villus. The emulsified fatty droplets give lymph its milky colour, hence name lacteal.
- The chylomicrons are carried through the lymphatic system to enter the bloodstream at the vena cava, and are then carried in the blood to all parts of the body. They are stored as triglycerides in adipose (fat) tissue.

- Fats are not properly broken down until they used for respiration in liver or muscle cells.

4. Digestion of Nucleic acids

- Pancreatic nuclease enzymes digest nucleic acids (DNA and RNA) to nucleotides in the duodenum.
- Membrane-bound nucleotidase enzymes in the epithelial cells of the ileum digest the nucleotides to sugar, base and phosphate, which are absorbed.

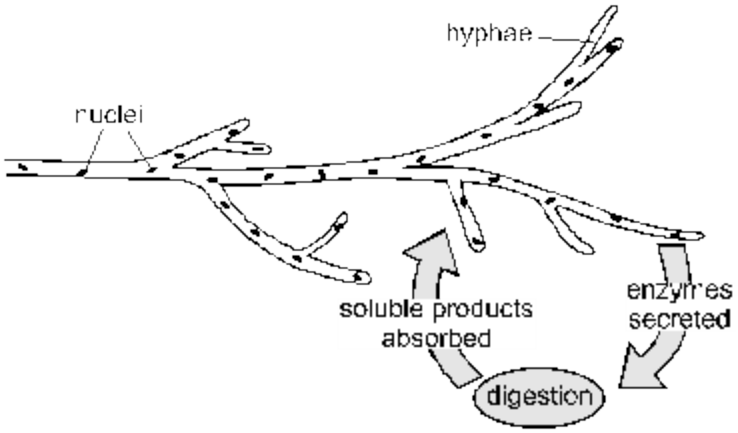
5. Other substances

Many substances in the diet are composed of small molecules that need little or no digestion. These include sugars, mineral ions, vitamins and water. These are absorbed by different transport mechanisms:

- Cholesterol and the fat-soluble vitamins (A, D, E, K) are absorbed into the epithelial cells of the ileum by lipid diffusion
- Mineral ions and water-soluble vitamins are absorbed by passive transport in the ileum
- Dietary monosaccharides are absorbed by active transport in the ileum
- Water is absorbed by osmosis in the ileum and colon.

Digestion in Fungi

Fungi are not consumers like animals, but are either saprophytes (decomposers), or pathogens. They therefore use saprophytic nutrition, which means they do not ingest their food, but use extracellular digestion. Fungi secrete digestive enzymes (carbohydrases, proteases and lipases) into the material that surrounds them and then absorb the soluble products (sugars, amino acids, etc).



Fungi are usually composed of long thin threads called hyphae. These grow quickly, penetrating dead material such as leaves, as well as growing underground throughout soil. The cotton wool appearance of bread mould growing on decaying bread is typical of a mass of hyphae, called a fungal mycelium. These thin hyphae give fungi a large surface area to volume ratio. They contain many nuclei, since they are formed from the fusion of many cells. (more on extracellular digestion)

EXTRA CELLULAR DIGESTION

Extra-cellular digestion in fungi:

- Body consists of thin threads (hyphae)
- Hyphae secrete enzymes that diffuse through wall onto food
- Enzymes hydrolyse materials in food to monomers
- Monomers then absorbed into hyphae by facilitated diffusion. and active transport

- In fungi (e.g. saprophytic fungi), cilia not involved in moving food

Summary Table

	Saprophytic fungus	Mammal
Cilia involved in moving food	Incorrect	Incorrect
Organism prduces digestive enzymes	Correct	Correct
Carbohydrates absorbed into cells as monomers such as glucose	Correct	Correct

THE HUMAN DIGESTIVE SYSTEM - KEY NOTES

THE MOUTH

- chewing makes a larger surface area of the food for the enzymes to attack.

- salivery amylase hydrolyses some starch to maltose.

THE STOMACH

- the walls of the stomach contain layers of muscle. the functions of which include: churning, mechanical digestion, mixing, and peristalsis.
- the gastric glands in the stomach wall secrete endopeptidase pepsin. however, it is secreted in its inactive form HCl in the stomach activates the enzyme.
- the enzyme is secreted in its inactive form in order to prevent it from digesting the walls of the stomach, while it is in storage in the gastric glands.
- once the enzyme has been activated, mucus, which coats the stomach walls, prevents them from being digested, and also protects the walls from acid.
- HCl in the stomach kills bacteria which are ingested along with food, and also created a low pH environment in which stomach enzymes work at their optimum rate.
- endopeptidases digest proteins into polypeptide chains by hydrolysing bonds in the centre of the protein molecule.
- food is released from the stomach by periodic relaxation of the pyloric sphincter muscle at the lower end of the stomach.

- after being released from the stomach, food enters the first part of the small intestine, known as the duodenum.

THE SMALL INTESTINE

- large Surface area
- moist surface
- thin (epithelial) surface/ short absorption pathway
- long/ folds (increasing surface area)
- villi
- microvilli
- lacteal
- capillary network in villus/ good blood supply
- mitochondria to supply ATP/ energy for active transport
- carrier proteins in membranes.
- the duodenum contains the following enzymes:

- amylase (from pancreas) hydrolyses starch to maltose.
- lipase (from pancreas) - for the digestion of lipids. lipids are hydrolysed to fatty acids and glycerol.
- endopeptidases (from pancreas) - for the digestion of proteins. these are hydrolysed to polypeptides.
- exopeptidases (from pancreas) - digest polypeptide chains to amino acids.
- both endo and exopeptidases are required for efficient digestion of polypeptides and proteins because endopeptidases act on the centre of polypeptide chains within proteins and hydrolyse them to smaller chains.
- this means that more 'ends' are created for the exopeptidases to act upon, in order to break down polypeptide chains to amino acids.
- many enzymes in the duodenum are secreted from the pancreas, and are carried to the duodenum by the hepato-pancreatic duct which also brings bile from the liver.
- maltases - the small intestine contains maltase as part of the intestinal fluid which forms a secretion which coats the walls of the small intestine epithelial cells. maltase acts on the disaccharide sugar maltose and hydrolyses the glycoside bonds between the units of glucose. the sugar is broken down to its simplest form glucose, and can then be absorbed.
- dipeptidases - the small intestine contains dipeptidases as part of the intestinal fluid which forms a secretion which coats the walls of the small intestine epithelial cells. Dipeptidases hydrolyses the peptide bonds between amino acids. the dipeptide is broken down into 2 amino acids, and can then be absorbed.
- the duodenum is the main site of absorption of all components of digestion, except water.
- food is moved along the duodenum by peristalsis (rhythmic contraction of the muscles of the intestinal wall, cause food to be pushed along the duodenum)
- segmentation in the duodenum produces a to and fro movement that causes mixing of the contents of the gut and digestive juices.

- segmentation also aids digestion by bringing products into contact with the mucosa –hence enabling absorption to occur.

ABSORPTION IN THE SMALL INTESTINE

- diffusion in capillaries
- active transport/ facilitated diffusion involved
- ATP used by active transport
- disaccharidases/Dipeptidases/enzymes in cell surface membrane
- glucose/ monomers/ monosaccharides actively transported into epithelial cells via protein carriers/ channels (in membrane)

- facilitated diffusion from epithelial cell/ towards blood

THE ROLE OF THE LIVER IN DIGESTION

- bile is a biological detergent, which is produced in the liver.
- in order for lipids to act upon triglycerides, the triglycerides must first be broken down into minute droplets to enable then to mix with lipases present in the pancreatic juice within the duodenum.

- in order to do this bile is secreted from the gall bladder.
- bile reduces the surface tension and increases the surface area /volume ratio. i.e., fats are emulsified.
- therefore, lipases act on a larger volume of material in a shorter time, ensuring that enzymes operate at their optimum rate.

- bile also neutralizes stomach acid, and provides the optimum pH for pancreatic digestive enzymes to work.

THE ROLE OF THE PANCREAS IN DIGESTION

- produces pancreatic juice.
- pancreatic juice contains many enzymes as detailled above.
- pancreatic juice is rich in sodium hydrogencarbonate, which:
- neutralizes acid chyme from the stomach.

- raises the pH to enable enzymes in the pancreatic juice to work.

THE LARGE INTESTINE

- the large intestine is made up of the following parts:
- ceacum and appendix – these are sack-like structures are at the junction of the small and large intestines.
- the colon and rectum- this is a muscular tube which contains large amounts of bacteria.
- peristalsis moves contents along the colon, and also compacts faeces.
- faeces are stored in the rectum.
- mucosa in the colon secretes mucus which lubricates the mucosa and protects it from enzymes action.
- the colon absorbs water and other soluble compounds.
- the colon absorbs vitamins and ions.
- bacteria contained in the colon, break down undigested food. this food is then absorbed of excreted as faeces.
- these bacteria synthesize vitamins B and K.
- faeces excreted via the anus. main components are:

- undigested food, bile pigments, bacteria, and dead cells from the small intestine.

TECHNIQUES

Contents

Biochemical Tests
Chromatography

Cell Fractionation
Enzyme Kinetics
Microscopy

1. Biochemical Tests

These five tests identify the main biologically important chemical compounds. For each test take a small amount of the substance to test, and shake it in water in a test tube. If the sample is a piece of food, then grind it with some water in a pestle and mortar to break up the cells and release the cell contents. Many of these compounds are insoluble, but the tests work just as well on a fine suspension.

- **Starch** (iodine test). To approximately 2 cm^3 of test solution add two drops of iodine/potassium iodide solution. A blue-black colour indicates the presence of starch as a starch-polyiodide complex is formed. Starch is only slightly soluble in water, but the test works well in a suspension or as a solid.
- **Reducing Sugars** (Benedict's test). All monosaccharides and most disaccharides (except sucrose) will reduce copper (II) sulphate, producing a precipitate of copper (I) oxide on heating, so they are called reducing sugars. Benedict's reagent is an aqueous solution of copper (II) sulphate, sodium carbonate and sodium citrate. To approximately 2 cm^3 of test solution add an equal quantity of Benedict's reagent. Shake, and heat for a few minutes at 95°C in a water bath. A precipitate indicates reducing sugar. The colour and density of the precipitate gives an indication of the amount of reducing sugar present, so this test is semi-quantitative. The original pale blue colour means no reducing sugar, a green precipitate means relatively little sugar; a brown or red precipitate means progressively more sugar is present.
- **Non-reducing Sugars** (Benedict's test). Sucrose is called a non-reducing sugar because it does not reduce copper sulphate, so there is no direct test for sucrose. However, if it is first hydrolysed (broken down) to its constituent monosaccharides (glucose and fructose), it will then give a positive Benedict's test. So sucrose is the only sugar that will give a negative Benedict's test before hydrolysis and a positive test afterwards. First test a sample for reducing sugars, to see if there are any present bef7ore hydrolysis. Then, using a separate sample, boil the test solution with dilute hydrochloric acid for a few minutes to hydrolyse the glycosidic bond. Neutralise the solution by gently adding small amounts of solid sodium hydrogen carbonate until it stops fizzing, then test as before for reducing sugars.
- **Lipids** (emulsion test). Lipids do not dissolve in water, but do dissolve in ethanol. This characteristic is used in the emulsion test. Do not start by dissolving the sample in water, but instead shake some of the test sample with about 4 cm^3 of ethanol. Decant the liquid into a test tube of water, leaving any undissolved substances behind. If there are lipids dissolved in the ethanol, they will precipitate in the water, forming a cloudy white emulsion.

- **Protein** (biuret test). To about 2 cm^3 of test solution add an equal volume of biuret solution, down the side of the test tube. A blue ring forms at the surface of the solution, which disappears on shaking, and the solution turns lilac-purple, indicating protein. The colour is due to a complex between nitrogen atoms in the peptide chain and Cu^{2+} ions, so this is really a test for peptide bonds.

2. Chromatography

Chromatography is used to separate pure substances from a mixture of substances, such as a cell extract. It is based on different substances having different solubilities in different solvents. A simple and common form of chromatography uses filter paper.

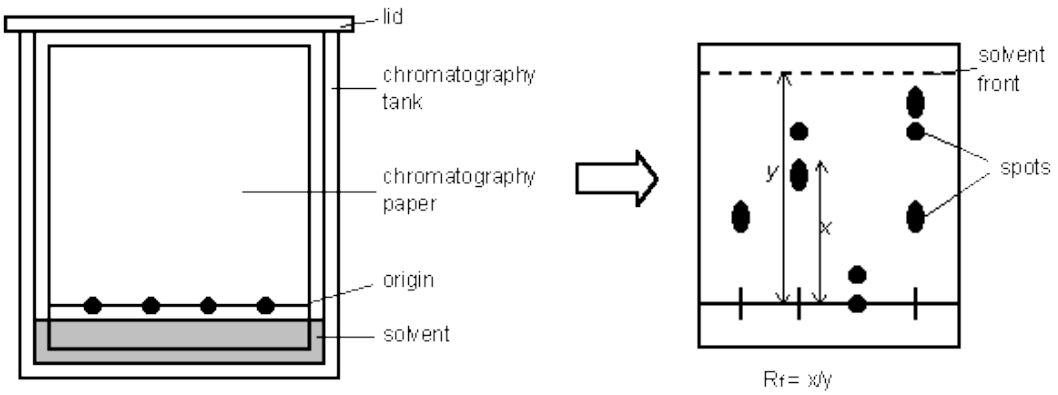
1. Pour some solvent into a chromatography tank and seal it, so the atmosphere is saturated

with solvent vapour. Different solvents are suitable for different tasks, but they are usually mixtures of water with organic liquids such as ethanol or propanone.

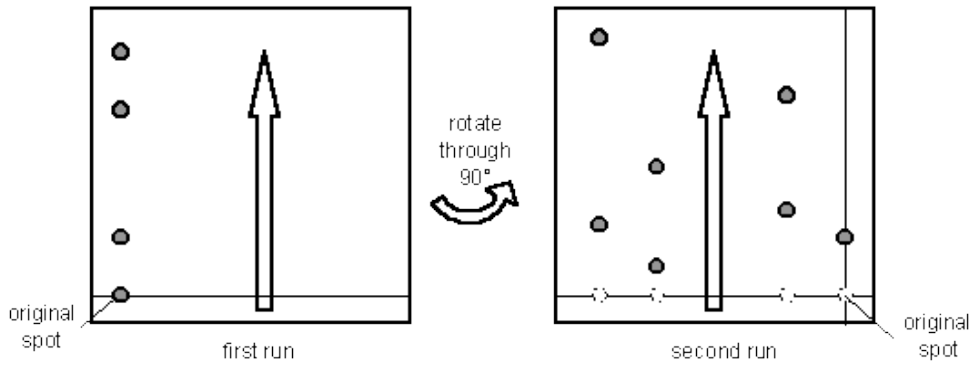
2. Place a drop of the mixture to be separated onto a sheet of chromatography paper near one end. This is the origin of the chromatogram. The spot should be small but concentrated. Repeat for any other mixtures. Label the spots with pencil, as ink may dissolve.
3. Place the chromatography sheet into the tank so that the origin is just above the level of solvent, and leave for several hours. The solvent will rise up the paper by capillary action carrying the contents of the mixture with it. Any solutes dissolved in the solvent will be partitioned between the organic solvent (the moving phase) and the water, which is held by the paper (the stationary phase). The more soluble a solute is in the solvent the further up the paper it will move.
4. When the solvent has nearly reached the top of the paper, the paper is removed and the position of the solvent front marked. The chromatogram may need to be developed to make the spots visible. For example amino acids stain purple with ninhydrin.
5. The chromatogram can be analysed by measuring the distance travelled by the solvent front, and the distance from the origin to the centre of each spot. This is used to calculate the R_f (relative front) value for each spot:

$$R_f = \frac{\text{distance moved by spot}}{\text{distance moved by solvent}}$$

An R_f value is characteristic of a particular solute in a particular solvent. It can be used to identify components of a mixture by comparing to tables of known R_f values.



Sometimes chromatography with a single solvent is not enough to separate all the constituents of a mixture. In this case the separation can be improved by two-dimensional chromatography, where the chromatography paper is turned through 90° and run a second time in a second solvent. Solutes that didn't separate in one solvent will separate in another because they have different solubilities.



There are many different types of chromatography.

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- Paper chromatography is the simplest, but does not always give very clean separation.

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- Thin layer chromatography (tlc) uses a thin layer of cellulose or silica coated onto a plastic or glass sheet. This is more expensive, but gives much better and more reliable separation.

–

- Column chromatography uses a glass column filled with a cellulose slurry. Large samples can be pumped through the column and the separated fractions can be collected for further experiments, so this is preparative chromatography as opposed to analytical chromatography.
- High performance liquid chromatography (HPLC) is an improved form of column chromatography that delivers excellent separation very quickly.

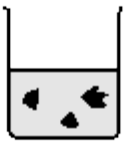
–

- Electrophoresis uses an electric current to separate molecules on the basis of charge. It can also be used to separate on the basis of molecular size, and as such is used in DNA sequencing.

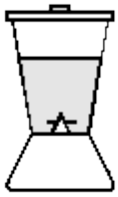
3. Cell Fractionation

This means separating different parts and organelles of a cell, so that they can be studied in detail. All the processes of cell metabolism (such as respiration or photosynthesis) have been studied in this way. The most common method of fractionating cells is to use differential centrifugation:

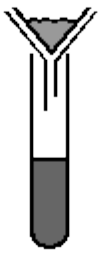
1. Cut tissue (eg liver, heart, leaf, etc) in ice-cold isotonic buffer. Cold to stop enzyme reactions, isotonic to stop osmosis, and buffer to stop pH changes.



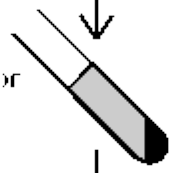
2. Grind tissue in a blender to break open cells.



3. Filter. This removes insoluble tissue (eg fat, connective tissue, plant cell walls, etc). This filtrate is now called a cell-free extract, and is capable of carrying out most of the normal cell reactions.

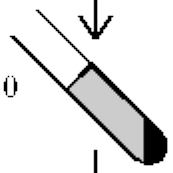


4. Centifuge filtrate at low speed (1 000 x g for 10 mins).



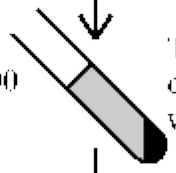
This pellets nuclei, which can be resuspended

5. Centifuge supernatant at medium speed (10 000 x g for 30 mins).



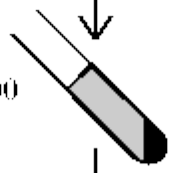
This pellets mitochondria and chloroplasts, which can be resuspended

6. Centifuge supernatant at high speed (100 000 x g for 1 hour).



This pellets ER, golgi and other membrane fragments, which can be resuspended

7. Centifuge supernatant at very high speed (300 000 x g for 3 hrs).



This pellets ribososmes, which can be resuspended

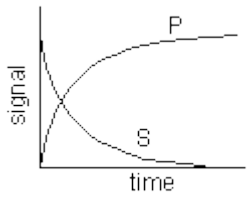
8. Supernatant is now organelle-free cytoplasm

A more sophisticated separation can be performed by density gradient centrifugation. In this, the cell-free extract is centrifuged in a dense solution (such as sucrose or caesium chloride). The fractions don't pellet, but instead separate out into layers with the densest fractions near the bottom of the tube. The desired layer can then be pipetted off. This is the technique used in the Meselson-Stahl experiment (module 2) and it is also used to separate the two types of ribosomes. The terms 70S and 80S refer to their positions in a density gradient.

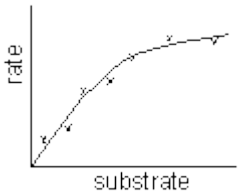
4. Enzyme Kinetics

This means measuring the rate of enzyme reactions.

- Firstly you need a signal to measure that shows the progress of the reaction. The signal should change with either substrate or product concentration, and it should preferably be something that can be measured continuously. Typical signals include colour changes, pH changes, mass changes, gas production, volume changes or turbidity changes. If the reaction has none of these properties, it can sometimes be linked to a second reaction which does generate one of these changes.



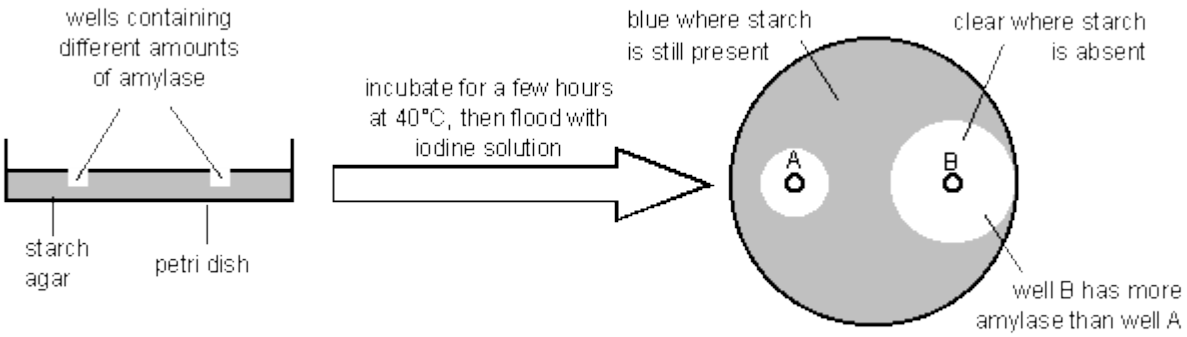
- If you mix your substrate with enzyme and measure your signal, you will obtain a time-course. If the signal is proportional to substrate concentration it will start high and decrease, while if the signal is proportional to product it will start low and increase. In both cases the time-course will be curved (actually an exponential curve).
  - How do you obtain a rate from this time-course? One thing that is not a good idea is to measure the time taken for the reaction, for as the time-course shows it is very difficult to say when the reaction ends: it just gradually approaches the end-point. A better method is to measure the initial rate - that is the initial slope of the time-course. This also means you don't need to record the whole time-course, but simply take one measurement a short time after mixing.



- Repeat this initial rate measurement under different conditions (such as different substrate concentrations) and then plot a graph of rate *vs.* the factor. Each point on this second graph is taken from a separate initial rate measurement (or better still is an average of several initial rate measurements under the same conditions). Draw a smooth curve through the points.

Be careful not to confuse the two kinds of graph (the time-course and rate graphs) when interpreting your data.

One useful trick is to dissolve the substrate in agar in an agar plate. If a source of enzyme is placed in the agar plate, the enzyme will diffuse out through the agar, turning the substrate into product as it goes. There must be a way to distinguish the substrate from the product, and the reaction will then show up as a ring around the enzyme source. The higher the concentration of enzyme, the higher the diffusion gradient, so the faster the enzyme diffuses through the agar, so the larger the ring in a given time. The diameter of the ring is therefore proportional to the enzyme concentration. This can be done for many enzymes, e.g. a protein agar plate can be used for a protease enzyme, or a starch agar plate can be used for the enzyme amylase.



5. Microscopy

Of all the techniques used in biology microscopy is probably the most important. The vast majority of living organisms are too small to be seen in any detail with the human eye, and cells and their organelles can only be seen with the aid of a microscope. Cells were first seen in 1665 by Robert Hooke (who named them after monks' cells in a monastery), and were studied in more detail by Leeuwehoek using a primitive microscope.

Units of measurement. The standard SI units of measurement used in microscopy are:

metre	m	= 1 m
millimetre	mm	= 10^{-3} m
micrometre	mμ	= 10^{-6} m
nanometre	nm	= 10^{-9} m
picometre	pm	= 10^{-12} m
angstrom	Å	= 10^{-10} m (obsolete)

Magnification and Resolving Power. By using more lenses microscopes can magnify by a larger amount, but this doesn't always mean that more detail can be seen. The amount of detail depends on the resolving power of a microscope, which is the smallest separation at which two separate objects

can be distinguished (or resolved). It is calculated by the formula:

$$\text{resolving power} = \frac{0.6\lambda}{n.a.}$$

where λ is the wavelength of light, and *n.a.* is the numerical aperture of the lens (which ranges from about 0.5 to 1.4). So the resolving power of a microscope is ultimately limited by the wavelength of light (400-600nm for visible light). To improve the resolving power a shorter wavelength of light is needed, and sometimes microscopes have blue filters for this purpose (because blue has the shortest wavelength of visible light).

Different kinds of Microscope.

Light Microscope. This is the oldest, simplest and most widely-used form of microscopy. Specimens are illuminated with light, which is focussed using glass lenses and viewed using the eye or photographic film. Specimens can be living or dead, but often need to be stained with a coloured dye to make them visible. Many different stains are available that stain specific parts of the cell such as DNA, lipids, cytoskeleton, etc. All light microscopes today are compound microscopes, which means they use several lenses to obtain high magnification. Light microscopy has a resolution of about 200 nm, which is good enough to see cells, but not the details of cell organelles. There has been a recent resurgence in the use of light microscopy, partly due to technical improvements, which have dramatically improved the resolution far beyond the theoretical limit. For example fluorescence microscopy has a resolution of about 10 nm, while interference microscopy has a resolution of about 1 nm.

Electron Microscope. This uses a beam of electrons, rather than electromagnetic radiation, to "illuminate" the specimen. This may seem strange, but electrons behave like waves and can easily be produced (using a hot wire), focussed (using electromagnets) and detected (using a phosphor screen or photographic film). A beam of electrons has an effective wavelength of less than 1 nm, so can be used to resolve small sub-cellular ultrastructure. The development of the electron microscope in the 1930s revolutionised biology, allowing organelles such as mitochondria, ER and membranes to be seen in detail for the first time.

The main problem with the electron microscope is that specimens must be fixed in plastic and viewed in a vacuum, and must therefore be dead. Other problems are that the specimens can be damaged by the electron beam and they must be stained with an electron-dense chemical (usually heavy metals like osmium, lead or gold). Initially there was a problem of artefacts (i.e. observed structures that were due to the preparation process and were not real), but improvements in technique have eliminated most of these.

There are two kinds of electron microscope. The transmission electron microscope (TEM) works much like a light microscope, transmitting a beam of electrons through a thin specimen and then focussing the electrons to form an image on a screen or on film. This is the most common form of electron microscope and has the best resolution. The scanning electron microscope (SEM) scans a fine beam of electron onto a specimen and collects the electrons scattered by the surface. This has poorer resolution, but gives excellent 3-dimentional images of surfaces.

- X-ray Microscope. This is an obvious improvement to the light microscope, since x-rays have wavelengths a thousand time shorter than visible light, and so could even be used to resolve

atoms. Unfortunately there are no good x-ray lenses, so an image cannot be focussed, and useable x-ray microscopes do not yet exist. However, x-rays can be used without focussing to give a diffraction pattern, which can be used to work out the structures of molecules, such as those of proteins and DNA.

- Scanning Tunnelling Microscope (or Atomic Force Microscope). This uses a very fine needle to scan the surface of a specimen. It has a resolution of about 10 pm, and has been used to observe individual atoms for the first time.

Comparison of Light and Electron Microscopes

	LIGHT MICROSCOPE	ELECTRON MICROSCOPE
illumination and source	light from lamp	electrons from hot wire
focusing	glass lenses	electromagnets
detection	eye or film	phosphor screen or film
magnification	1 500 x	500 000 x
resolution	200 nm	1 nm
specimen	living or dead	dead
staining	coloured dyes	heavy metals
cost	cheap to expensive	very expensive

MICROSCROPY NOTES

Preparation of samples

- Fixation: Chemicals preserve material in a life like condition. Does not distort the specimen.
- Dehydration: Water removed from the specimen using ethanol. Particularly important for electron microscopy because water molecules deflect the electron beam which blurs the image.
- Embedding: Supports the tissue in wax or resin so that it can be cut into thin sections. Sectioning Produces very thin slices for mounting. Sections are cut with a microtome or an ulramicrotome to make them either a few micrometres (light microscopy) or nanometres (electron microscopy) thick.
- Staining: Most biological material is transparent and needs staining to increase the contrast between different structures. Different stains are used for different types of tissues. Methylene blue is often used for animal cells, while iodine in KI solution is used for plant tissues.

- Mounting: Mounting on a slide protects the material so that it is suitable for viewing over a long period.

Magnification and Resolution

Magnification is how much bigger a sample appears to be under the microscope than it is in real life.

Overall magnification = Objective lens x Eyepiece lens

Resolution is the ability to distinguish between two points on an image.

- The resolution of an image is limited by the wavelength of radiation used to view the sample.
- This is because when objects in the specimen are much smaller than the wavelength of the radiation being used, they do not interrupt the waves, and so are not detected.
- The wavelength of light (min. – violet is 400nm) is much larger than the wavelength of electrons, so the resolution of the light microscope is a lot lower.
- The actual resolution is often half the size of the wavelength of radiation used. Thus, for the light microscope the maximum resolution is about 200nm.
- In other words, if two objects in the specimen are closer than 200nm in real life, then they will only show up as one object on the image.
- Using a microscope with a more powerful magnification will **not** increase this resolution any further. It will increase the size of the image, but objects closer than 200nm will still only be seen as one point.

Transmission and Scanning Electron Microscopes

- **Transmission electron microscopes** pass a beam of electrons through the specimen. The electrons that pass through the specimen are detected on a fluorescent screen on which the image is displayed.
- Thin sections of specimen are needed for transmission electron microscopy as the electrons have to pass through the specimen for the image to be produced.

- **Scanning electron microscopes** pass a beam of electrons over the surface of the specimen in the form of a ‘scanning’ beam.
- Electrons are reflected off the surface of the specimen as it has been previously coated in heavy metals.
- It is these reflected electron beams that are focussed of the fluorescent screen in order to make up the image.
- Larger, thicker structures can thus be seen under the scanning electron microscope as the electrons do not have to pass through the sample in order to form the image.
- However the resolution of the scanning electron microscope is lower than that of the transmission electron microscope.

Comparison of the light and electron microscope

LIGHT MICROSCOPE	ELECTRON MICROSCOPE
Cheap to purchase (£100 – 500)	Expensive to buy (over £ 1 000 000).
Cheap to operate.	Expensive to produce electron beam.
Small and portable.	Large and requires special rooms.
Simple and easy sample preparation.	Lengthy and complex sample prep.
Material rarely distorted by preparation.	Preparation distorts material.
Vacuum is not required.	Vacuum is required.
Natural colour of sample maintained.	All images in black and white.
Magnifies objects only up to 2000 times	Magnifies over 500 000 times.

Basic Principles of Light and Electron Microscopy

Light Microscopy

- Light is produced from either an internal or external light source and passes through the iris diaphragm, a hole of variable size which controls the amount of light reaching the specimen.
- The light then passes through the condenser which focuses the light onto the specimen.
- The slide is held on the stage at 90 degrees to the path of light which next travels through the specimen.
- The objective lens magnifies the image of the specimen before the light travels through the barrel of the microscope.
- The light finally passes through the eyepiece lens and into the viewer's eye which sends impulses to the brain which in turn interprets the image.

Electron Microscopy

- A negatively charged platinum metal electrode (the cathode) emits a beam of high velocity negatively charged electrons.
- The electromagnets on the side of the barrel focus the beam of electrons on the specimen in the same way that the glass lenses on a light microscope focus the beams of light.
- The specimen is introduced via an air lock so as to maintain the internal vacuum conditions.
- The transmitted or reflected beam of electrons, depending on type of microscope are focused by the electromagnets onto a fluorescent screen to produce the image which is then viewed by the operator.

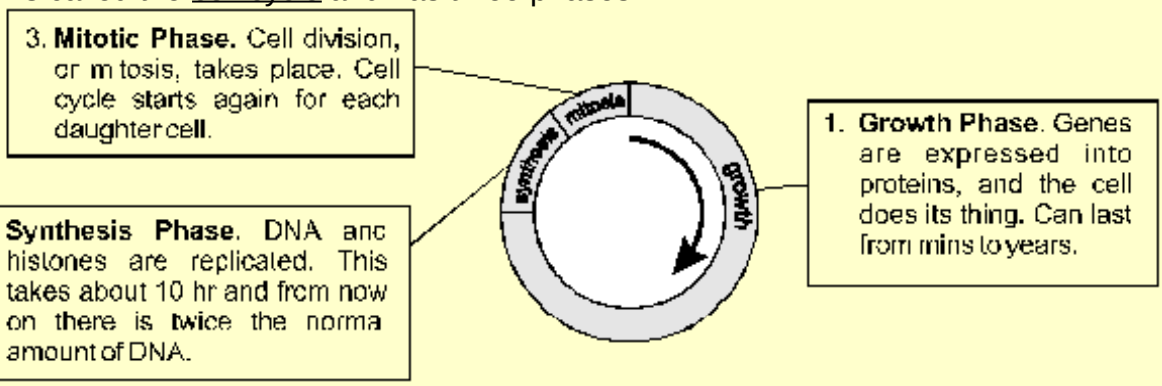
MODULE 2

CELL CYCLES: Contents

- The Cell Cycle
- Mitosis
- Asexual Reproduction: Natural
- Asexual Reproduction: Artificial
- Sexual Reproduction
- Gametes

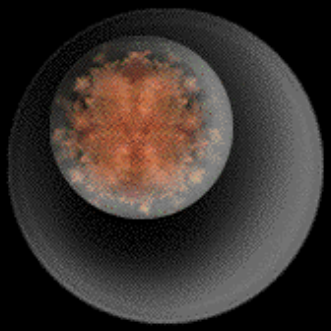
The Cell Cycle

The life of a cell is called the cell cycle and has three phases:

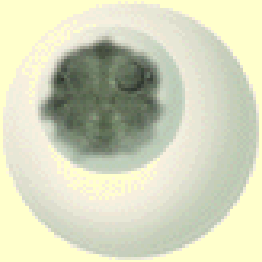
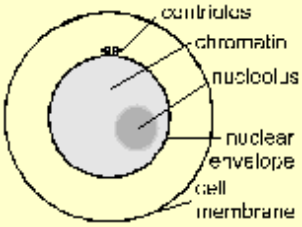
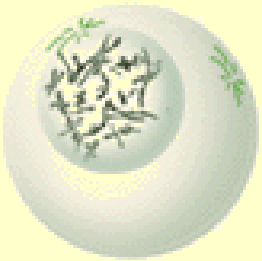
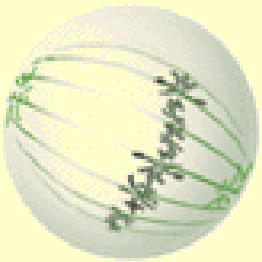
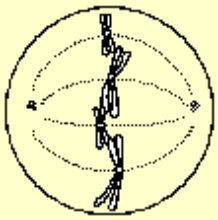
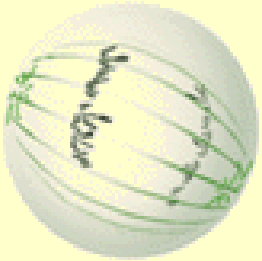
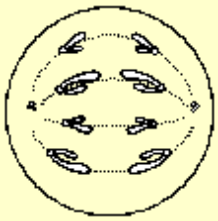


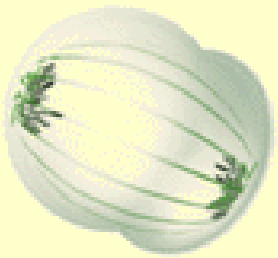
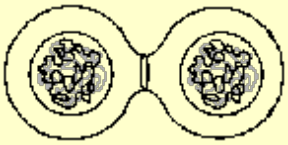
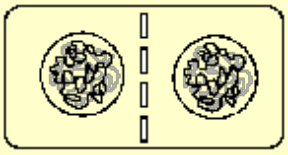
In different cell types the cell cycle can last from hours to years. E.g. bacterial cells can divide every 30 minutes under suitable conditions, skin cells divide about every 12 hours on average, liver cells every 2 years.
The mitotic phase can be sub-divided into four phases (prophase, metaphase, anaphase and telophase). Mitosis is strictly nuclear division, and is followed by cytoplasmic division, or cytokinesis, to complete cell division. The growth and synthesis phases are collectively called interphase (i.e. in between cell division). Mitosis results in two "daughter cells", which are genetically identical to each other, and is used for growth and asexual reproduction. The details of each of these phases follows.

Cell Division by Mitosis



In this animation the stages of mitosis can clearly be seen - it's important to realise that cell division is a continuous process and that the stages flow into each other.

Interphase			• chromatin not visible • DNA replicated
Prophase			• chromosomes condensed and visible • centrioles at opposite poles of cell • phase ends with the breakdown of the nuclear membrane
Metaphase			• chromosomes align along equator of cell • spindle fibres (microtubules) connect centrioles to chromosomes
Anaphase			• centromeres split, allowing chromatids to separate • chromatids move towards poles
Telophase			• spindle fibres

			disperse • nuclear membranes form
Cytokinesis (division of cytoplasm)			• In animal cells a ring of filaments form round the equator of the cell, and then tighten to split the cell in two.
			• In plant cells a new cell wall is laid down inside the existing cell splitting the cell into two

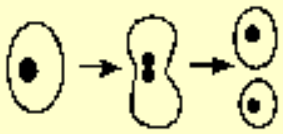
Asexual Reproduction

Asexual reproduction is the production of offspring from a single parent using mitosis. Therefore the offspring are genetically identical to each other and to their "parent"- i.e. they are clones. Asexual reproduction can be either natural or artificial.

	Methods of Asexual Reproduction	
	Natural Methods	Artificial Methods
Microbes	binary fission, budding, spores, fragmentation	cell culture, fermenters
Plants	vegetative propagation, parthenogenesis	cuttings, grafting, tissue culture
Animals	budding, fragmentation, parthenogenesis	embryo splitting, somatic cell cloning

Natural Methods

Binary Fission. The simplest and fastest method of asexual reproduction. The nucleus divides by mitosis and the cell splits into two.

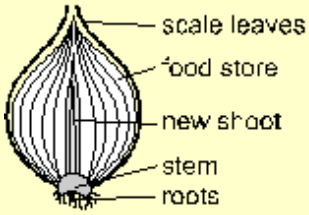


Budding. A small copy of the parent develops as an outgrowth, or bud, from the parent, and then is released as a separate individual.

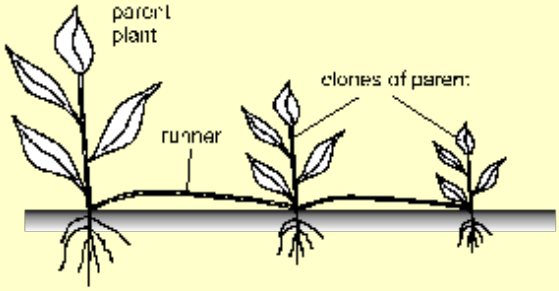
Spores. These are simply specialised cells that are released from the parent (usually in large numbers) to be dispersed. Each spore can grow into a new individual.

Vegetative Reproduction. (note also the name of an artificial technique) This term describes all the natural methods of asexual reproduction used by plants. A bud grows from a vegetative part of the plant (usually the stem) and develops into a complete new plant, which eventually becomes detached from the parent plant. There are numerous forms of vegetative reproduction, including:

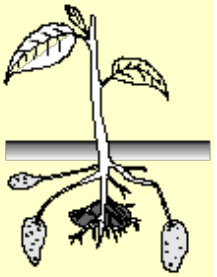
- bulbs (e.g. daffodil)
- rhizomes (e.g. couch grass)
- runners (e.g. strawberry)
- tubers (e.g. potato)



Bulb



Runners



Tubers

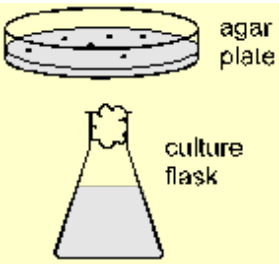
Many of these methods are also perenating organs, which means they contain a food store and are used for survival over winter as well as for asexual reproduction. Since vegetative reproduction relies entirely on mitosis, all offspring are clones of the parent.

Parthenogenesis. This is used by some plants (e.g. citrus fruits) and some invertebrate animals (e.g. honeybees & aphids) as an alternative to sexual reproduction. Egg cells simply develop into adult clones without being fertilised. These clones may be haploid, or the chromosomes may replicate to form diploid cells.

Artificial Methods: (Plants)

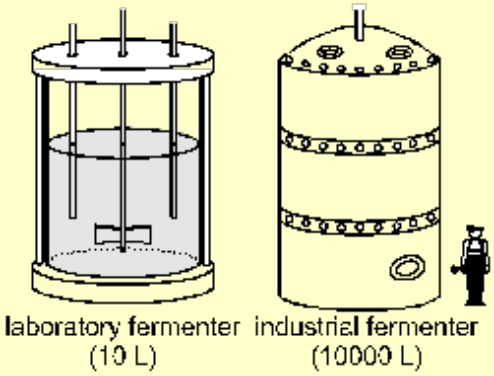
Cloning is of great commercial importance, as brewers, pharmaceutical companies, farmers and plant growers all want to be able to reproduce "good" organisms exactly. Natural methods of asexual reproduction can be used for some organisms (such as potatoes and strawberries), but many important plants and animals do not reproduce asexually, so artificial methods have to be used.

Cell Culture. Microbes can be cloned very easily in the lab using their normal asexual reproduction. Microbial cells can be isolated and identified by growing them on a solid medium in an

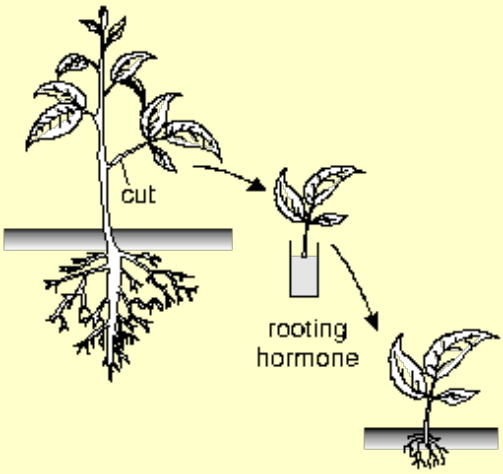


agar plate, and can then be grown up on a small scale in a liquid medium in a culture flask.

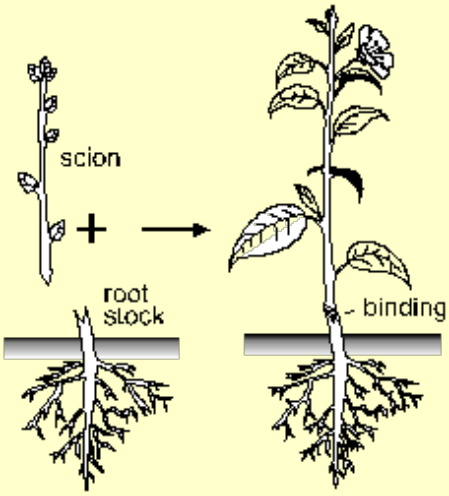
Fermenters. In biotechnology, fermenters are vessels used for growing microbes on a large scale. Fermenters must be stirred, aerated and thermostated, materials can added or removed during the fermentation, and the environmental conditions (such as pH, O_2, pressure and temperature) must be constantly monitored using probes. This will ensure the maximum growth rate of the microbes.



Cuttings. A very old method of cloning plants. Part of a plant stem is cut off and simply replanted in wet soil. Each cutting produces roots and grows into a complete new plant, so the original plant can be cloned many times. Rooting is helped if the cuttings are dipped in rooting hormone (auxin). Many flowering plants, such as geraniums are reproduced commercially by cuttings.

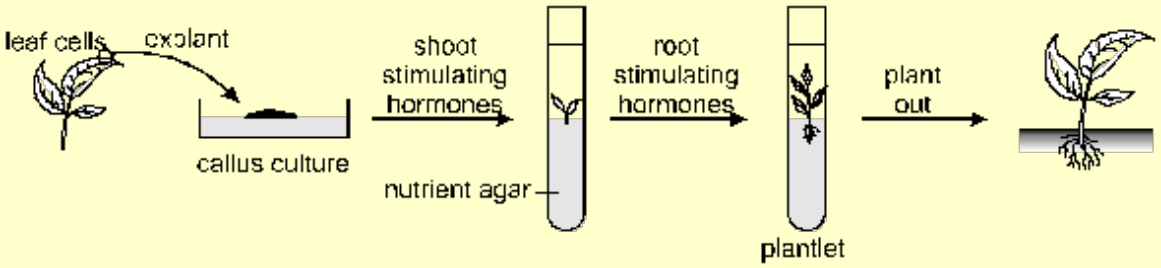


Grafting. Another ancient technique, used for plant species that cannot grow roots from cuttings. Instead they can often be cloned by grafting a stem



cutting onto the lower part of an existing plant.

Tissue Culture (or micropropagation). A more modern way of cloning plants. Small samples of plant tissue are grown on agar plates in the laboratory in much the same way that bacteria are grown. The plant tissue is separated into individual cells, each which can grow into a mass of cells called a callus, and if the correct plant hormones are added these cells can develop into whole plantlets, which can eventually be planted outside, where they will grow into normal-sized plants. Conditions must be kept sterile to prevent infection by microbes.



Micropropagation is used on a large scale for many plants including fruit trees, sugar cane and banana. The advantages are:

- thousands of clones of a good plant can be made quickly and in a small space
- disease-free plants can be grown from a few disease-free cells
- the technique works for plants species that cannot be asexually propagated by other means
- a single cell can be genetically modified and turned into many identical plants

Although some animal cells can be grown in culture, they cannot be grown into complete animals, so tissue culture cannot be used for cloning animals.

Artificial Methods: (Animals)

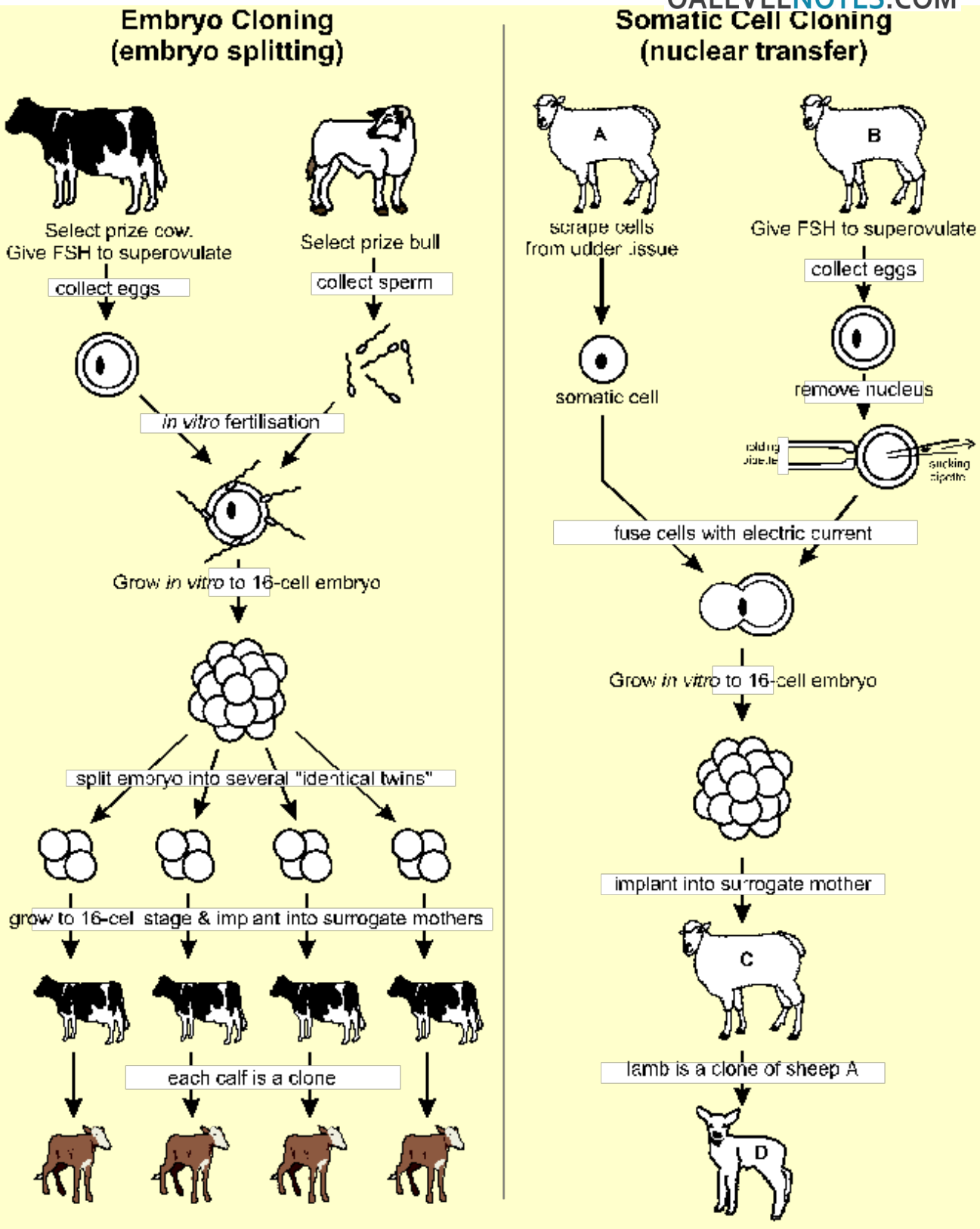
Embryo Cloning (or Embryo Splitting). The most effective technique for cloning animals is to duplicate embryo cells before they have irreversibly differentiated into tissues. It is difficult and quite expensive, so is

only worth it for commercially-important farm animals, such as prize cows, or genetically engineered animals. A female animal is fed a fertility drug so that she produces many mature eggs (superovulation). The eggs are then removed from the female's ovaries. The eggs are fertilised *in vitro* (IVF) using selected sperm from a prize male. The fertilised eggs (zygotes) are allowed to develop *in vitro* for a few days until the embryo is at the 16-cell stage. This young embryo can be split into 16 individual cells, which will each develop again into an embryo. (This is similar to the natural process when a young embryo splits to form identical twins.) The identical embryos can then be transplanted into the uterus of surrogate mothers, where they will develop and be born normally.

Could humans be cloned this way? Almost certainly yes. A human embryo was split and cloned to the stage of a few cells in the USA in 1993, just to show that it is possible. However experiments with human embryos are now banned in most countries including the UK for ethical reasons.

Nuclear Transfer. The problem with embryo cloning is that you don't know the characteristics of the animal you are cloning. By selecting good parents you hope it will have good characteristics, but you will not know until the animal has grown. It would be far better to clone a mature animal, whose characteristics you know. Until recently it was thought impossible to grow a new animal from the somatic cells of an existing animal (in contrast to plants). However, techniques have gradually been developed to do this most recently with sheep (the famous "Dolly") in 1996.

The cell used for Dolly was from the skin of the udder, so was a fully differentiated somatic cell. This cell was fused with a unfertilised egg cell which had had its nucleus removed. This combination of a diploid nucleus in an unfertilised egg cell was a bit like a zygote, and it developed into an embryo. The embryo was implanted into the uterus of a surrogate mother, and developed into an apparently normal sheep, Dolly.

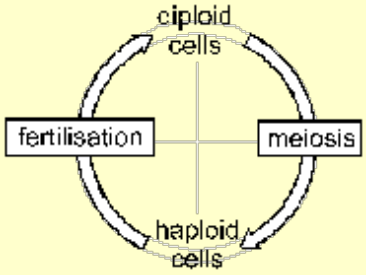
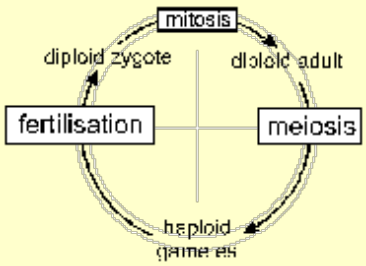
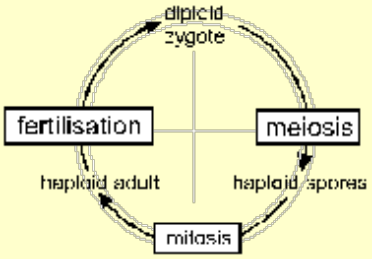
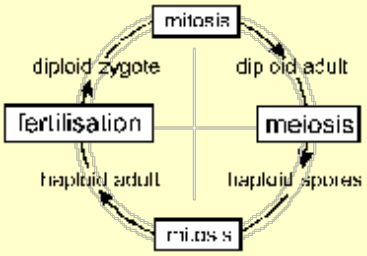


Sexual Reproduction

Sexual reproduction is the production of offspring from two parent using gametes. The cells of the offspring have two sets of chromosomes (one from each parent), so are diploid. Sexual reproduction involves two stages:

- Meiosis- the special cell division that makes haploid gametes
- Fertilisation- the fusion of two gametes to form a diploid zygote

These two stages of sexual reproduction can be illustrated by a sexual life cycle:

All sexually-reproducing species have the basic life cycle shown on the right, alternating between diploid and haploid forms. In addition, they will also use mitosis to grow into adult organisms, the details vary with different organisms.	
In the animal kingdom (including humans), and in flowering plants the dominant, long-lived adult form is diploid, and the haploid gamete cells are only formed briefly.	
In the fungi kingdom the long-lived adult form is haploid. Haploid spores undergo mitosis and grow into complete adults (including large structures like mushrooms). At some stage two of these haploid cells fuse to form a diploid zygote, which immediately undergoes meiosis to reestablish the haploid state and complete the cycle.	
In the plant kingdom the life cycle shows alternation of generations. Plants have two distinct adult forms; one diploid and the other haploid.	

Meiosis

Meiosis is a form of cell division. It starts with DNA replication, like mitosis, but then proceeds with two divisions one immediately after the other. Meiosis therefore results in four daughter cells rather than the two cells formed by mitosis. It differs from mitosis in two important aspects:

- The chromosome number is halved from the diploid number (2n) to the haploid number (n). This is necessary so that the chromosome number remains constant from generation to generation. Haploid cells have one copy of each chromosome, while diploid cells have homologous pairs of each chromosome.
- The chromosomes are re-arranged during meiosis to form new combinations of genes. This genetic recombination is vitally important and is a major source of genetic variation. It means for example that of all the millions of sperm produced by a single human male, the probability is that no two will be identical.

You don't need to know the details of meiosis at this stage (It's covered in module 4).

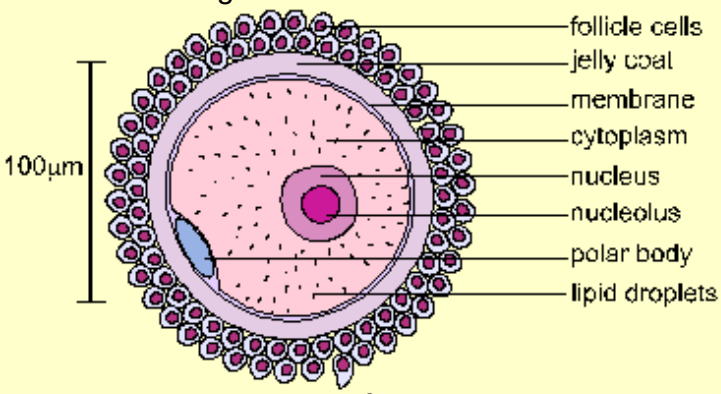
Gametes

The usual purpose of meiosis is to form gametes- the sex cells that will fuse together to form a new diploid individual.

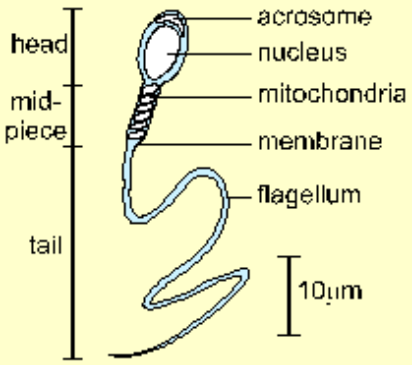
In all plants and animals the gametes are different sizes. This is called heterogamy.

Summary table (you need to learn this)
Female gametes (ova or eggs in animals, ovules in plants) are produced in fairly small numbers. Human females for example release about 500 ova in a lifetime. They are the larger gametes and tend to be stationary. They often contain food reserves (lipids, proteins, carbohydrates) to nourish the embryo after fertilisation.
Male gametes are produced in very large numbers. Human males for example release about 100 million sperm in one ejaculation. They are the smaller gametes and can move. If they can propel themselves they are called motile (e.g. animal sperm). If they can easily be carried by the wind or animals they are called mobile (e.g. plant pollen).

These diagrams of human gametes illustrate the differences between male and female.



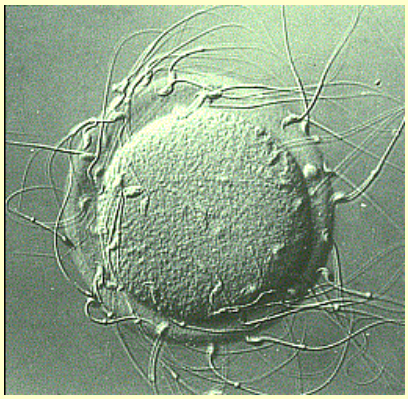
A Human Ovum



A Human Sperm

Fertilisation

Fertilisation is the fusion of two gametes to form a zygote.



In humans this takes place near the top of the oviduct. Hundreds of sperm reach the egg (shown in this photo). When a sperm reaches the ovum cell the two membranes fuse and the sperm nucleus enters the cytoplasm of the ovum. This triggers a series of reactions in the ovum that cause the jelly coat to thicken and harden, preventing any other sperm from entering the ovum. The sperm and egg nuclei then fuse, forming a diploid zygote.

In plants fertilisation takes place in the ovary at the base of the carpel. The haploid male nuclei travel down the pollen tube from the pollen grain on the stigma to the ovules in the ovary. In the ovule two fusions between male and female nuclei take place: one forms the zygote (which will become the embryo) while the other forms the endosperm (which will become the food store in the seed). This double fertilisation is unique to flowering

plants.

The Advantages of Sex

For most of the history of life on Earth, organisms have reproduced only by asexual reproduction. Each individual was a genetic copy (or clone) of its "parent", and the only variation was due to random genetic mutation. The development of sexual reproduction in the eukaryotes around one billion years ago led to much greater variation and diversity of life. Sexual reproduction is slower and more complex than asexual, but it has the great advantage of introducing genetic variation (due to genetic recombination in meiosis and random fertilisation). This variation allows species to adapt to their environment and so to evolve. This variation is clearly such an advantage that practically all species can reproduce sexually. Some organisms can do both, using sexual reproduction for genetic variety and asexual reproduction to survive harsh times.

MODULE 2

NUCLEIC ACIDS: Contents

- Nucleotides
- DNA Structure
- DNA Function
- RNA
- Replication
- Transcription
- Translation
- Mutations

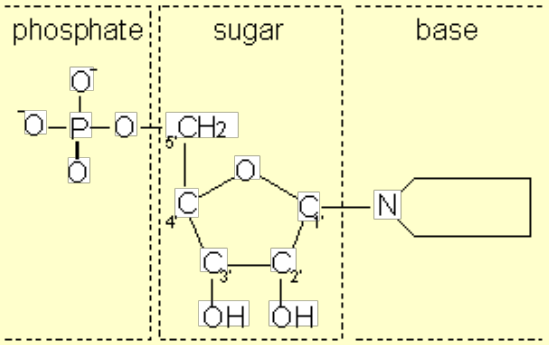
DNA:

DNA and its close relative RNA are perhaps the most important molecules in biology. They contains the instructions that make every single living organism on the planet. DNA stands for deoxyribonucleic acid and RNA for ribonucleic acid. They are polymers (long chain molecules) made from nucleotides.

Nucleotides

Nucleotides have three parts to them:

- a phosphate group, which is negatively charged.
- a pentose sugar, which has 5 carbon atoms in it. In RNA the sugar is ribose. In DNA the sugar is deoxyribose.
- a nitrogenous base. There are five different bases (you don't need to know their structures). The bases are usually known by there first letters only, you don't need to learn the full names. The base thymine is found in DNA only and the base uracil is found in RNA only.



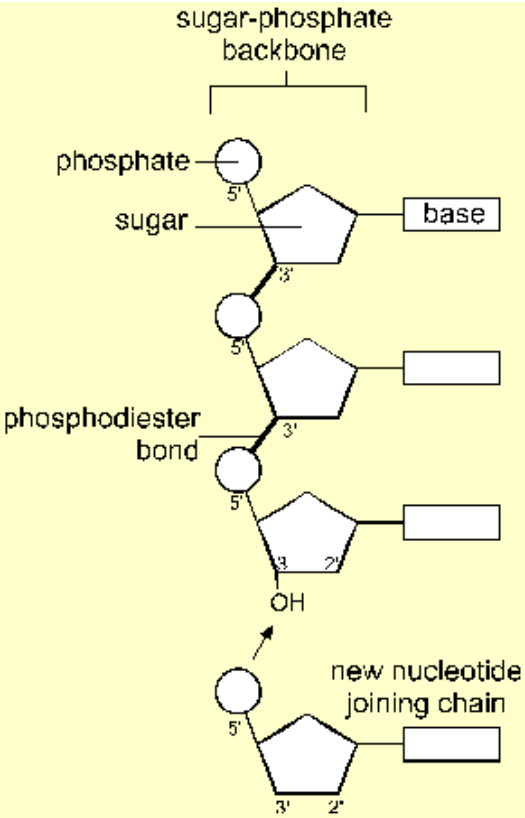
The Bases:

Adenine (A), Thymine (T), Cytosine (C), Guanine (G) and Uracil (U)

Nucleotide Polymerisation:

Nucleotides polymerise by forming bonds between the carbon of the sugar and an oxygen atom of the phosphate. The bases do not take part in the polymerisation, so the chain is held together by a sugar-phosphate backbone with the bases extending off it. This means that the nucleotides can join together in any order along the chain. Many nucleotides form a polynucleotide.

A polynucleotide has a free phosphate group at one

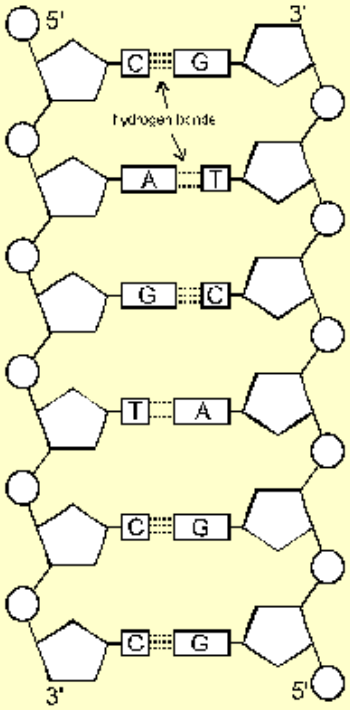


end and a free OH group at the other end.

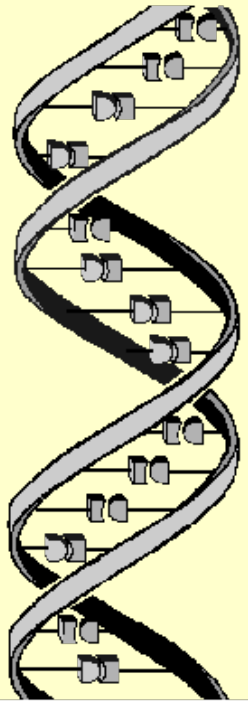
Structure of DNA:

The main features of the three-dimensional structure of DNA are:

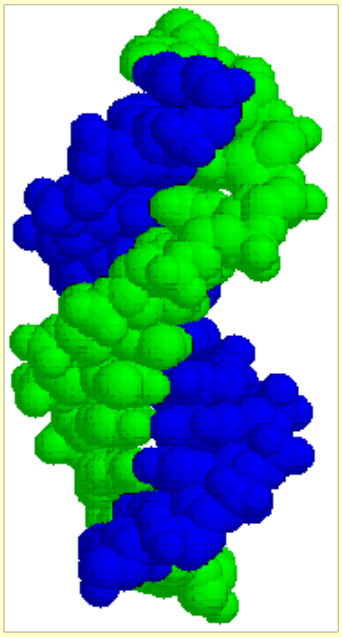
- DNA is double-stranded, so there are two polynucleotide stands alongside each other.
- The two strands are wound round each other to form a double helix.
- The two strands are joined together by hydrogen bonds between the bases. The bases therefore form base pairs, which are like rungs of a ladder.
- The base pairs are specific. A only binds to T (and T with A), and C only binds to G (and G with C). These are called complementary base pairs. This means that whatever the sequence of bases along one strand, the sequence of bases on the other strand must be complementary to it. (Incidentally, complementary, which means matching, is different from complimentary, which means being nice.)



DNA showing the complementary base pairing between antiparallel strands



DNA showing the double helix

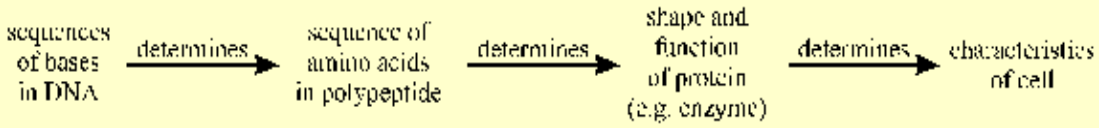


spacefilling model of the double helix

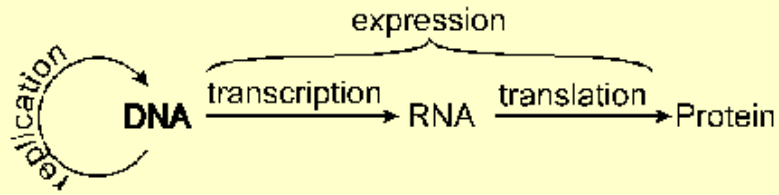
Function of DNA

DNA is the genetic material, and genes are made of DNA. DNA therefore has two essential functions: replication and expression.

- Replication means that the DNA, with all its genes, must be copied every time a cell divides.
- Expression means that the genes on DNA must control characteristics. A gene is a section of DNA that codes for a particular protein. Characteristics are controlled by genes through the proteins they code for, like this:



Expression can be split into two parts: transcription (making RNA) and translation (making proteins). These two functions are shown in this diagram.



No one knows exactly how many genes we humans have to control all our characteristics, the latest estimates are 60-80,000. The sum total of all the genes in an organism is called the genome.

Genes only seem to comprise about 2% of the DNA in a cell. The majority of the DNA does not form genes and doesn't seem to do anything. The purpose of this junk DNA remains a mystery!

RNA

RNA is a nucleic acid like DNA, but with 4 differences:

- RNA has the sugar ribose instead of deoxyribose
- RNA has the base uracil instead of thymine
- RNA is usually single stranded
- RNA is usually shorter than DNA

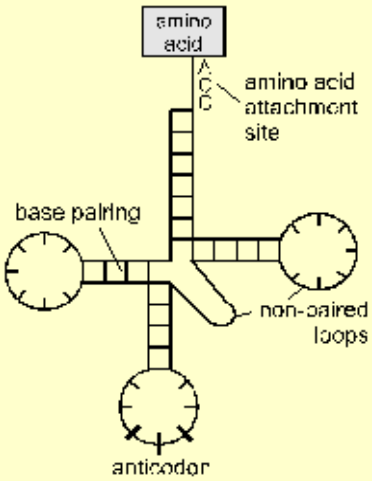
Messenger RNA (mRNA)

mRNA carries the "message" that codes for a particular protein from the nucleus (where DNA is) to the cytoplasm (where proteins are synthesised). It is single stranded and just long enough to contain one gene only.

Ribosomal RNA (rRNA)

A structural molecule part of ribosomes - details are not required

Transfer RNA (tRNA)



- tRNA matches amino acids to their codon.
- tRNA is only about 80 nucleotides long, and it folds up by complementary base pairing to form a clover-leaf structure. At one end of the molecule there is an amino acid binding site. On the middle loop there is a triplet nucleotide sequence called the anticodon.
- There are 64 different tRNA molecules, each with a different anticodon sequence complementary to the 64 different codons on mRNA.

The Genetic Code

The sequence of bases on DNA codes for the sequence of amino acids in proteins. But there are 20 different amino acids and only 4 different bases, so the bases are read in groups of 3. This gives 64 combinations, more than enough to code for 20 amino acids. A group of three bases coding for an amino acid is called a codon, and the meaning of each of the 64 codons is called the genetic code.

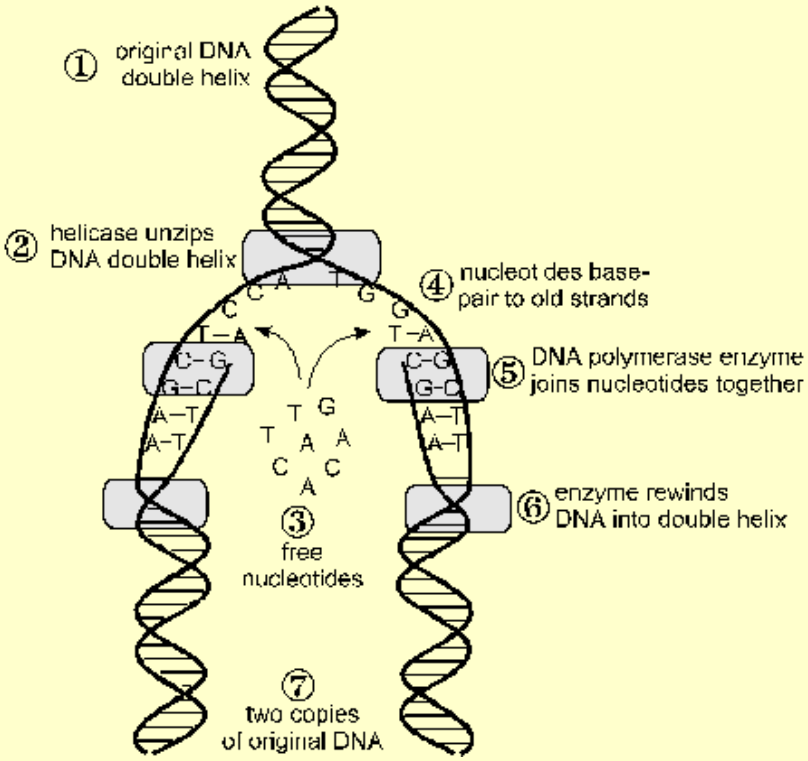
The Genetic Code (mRNA codons)			
UUU, UUC: phe UUA, UUG: leu	CUU, CUC, CUA, CUG: leu	AUU, AUC, AUA: ile AUG: start/met	GUU, GUC, GUA, GUG: val
UCU, UCC, UCA, UCG: ser	CCU, CCC, CCA, CCG: pro	ACU, ACC, ACA, ACG: thr	GCU, GCC, GCA, GCG: ala
UAU, UAC: tyr UAA: stop UAG: stop	CAU, CAC: his CAA, CAG: gln	AAU, AAC: asn AAA, AAG: lys	GAU, GAC: asp GAA, GAG: glu
UGU, UGC: cys UGA: stop UGG: trp	CGU, CGC, CGA, CGG: arg	AGU, AGC: ser AGA, AGG: arg	GGU, GGC, GGA, GGG: gly

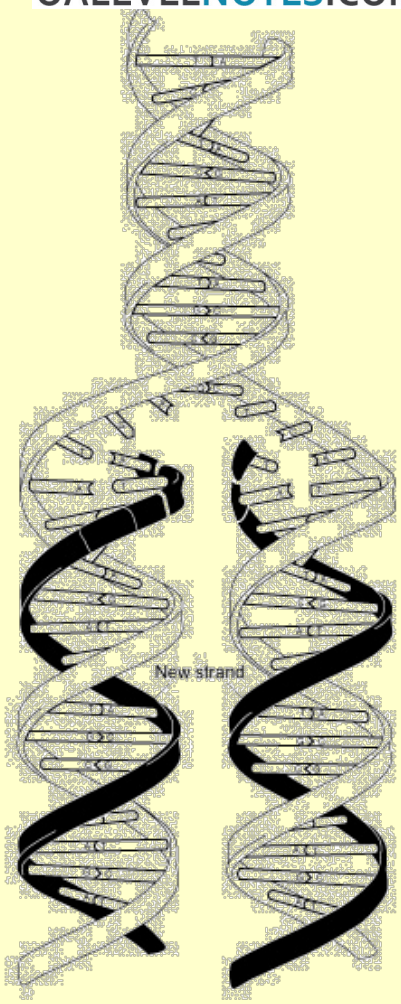
There are several interesting points from this code (which by the wat you do not need to know):

- The code is degenerate, i.e. there is often more than one codon for an amino acid. The degeneracy is on the third base of the codon, which is therefore less important than the others.
- One codon means "start" i.e. the start of the gene sequence. It is AUG.
- Three codons mean "stop" i.e. the end of the gene sequence. They do not code for amino acids.
- The code is only read in one direction along the mRNA molecule.

Replication - DNA Synthesis

DNA is copied, or replicated, before every cell division, so that one identical copy can go to each daughter cell. The double helix unzips and two new strands are built up by complementary base-pairing onto the two old strands.





1. Replication starts at a specific sequence on the DNA molecule.
2. An enzyme unwinds and unzips DNA, breaking the hydrogen bonds that join the base pairs, and forming two separate strands.
3. The new DNA is built up from the four nucleotides (A, C, G and T) that are abundant in the nucleoplasm.
4. These nucleotides attach themselves to the bases on the old strands by complementary base pairing. Where there is a T base, only an A nucleotide will bind, and so on.
5. The enzyme DNA polymerase joins the new nucleotides to each other by strong covalent bonds, forming the sugar-phosphate backbone.
6. A winding enzyme winds the new strands up to form double helices.
7. The two new molecules are identical to the old molecule.

The Meselson-Stahl Experiment

This replication mechanism is sometimes called semi-conservative replication, because each new DNA molecule contains one new strand and one old strand. There was an alternative theory which suggested that a "photocopy" of the original DNA was made, leaving the original DNA conserved (conservative replication). The proof that the semi-conservative method was the correct method came from an experiment performed by Meselson and Stahl using the bacterium *E. coli* together with the technique of density gradient centrifugation, which separates molecules on the basis of their density.

1. Grow bacteria on medium with normal $^{14}NH_4$ → purify DNA and centrifuge → light DNA; CsCl solution

These first two steps are a calibration. They show that the method can distinguish between DNA containing ^{14}N and that containing ^{15}N.

2. Grow bacteria for many generations on medium with ^{15}NH → purify DNA and centrifuge → heavy DNA

3. Return to $^{14}NH_4$ medium for 20 mins (one generation) → purify DNA and centrifuge → intermediate DNA

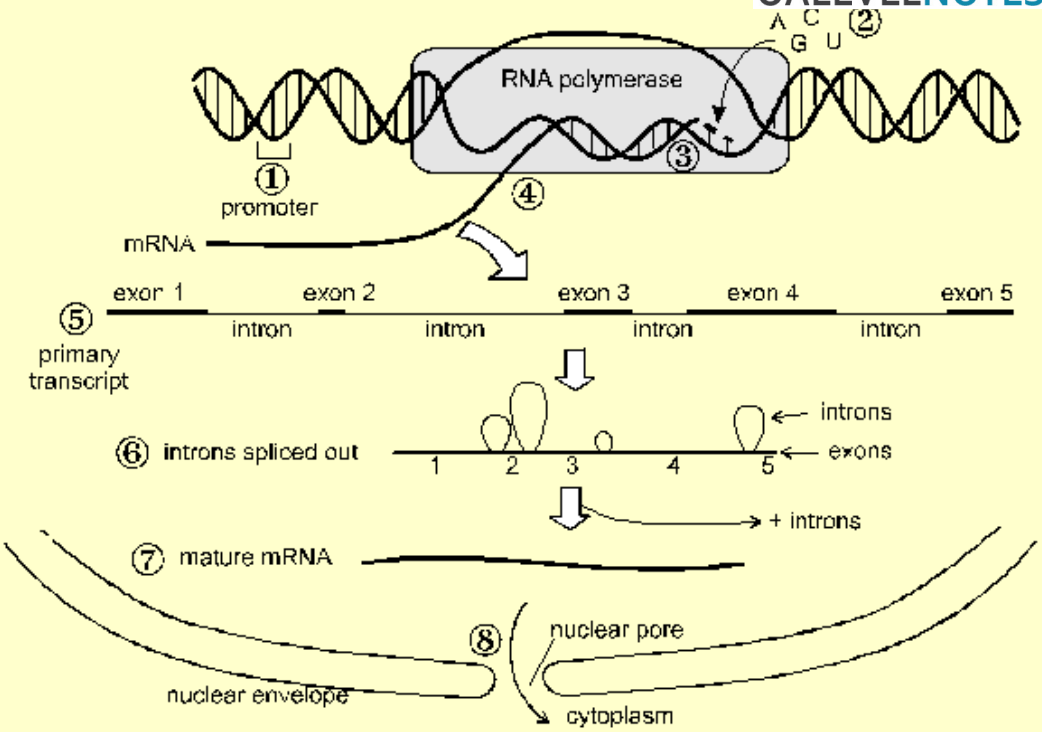
This is the crucial step. The DNA has replicated just once in ^{14}N medium. The resulting DNA is not heavy or light, but exactly half way between the two. This rules out conservative replication.

4. Grow on $^{14}NH_4$ medium for 40 mins (two generations) → purify DNA and centrifuge → light DNA; intermediate DNA

After two generations the DNA is either light or half-and-half. This rules out dispersive replication. The results are all explained by semi-conservative replication.

Transcription - RNA Synthesis

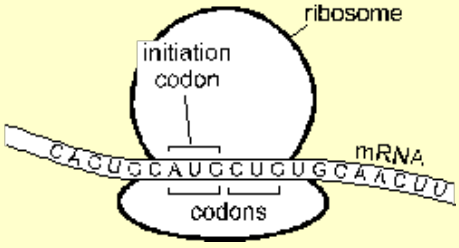
DNA never leaves the nucleus, but proteins are synthesised in the cytoplasm, so a copy of each gene is made to carry the "code" from the nucleus to the cytoplasm. This copy is mRNA, and the process of copying is called transcription.



1. The start of each gene on DNA is marked by a special sequence of bases.
2. The RNA molecule is built up from the four ribose nucleotides (A, C, G and U) in the nucleoplasm. The nucleotides attach themselves to the bases on the DNA by complementary base pairing, just as in DNA replication. However, only one strand of RNA is made.
3. The new nucleotides are joined to each other by covalent bonds by the enzyme RNA polymerase
4. The initial mRNA contains some regions that are not part of the protein code. These are called introns
5. The introns are cut out by enzymes
6. The result is a shorter mature RNA.
7. The mRNA diffuses out of the nucleus through a nuclear pore into the cytoplasm.

Translation - Protein Synthesis

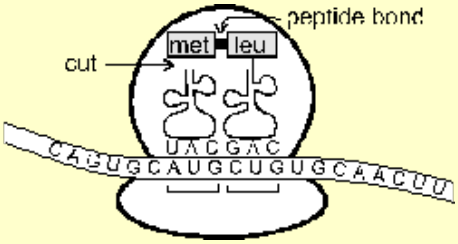
1. A ribosome attaches to the mRNA at an initiation codon (AUG). The ribosome encloses two codons.



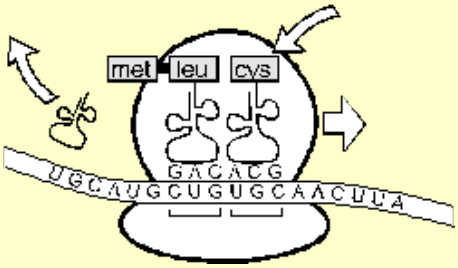
2. met-tRNA diffuses to the ribosome and attaches to the mRNA initiation codon by complementary base pairing.

3. The next amino acid-tRNA attaches to the adjacent mRNA codon (leu in this case).

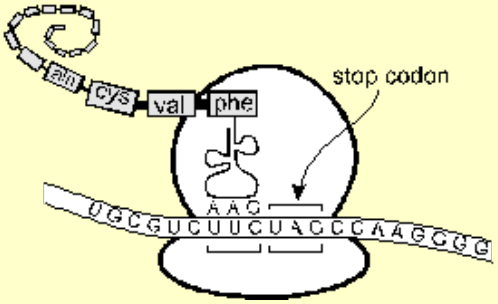
4. The bond between the amino acid and the tRNA is cut and a peptide bond is formed between the two amino acids.



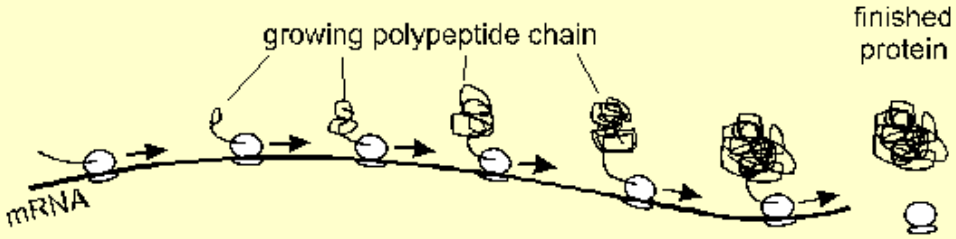
5. The ribosome moves along one codon so that a new amino acid-tRNA can attach. The free tRNA molecule leaves to collect another amino acid. The cycle repeats from step 3.



6. The polypeptide chain elongates one amino acid at a time, and peels away from the ribosome, folding up into a protein as it goes. This continues for hundreds of amino acids until a stop codon is reached.



A single piece of mRNA can be translated by many ribosomes simultaneously. A group of ribosomes all attached to one piece of mRNA is called a polysome.

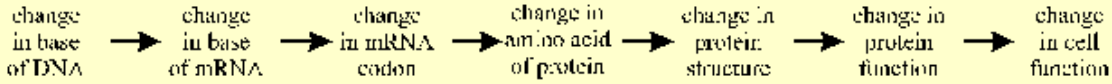


Post-Translational Modification

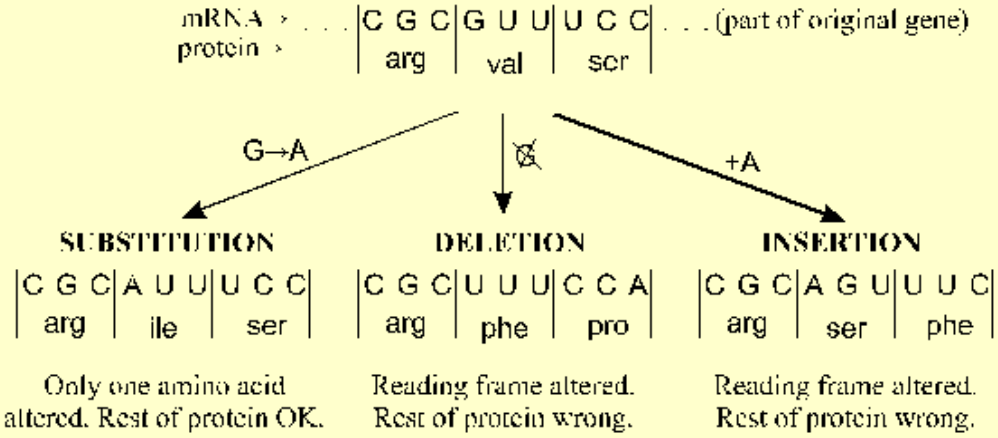
In eukaryotes, proteins often need to be altered before they become fully functional. Modifications are carried out by other enzymes and include: chain cutting, adding sugars (to make glycoproteins) or lipids (to make lipoproteins). These changes occur in the Golgi Apparatus

Mutations

Mutations are changes in genes, which are passed on to daughter cells. DNA is a very stable molecule, and it doesn't suddenly change without reason, but bases can change when DNA is being replicated. Normally replication is extremely accurate but very occasionally mistakes do occur (such as a T-C base pair). Changes in DNA can lead to changes in cell function like this:



There are basically three kinds of gene mutation, shown in this diagram:



The actual effect of a single mutation depends on many factors:

- A substitution on the third base of a codon may have no effect because the third base is less important (e.g. all codons beginning with CC code for proline).
- If a single amino acid is changed to a similar one, then the protein structure and function may be unchanged, but if an amino acid is changed to a very different one, then the structure and function of the protein will be very different.
- If the changed amino acid is at the active site of the enzyme then it is more likely to affect enzyme function than if it is part of the supporting structure.
- Additions and Deletions are Frame shift mutations and are far more serious than substitutions because more of the protein is altered.
- If a frame-shift mutation is near the end of a gene it will have less effect than if it is near the start of the gene
- If the mutation is in a gene that is not expressed in this cell (e.g. the insulin gene in a red blood cell) then it won't matter.
- Some proteins are simply more important than others. For instance non-functioning receptor proteins in the tongue may lead to a lack of taste but is not life-threatening, whereas non-functioning haemoglobin is fatal.
- Some cells are more important than others. Mutations in somatic cells (i.e. non-reproductive body cells) will only affect cells that derive from that cell, so will probably have a small local effect like a birthmark (although they can cause widespread effects like diabetes or cancer). Mutations in germ cells (i.e. reproductive cells) will affect every single cell of the resulting organism as well as its offspring. These mutations are one source of genetic variation.

As a result of a mutation there are three possible phenotypic effects:

- Most mutations have no observable (phenotypic) effect.
- Of the mutations that have a phenotypic effect, most will have a negative effect. Most of the proteins in cells are enzymes, and most changes in enzymes will stop them working. When an enzyme stops working, a metabolic block can occur, when a reaction in cell doesn't happen, so the cell's function is changed. An example of this is the genetic disease phenylketonuria (PKU), caused by a mutation in the gene for an enzyme. This causes a metabolic block in the pathway involving the amino acid phenylalanine, which builds up, causing mental retardation.

- Very rarely a mutation can have a beneficial phenotypic effect, such as making an enzyme work faster, or a structural protein stronger, or a receptor protein more sensitive. Although rare beneficial mutations are important as they drive evolution.

These kinds of mutation are called point or gene mutations because they affect specific points within a gene. There are other kinds of mutation that can affect many genes at once or even whole chromosomes. These chromosome mutations can arise due to mistakes in cell division. A well-known example is Down syndrome (trisonomy 21) where there are three copies of chromosome 21 instead of the normal two.

Mutation Rates and Mutagens

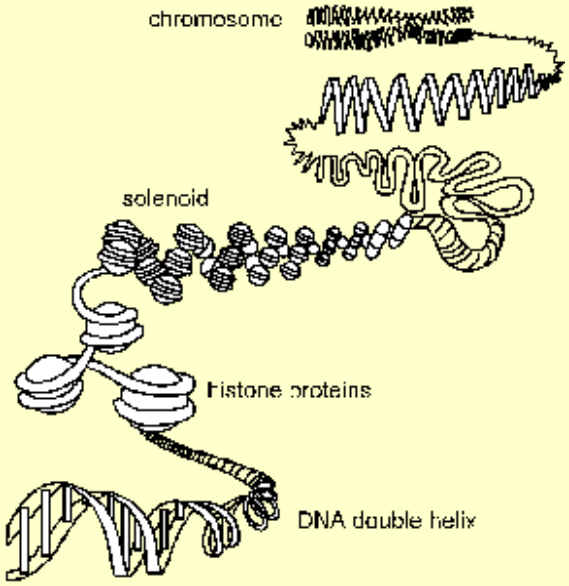
Mutations are normally very rare, which is why members of a species all look alike and can interbreed. However the rate of mutations is increased by chemicals or by radiation. These are called mutagenic agents or mutagens, and include:

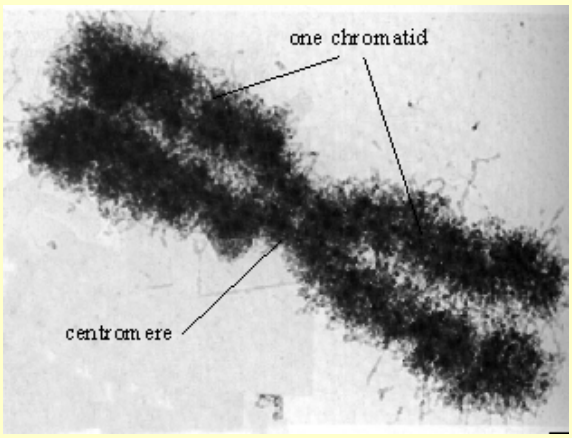
- High energy ionising radiation such as x-rays, ultraviolet rays, rays from radioactive sources all ionise the bases so that they don't form the correct base pairs.
- Intercalating chemicals such as mustard gas (used in World War 1), which bind to DNA separating the two strands.
- Chemicals that react with the DNA bases such as benzene and tar in cigarette smoke.

DNA and Chromosomes

The DNA molecule in a single human cell is about 1m long so in order to fit into the cell the DNA is cut into shorter lengths and each length is tightly wrapped up with histone proteins to form a complex called chromatin. During most of the life of a cell the chromatin is dispersed throughout the nucleus and cannot be seen with a light microscope.

Just before cell division the DNA is replicated so there is temporarily twice the normal amount DNA. Following replication the chromatin then coils up even tighter to form short fat bundles called chromosomes. These are about 100 000 times shorter than fully stretched DNA and are thick enough to be seen under the microscope. Each chromosome is roughly X-shaped because it contains two replicated copies of the DNA. The two arms of the X are therefore identical. They are called chromatids, and are joined at the centromere. (Do not confuse the two chromatids with the two strands of DNA.) The complex folding of DNA into chromosomes is shown below.





micrograph of a single chromosome

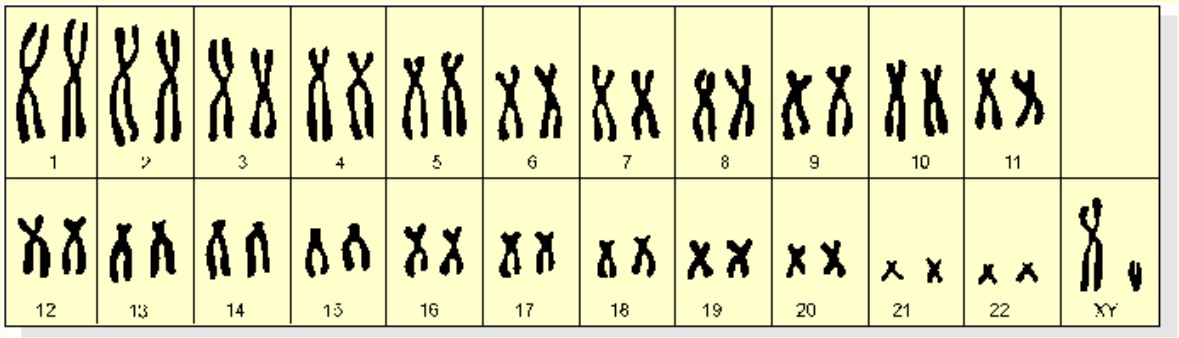
- Chromatin DNA + histones at any stage of the cell cycle

- Chromosome compact X-shaped form of chromatin formed (and visible) during mitosis
- Chromatid single arm of an X-shaped chromosome

Since the DNA molecule extends from one end of a chromosome to the other, and the genes are distributed along the DNA, then each gene has a defined position on a chromosome. This position is called the locus of the gene.

Karyotypes and Homologous Chromosomes

If a dividing cell is stained with a special fluorescent dye and examined under a microscope during cell division, the individual chromosomes can be distinguished. They can then be photographed and studied. This is a difficult and skilled procedure, and it often helps if the chromosomes are cut out and arranged in order of size.



This display is called a karyotype, and it shows several features:

- Different species have different number of chromosomes, but all members of the same species have the same number. Humans have 46.
- Each chromosome has a characteristic size, shape and banding pattern, which allows it to be identified and numbered. The chromosomes are numbered from largest to smallest.
- Chromosomes come in pairs, with the same size, shape and banding pattern, called homologous pairs ("same shaped"). So there are two chromosome number 1s, two chromosome number 2s, etc, and humans really have 23 pairs of chromosomes. Homologous chromosomes are a result of sexual reproduction, and the homologous pairs are the maternal and paternal versions of the same chromosome, so they have the same sequence of genes
- 1 pair of chromosomes is different in males and females. These are the sex chromosomes, and are non-homologous in one of the sexes. In humans sex chromosomes are homologous in females (XX) and non-homologous in males (XY). (In birds it is the other way round!) The non-sex chromosomes are sometimes called autosomes, so humans have 22 pairs of autosomes, and 1 pair of sex chromosomes.

Try some comprehension questions

MODULE 2

Genetic Engineering: Contents

- Techniques
  - Restriction Enzymes/DNA Ligase
  - Vectors/Plasmids
  - Gene Transfer
  - Genetic Markers
  - PCR
  - DNA probes
  - Electrophoresis
  - DNA Sequencing
- Applications
  - Gene Products
  - New Phenotypes
  - Gene Therapy

Genetic Engineering

Genetic engineering, also known as recombinant DNA technology, means altering the genes in a living organism to produce a Genetically Modified Organism (GMO) with a new genotype. Various kinds of genetic modification are possible: inserting a foreign gene from one species into another, forming a transgenic organism; altering an existing gene so that its product is changed; or changing gene expression so that it is translated more often or not at all.

Techniques of Genetic Engineering

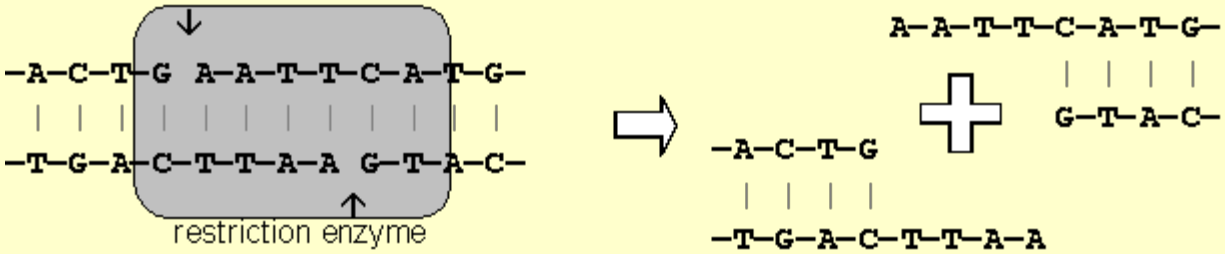
Genetic engineering is a very young discipline, and is only possible due to the development of techniques from the 1960s onwards. These techniques have been made possible from our greater understanding of DNA and how it functions following the discovery of its structure by Watson and Crick in 1953. Although the final goal of genetic engineering is usually the expression of a gene in a host, in fact most of the techniques and time in genetic engineering are spent isolating a gene and then cloning it. This table lists the techniques

that we'll look at in detail.

Technique	Purpose
Restriction Enzymes	To cut DNA at specific points, making small fragments
DNA Ligase	To join DNA fragments together
Vectors	To carry DNA into cells and ensure replication
Plasmids	Common kind of vector
Genetic Markers	To identify cells that have been transformed
PCR	To amplify very small samples of DNA
cDNA	To make a DNA copy of mRNA
DNA probes	To identify and label a piece of DNA containing a certain sequence
Gene Synthesis	To make a gene from scratch
Electrophoresis	To separate fragments of DNA
DNA Sequencing	To read the base sequence of a length of DNA

Restriction Enzymes

These are enzymes that cut DNA at specific sites. They are properly called restriction endonucleases because they cut the bonds in the middle of the polynucleotide chain. Most restriction enzymes make a staggered cut in the two strands, forming sticky ends.



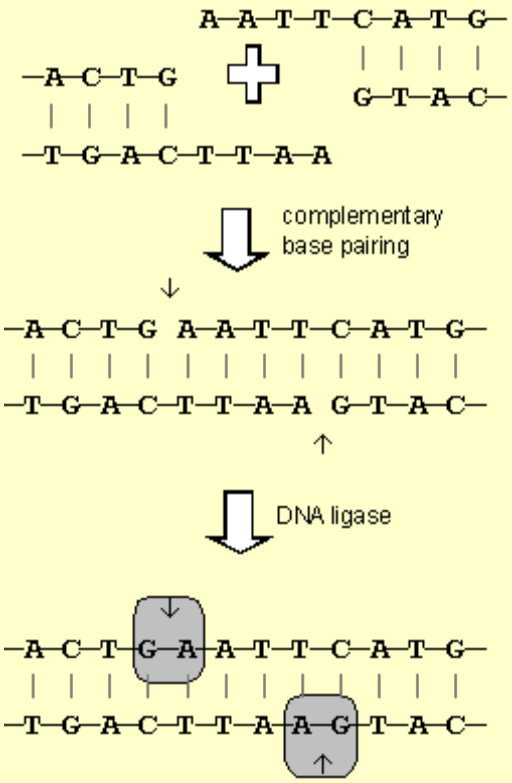
The cut ends are "sticky" because they have short stretches of single-stranded DNA. These sticky ends will stick (or anneal) to another piece of DNA by complementary base pairing, but only if they have both been cut with the same restriction enzyme. Restriction enzymes are highly specific, and will only cut DNA at specific base sequences, 4-8 base pairs long.

Restriction enzymes are produced naturally by bacteria as a defence against viruses (they "restrict" viral growth), but they are enormously useful in genetic engineering for cutting DNA at precise places ("molecular scissors"). Short lengths of DNA cut out by restriction enzymes are called restriction fragments. There are thousands of different restriction enzymes known, with over a hundred different recognition sequences. Restriction enzymes are named after the bacteria species they came from, so *Eco*R1 is from *E. coli* strain R.

DNA Ligase

This enzyme repairs broken DNA by joining two nucleotides in a DNA strand. It is commonly used in genetic engineering to do the reverse of a restriction enzyme, i.e. to join together complementary restriction fragments.

The sticky ends allow two complementary



restriction fragments to anneal, but only by weak hydrogen bonds, which can quite easily be broken, say by gentle heating. The backbone is still incomplete.

DNA ligase completes the DNA backbone by forming covalent bonds. Restriction enzymes and DNA ligase can therefore be used together to join lengths of DNA from different sources.

Vectors

In biology a vector is something that carries things between species. E.g. the mosquito is a vector that carries the malaria parasite into humans. In genetic engineering a vector is a length of DNA that carries the gene we want into a host cell. A vector is needed because a length of DNA containing a gene on its own won't actually do anything inside a host cell. Since it is not part of the cell's normal genome it won't be replicated when the cell divides, it won't be expressed, and in fact it will probably be broken down pretty quickly. A vector gets round these problems by having these properties:

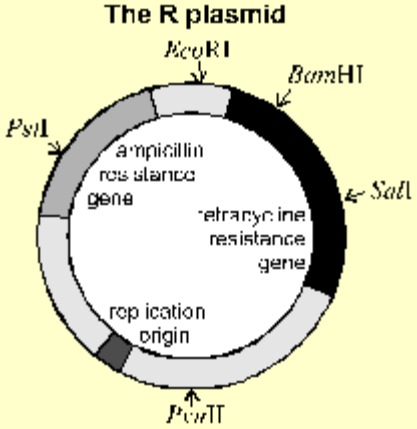
- It is big enough to hold the gene we want
- It is circular (or more accurately a closed loop), so that it is less likely to be broken down
- It contains control sequences, such as a transcription promoter, so that the gene will be replicated or expressed.
- It contain marker genes, so that cells containing the vector can be identified.

Type of vector	Max length of DNA insert
Plasmid	10 kbp
Virus or phage	30 kbp

Plasmids (the most common vectors)

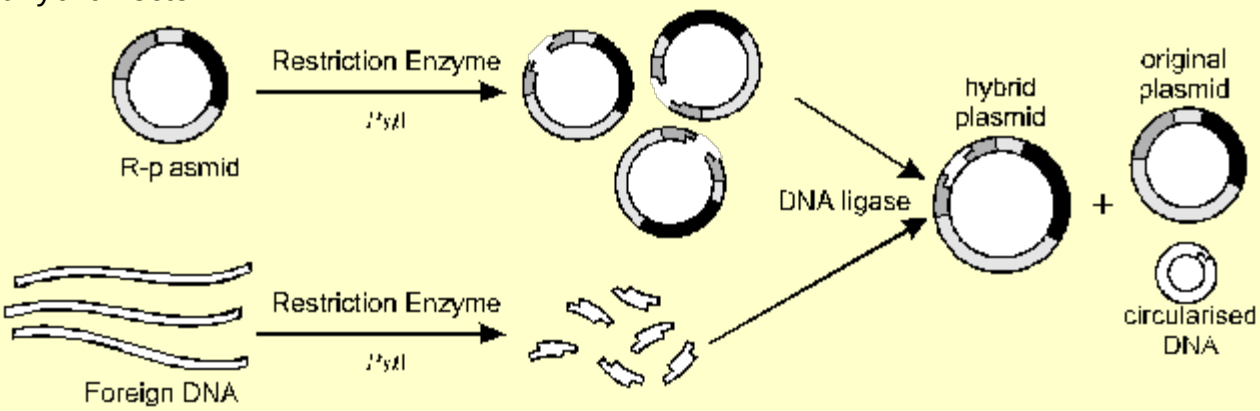
Plasmids are by far the most common kind of vector, so we shall look at how they are used in some detail. Plasmids are short circular bits of DNA found naturally in bacterial cells. A typical plasmid contains 3-5 genes and there are around 10 copies of a plasmid in a bacterial cell. Plasmids are copied when the cell divides, so the plasmid genes are passed on to all daughter cells. They are also used naturally for exchange of genes

between bacterial cells (the nearest they get to sex), so bacterial cells will take up a plasmid. Because they are so small, they are easy to handle in a test tube, and foreign genes can quite easily be incorporated into them using restriction enzymes and DNA ligase.



One of the most common plasmids used is the R-plasmid (or pBR322). This plasmid contains a replication origin, several recognition sequences for different restriction enzymes (with names like *Eco*RI), and two marker genes, which confer resistance to different antibiotics (ampicillin and tetracycline).

The diagram below shows how DNA fragments can be incorporated into a plasmid using restriction and ligase enzymes. The restriction enzyme used here (*Pst*I) cuts the plasmid in the middle of one of the marker genes (we'll see why this is useful later). The foreign DNA anneals with the plasmid and is joined covalently by DNA ligase to form a hybrid vector (in other words a mixture or hybrid of bacterial and foreign DNA). Several other products are also formed: some plasmids will simply re-anneal with themselves to re-form the original plasmid, and some DNA fragments will join together to form chains or circles. Theses different products cannot easily be separated, but it doesn't matter, as the marker genes can be used later to identify the correct hybrid vector.

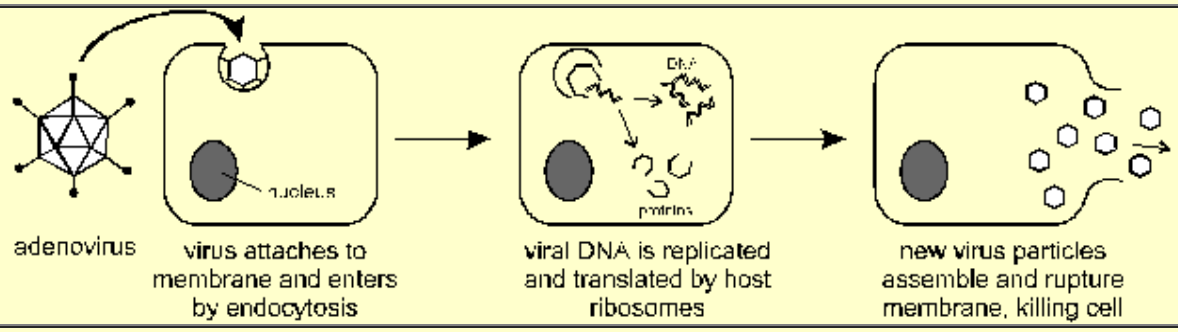
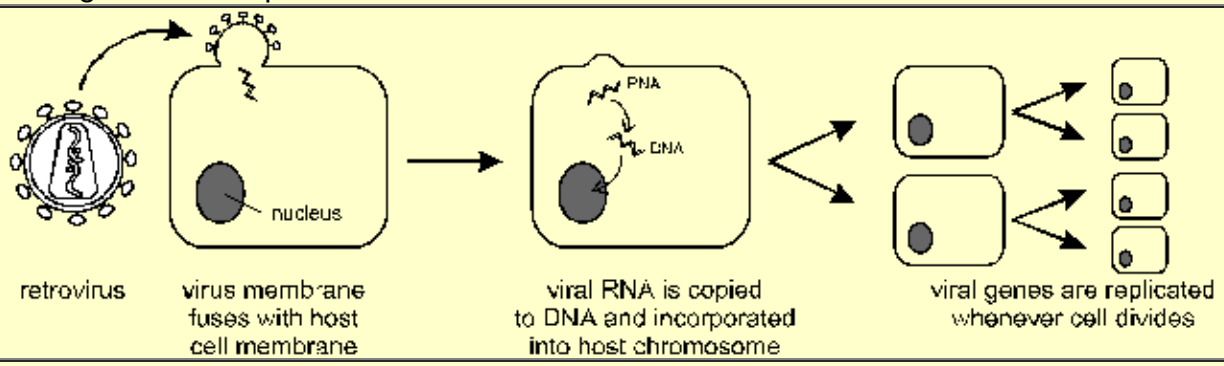


Gene Transfer

Vectors containing the genes we want must be incorporated into living cells so that they can be replicated or expressed. The cells receiving the vector are called host cells, and once they have successfully incorporated the vector they are said to be transformed. Vectors are large molecules which do not readily cross cell membranes, so the membranes must be made permeable in some way. There are different ways of doing this depending on the type of host cell. The most important one have the symbol the others are less commonly used

- Heat Shock. Cells are incubated with the vector in a solution containing calcium ions at 0°C. The temperature is then suddenly raised to about 40°C. This heat shock causes some of the cells to take up the vector, though no one knows why. This works well for bacterial and animal cells.
- Electroporation. Cells are subjected to a high-voltage pulse, which temporarily disrupts the membrane and allows the vector to enter the cell. This is the most efficient method of delivering genes to bacterial cells.
- Viruses. The vector is first incorporated into a virus, which is then used to infect cells, carrying the foreign gene along with its own genetic material. Since viruses rely on getting their DNA into host cells for their survival they have evolved many successful methods, and so are an obvious choice for gene delivery. The virus must first be genetically engineered to make it safe, so that it can't reproduce itself

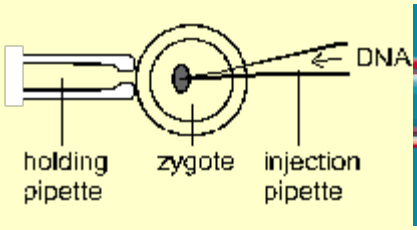
or make toxins. Three viruses are commonly used:

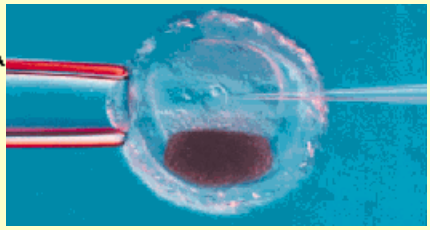
<table>
<tr><th>Type of Virus</th><th>Details</th></tr>
<tr><td>Bacteriophages</td><td>(also called phages) are viruses that infect bacteria. They are an effective way of delivering large genes into bacteria cells in culture.</td></tr>
<tr><td rowspan="2">Adenoviruses</td><td>are human viruses that causes respiratory diseases including the common cold. Their genetic material is double-stranded DNA, and they are ideal for delivering genes to living patients in gene therapy. Their DNA is not incorporated into the host's chromosomes, so it is not replicated, but their genes are expressed. The adenovirus is genetically altered so that its coat proteins are not synthesised, so new virus particles cannot be assembled and the host cell is not killed..</td></tr>
<tr><td> 
</td></tr>
<tr><td rowspan="2">Retroviruses</td><td>are a group of human viruses that include HIV. They are enclosed in a lipid membrane and their genetic material is double-stranded RNA. On infection this RNA is copied to DNA and the DNA is incorporated into the host's chromosome. This means that the foreign genes are replicated into every daughter cell. After a certain time, the dormant DNA is switched on, and the genes are expressed in the host cells.</td></tr>
<tr><td> 
</td></tr>
</table>

- Plant Tumours. This method has been used successfully to transform plant cells, which are perhaps the hardest to do. The gene is first inserted into the plasmid of a soil bacterium, and then plants are infected with the bacterium. The bacterium inserts the plasmid into the plant cells' chromosomal DNA and causes a "crown gall" tumour. These tumour cells can be cultured in the laboratory.

- Gene Gun. This technique fires microscopic gold particles coated with the foreign DNA at the cells using a compressed air gun. It is designed to overcome the problem of the strong cell wall in plant tissue.

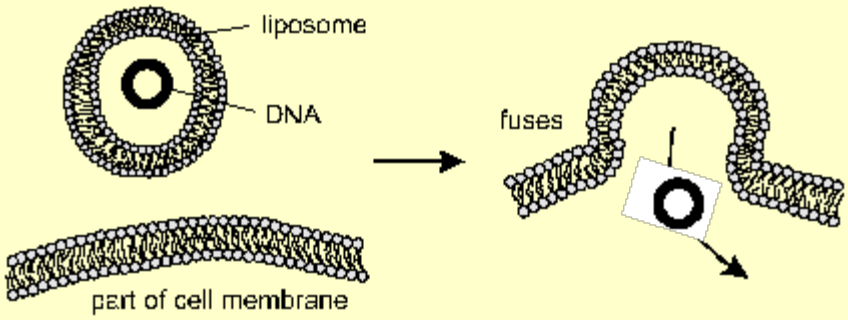
- Micro-Injection. A cell is held on a pipette under a microscope and the foreign DNA is injected directly into the nucleus using an incredibly fine micro-pipette. Used where there are only a very few cells available, such as fertilised animal egg cells.





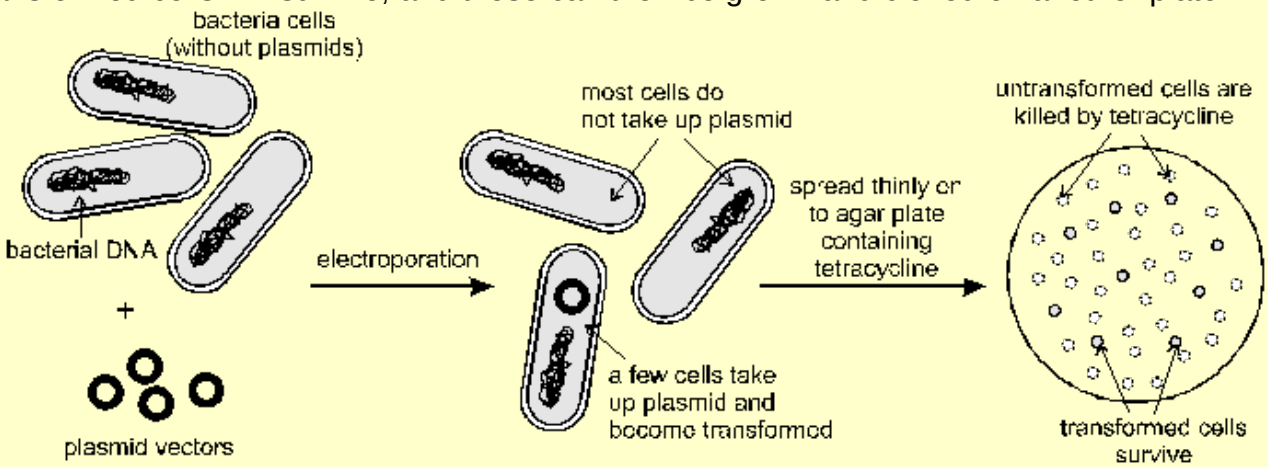
- Liposomes. Vectors can be encased in liposomes, which are small membrane vesicles (see module 1).

The liposomes fuse with the cell membrane (and sometimes the nuclear membrane too), delivering the DNA into the cell. This works for many types of cell, but is particularly useful for delivering genes to cell *in vivo* (such as in gene therapy).



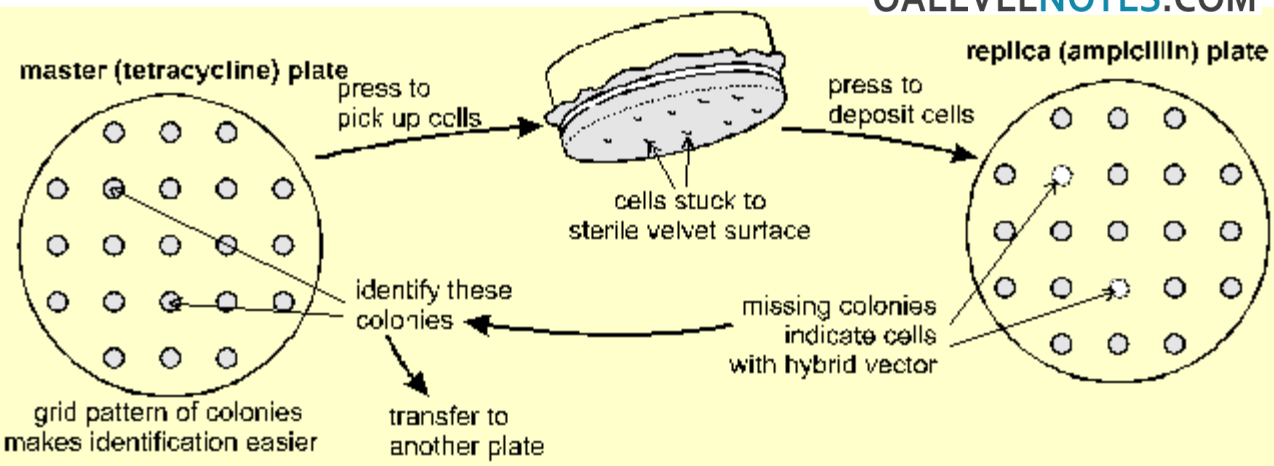
Genetic Markers

These are needed to identify cells that have successfully taken up a vector and so become transformed. With most of the techniques above less than 1% of the cells actually take up the vector, so a marker is needed to distinguish these cells from all the others. A common marker, used in plasmids, is a gene for resistance to an antibiotic such as tetracycline. Bacterial cells taking up this plasmid are resistant to this antibiotic. So if the cells are grown on a medium containing tetracycline all the normal untransformed cells (99%) will die. Only the 1% transformed cells will survive, and these can then be grown and cloned on another plate.



Replica Plating

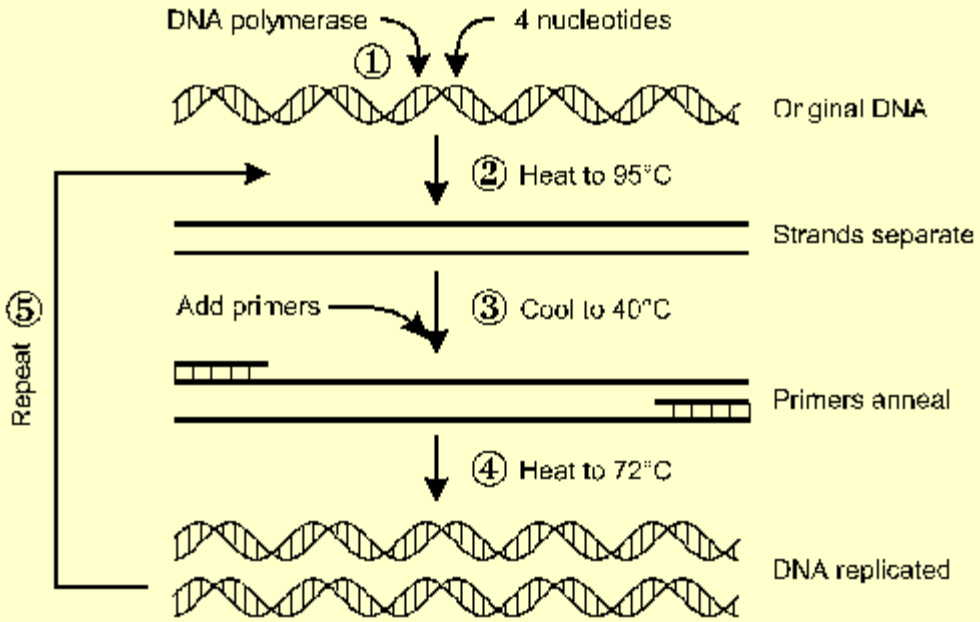
Replica plating is a simple technique for making an exact copy of an agar plate. A pad of sterile cloth the same size as the plate is pressed on the surface of an agar plate with bacteria growing on it. Some cells from each colony will stick to the cloth. If the cloth is then pressed onto a new agar plate, some cells will be deposited and colonies will grow in exactly the same positions on the new plate. This technique has a number of uses, but the most common use in genetic engineering is to help solve another problem in identifying transformed cells. This problem is to distinguish those cells that have taken up a hybrid plasmid vector (with a foreign gene in it) from those cells that have taken up plasmids without the gene. This is where the second marker gene (for resistance to ampicillin) is used. If the foreign gene is inserted into the middle of this marker gene, the marker gene is disrupted and won't make its proper gene product. So cells with the hybrid plasmid will be killed by ampicillin, while cells with the normal plasmid will be immune to ampicillin. Since this method of identification involves killing the cells we want, we must first make a master agar plate and then make a replica plate of this to test for ampicillin resistance.



Once the colonies of cells containing the correct hybrid plasmid vector have been identified, the appropriate colonies on the master plate can be selected and grown on another plate.

Polymerase Chain Reaction (PCR)

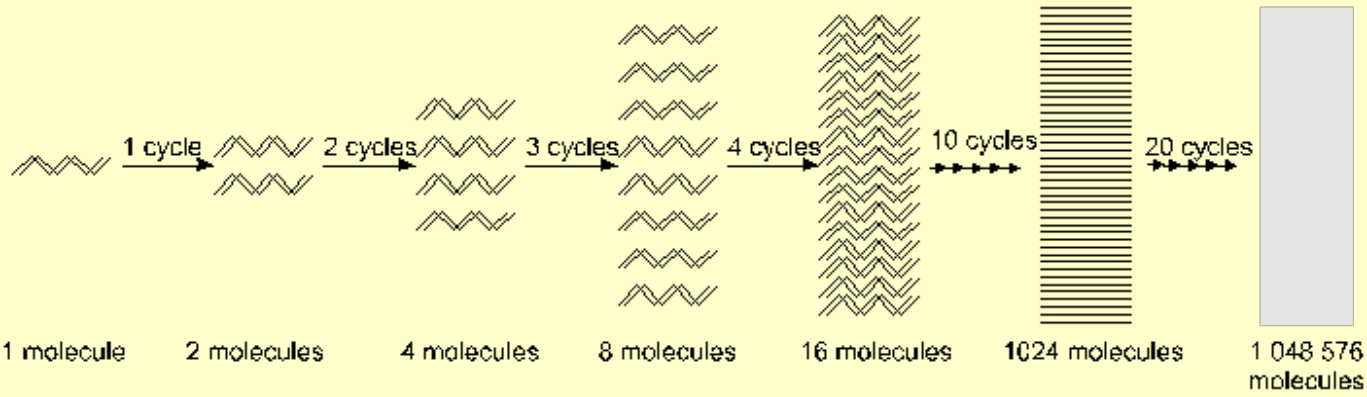
Genes can be cloned by cloning the bacterial cells that contain them, but this requires quite a lot of DNA in the first place. PCR can clone (or amplify) DNA samples as small as a single molecule. It is a newer technique, having been developed in 1983 by Kary Mullis, for which discovery he won the Nobel prize in 1993. The polymerase chain reaction is simply DNA replication in a test tube. If a length of DNA is mixed with the four nucleotides (A, T, C and G) and the enzyme DNA polymerase in a test tube, then the DNA will be replicated many times.



1. Start with a sample of the DNA to be amplified, and add the four nucleotides and the enzyme DNA polymerase.
2. Normally (*in vivo*) the DNA double helix would be separated by the enzyme helicase, but in PCR (*in vitro*) the strands are separated by heating to 95°C for two minutes. This breaks the hydrogen bonds.
3. DNA polymerisation always requires short lengths of DNA (about 20 bp long) called primers, to get it started. *In vivo* the primers are made during replication by DNA polymerase, but *in vitro* they must be synthesised separately and added at this stage. This means that a short length of the sequence of the DNA must already be known, but it does have the advantage that only the part between the primer sequences is replicated. The DNA must be cooled to 40°C to allow the primers to anneal to their complementary sequences on the separated DNA strands.
4. The DNA polymerase enzyme can now extend the primers and complete the replication of the rest of

the DNA. The enzyme used in PCR is derived from the thermophilic bacterium *Thermus aquaticus*, which grows naturally in hot springs at a temperature of 90°C, so it is not denatured by the high temperatures in step 2. Its optimum temperature is about 72°C, so the mixture is heated to this temperature for a few minutes to allow replication to take place as quickly as possible.

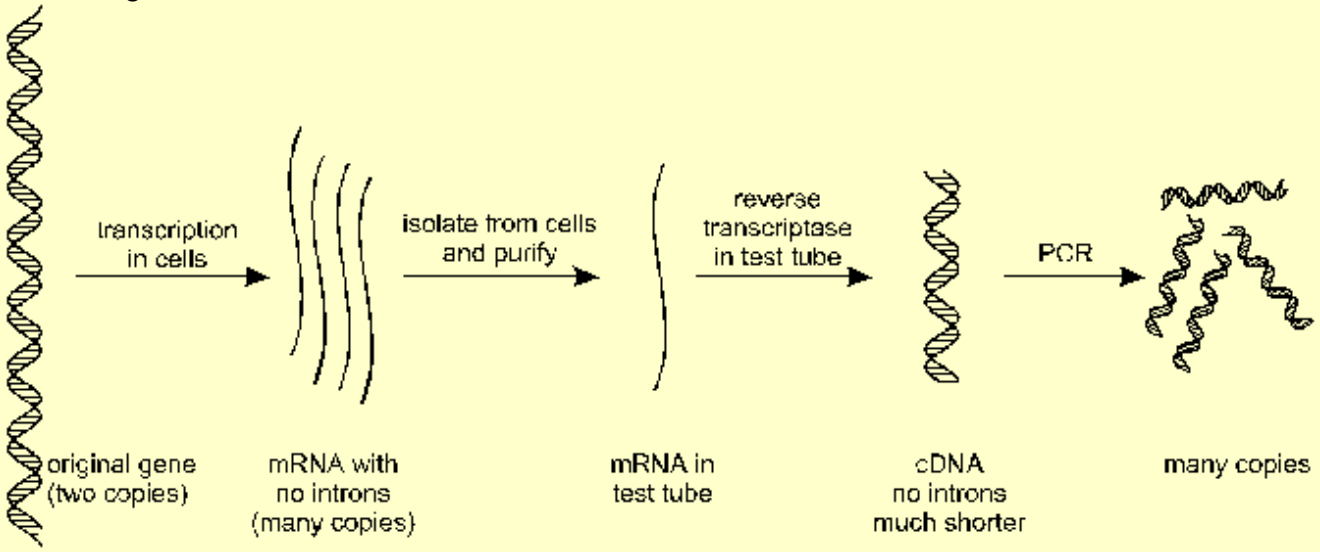
5. Each original DNA molecule has now been replicated to form two molecules. The cycle is repeated from step 2 and each time the number of DNA molecules doubles. This is why it is called a chain reaction, since the number of molecules increases exponentially, like an explosive chain reaction. Typically PCR is run for 20-30 cycles.



PCR can be completely automated, so in a few hours a tiny sample of DNA can be amplified millions of times with little effort. The product can be used for further studies, such as cloning, electrophoresis, or gene probes. Because PCR can use such small samples it can be used in forensic medicine (with DNA taken from samples of blood, hair or semen), and can even be used to copy DNA from mummified human bodies, extinct woolly mammoths, or from an insect that's been encased in amber since the Jurassic period. One problem of PCR is having a pure enough sample of DNA to start with. Any contaminant DNA will also be amplified, and this can cause problems, for example in court cases.

Complementary DNA

Complementary DNA (cDNA) is DNA made from mRNA. This makes use of the enzyme reverse transcriptase, which does the reverse of transcription: it synthesises DNA from an RNA template. It is produced naturally by a group of viruses called the retroviruses (which include HIV), and it helps them to invade cells. In genetic engineering reverse transcriptase is used to make an artificial gene of cDNA as shown in this diagram.



Complementary DNA has helped to solve different problems in genetic engineering:

It makes genes much easier to find. There are some 70 000 genes in the human genome, and finding one

gene out of this many is a very difficult (though not impossible) task. However a given cell only expresses a few genes, so only makes a few different kinds of mRNA molecule. For example the b cells of the pancreas make insulin, so make lots of mRNA molecules coding for insulin. This mRNA can be isolated from these cells and used to make cDNA of the insulin gene.

DNA Probes

These are used to identify and label DNA fragments that contain a specific sequence. A probe is simply a short length of DNA (20-100 nucleotides long) with a label attached. There are two common types of label used:

- a radioactively-labelled probe (synthesised using the isotope ^{32}P) can be visualised using photographic film (an autoradiograph).
- a fluorescently-labelled probe will emit visible light when illuminated with invisible ultraviolet light. Probes can be made to fluoresce with different colours.

Probes are always single-stranded, and can be made of DNA or RNA. If a probe is added to a mixture of different pieces of DNA (e.g. restriction fragments) it will anneal (base pair) with any lengths of DNA containing the complementary sequence. These fragments will now be labelled and will stand out from the rest of the DNA. DNA probes have many uses in genetic engineering:

- To identify restriction fragments containing a particular gene out of the thousands of restriction fragments formed from a genomic library. This use is described in shotguning below.
- To identify the short DNA sequences used in DNA fingerprinting.
- To identify genes from one species that are similar to those of another species. Most genes are remarkably similar in sequence from one species to another, so for example a gene probe for a mouse gene will probably anneal with the same gene from a human. This has aided the identification of human genes.
- To identify genetic defects. DNA probes have been prepared that match the sequences of many human genetic disease genes such as muscular dystrophy, and cystic fibrosis. Hundreds of these probes can be stuck to a glass slide in a grid pattern, forming a DNA microarray (or DNA chip). A sample of human DNA is added to the array and any sequences that match any of the various probes will stick to the array and be labelled. This allows rapid testing for a large number of genetic defects at a time.

Shotguning

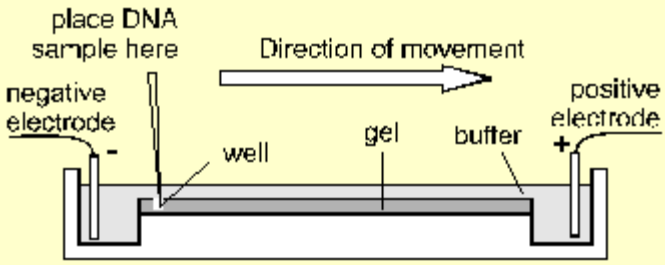
This is used to find one particular gene in a whole genome, a bit like finding the proverbial needle in a haystack. It is called the shotgun technique because it starts by indiscriminately breaking up the genome (like firing a shotgun at a soft target) and then sorting through the debris for the particular gene we want. For this to work a gene probe for the gene is needed, which means at least a short part of the gene's sequence must be known.

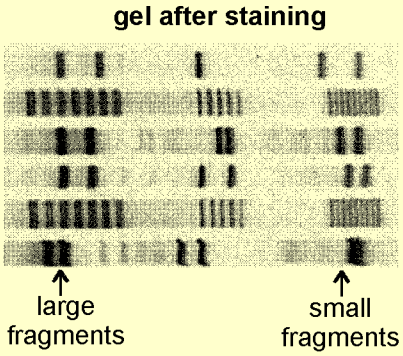
Antisense Genes

These are used to turn off the expression of a gene in a cell. The principle is very simple: a copy of the gene to be switch off is inserted into the host genome the "wrong" way round, so that the complementary (or antisense) strand is transcribed. The antisense mRNA produced will anneal to the normal sense mRNA forming double-stranded RNA. Ribosomes can't bind to this, so the mRNA is not translated, and the gene is effectively "switched off".

Electrophoresis

This is a form of chromatography used to separate different pieces of DNA on the basis of their length. It might typically be used to separate restriction fragments. The DNA samples are placed into wells at one end of a thin slab of gel (usually made of agarose) and covered in a buffer solution. An electric current is passed through the gel. Each nucleotide in a molecule of DNA contains a negatively-charged phosphate group, so DNA is attracted to the anode (the positive electrode). The molecules have to diffuse through the gel, and smaller lengths of DNA move faster than larger lengths, which are retarded by the gel. So the smaller the length of the DNA molecule, the further down the gel it will move in a given time. At the end of the run the current is turned off.





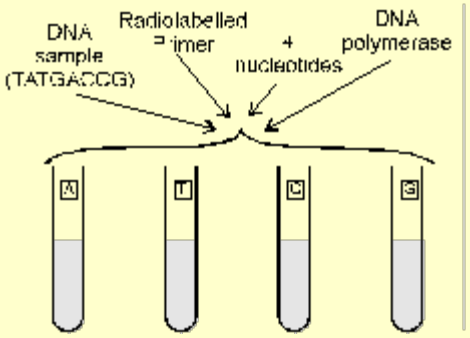
Unfortunately the DNA on the gel cannot be seen, so it must be visualised. There are three common methods for doing this:

- The gel can be stained with a chemical that specifically stains DNA, such as ethidium bromide. The DNA shows up as blue bands.
- The DNA samples at the beginning can be radiolabelled with a radioactive isotope such as ^{32}P. Photographic film is placed on top of the finished gel in the dark, and the DNA shows up as dark bands on the film. This method is extremely sensitive.
- The DNA fragments at the beginning can be labelled with a fluorescent molecule. The DNA fragments show up as coloured lights when the finished gel is illuminated with invisible ultraviolet light.

DNA Sequencing

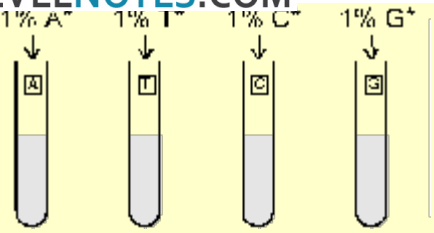
This means reading the base sequence of a length of DNA. Once this is known the amino acid sequence of the protein that the DNA codes for can also be determined, using the genetic code table. The sequence can also be compared with DNA sequences from other individuals and even other species to work out relationships.

DNA sequencing is based on a beautifully elegant technique developed by Fred Sanger, and now called the Sanger method.



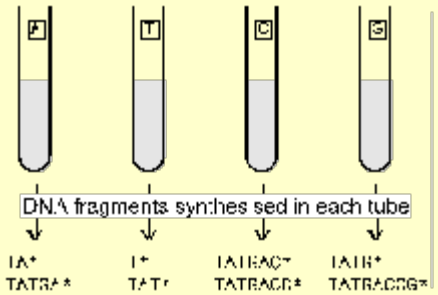
- Label 4 test tubes labelled A, T, C and G. Into each test tube add: a sample of the DNA to be sequenced (containing many millions of individual molecules) a radioactive primer (so the DNA can be visualised later on the gel), the four DNA nucleotides and the enzyme DNA polymerase.

- In each test tube add a small amount of a special modified dideoxy nucleotide that cannot form a bond and so stops further synthesis of DNA. Tube A has dideoxy A (A*), tube T has dideoxy T (T*), tube C has dideoxy C

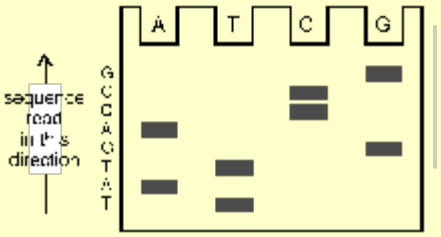


(C*) and tube G has dideoxy G (G*). The dideoxy nucleotides are present at about 1% of the concentration of the normal nucleotides.

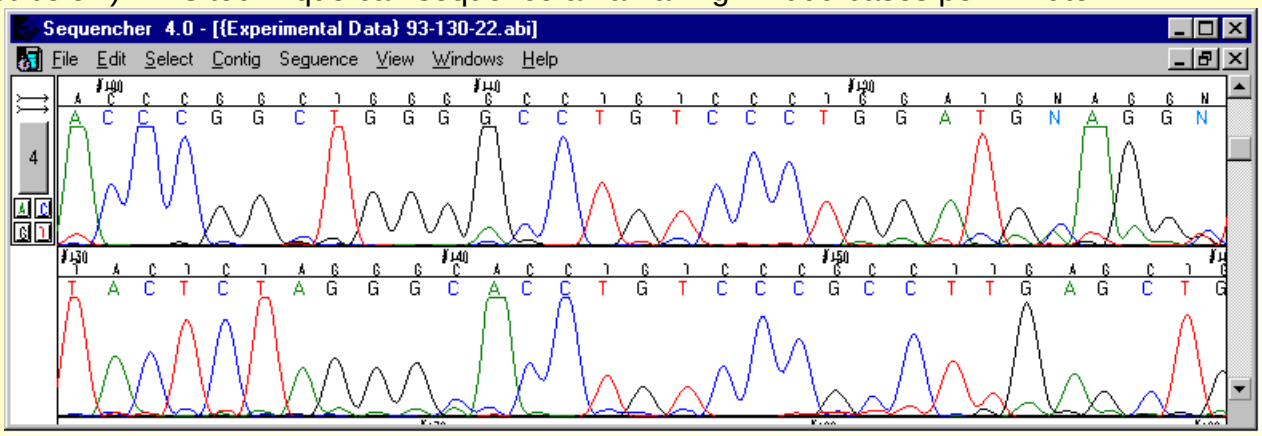
- Let the DNA polymerase synthesise many copies of the DNA sample. From time to time at random a dideoxy nucleotide will be added to the growing chain and synthesis of that chain will then stop. A range of DNA molecules will be synthesised ranging from full length to very short. The important point is that in tube A, all the fragments will stop at an A nucleotide. In tube T, all the fragments will stop at a T nucleotide , and so on.



- The contents of the four tubes are now run side by side on an electrophoresis gel, and the DNA bands are visualised by autoradiography. Since the fragments are now sorted by length the sequence can simply be read off the gel starting with the smallest fragment (just one nucleotide) at the bottom and reading upwards.



There is now a modified version of the Sanger method called cycle sequencing, which can be completely automated. The primers are not radiolabelled, but instead the four dideoxy nucleotides are fluorescently labelled, each with a different colour (A* is green, T* is red, C* is blue and G* is yellow). The polymerisation reaction is done in a single tube, using PCR-like cycles to speed up the process. The resulting mixture is separated using capillary electrophoresis, which gives good separation in a single narrow gel. The gel is read by a laser beam and the sequence of colours is converted to a DNA sequence by computer program (like the screenshot below). This technique can sequence an amazing 12 000 bases per minute.



Thousands of genes have been sequenced using these methods and the entire genomes of several organisms have also been sequenced. A huge project is underway to sequence the human genome, and it delivered a draft sequence in June 2000. The complete 3 billion base sequence should be complete by 2003. This information will give us unprecedented knowledge about ourselves, and is likely to lead to dramatic medical and scientific advances.

Applications of Genetic Engineering

We have now looked at some of the many techniques used by genetic engineers. What can be done with these techniques? By far the most numerous applications are still as research tools, and the techniques above are helping geneticists to understand complex genetic systems. Despite all the hype, genetic engineering still has very few successful commercial applications, although these are increasing each year. The applications so far can usefully be considered in three groups.

- Gene Products — using genetically modified organisms (usually microbes) to produce chemicals, usually for medical or industrial applications.
- New Phenotypes — using gene technology to alter the characteristics of organisms (usually farm animals or crops)
- Gene Therapy — using gene technology on humans to treat a disease

Gene Products

The biggest and most successful kind of genetic engineering is the production of gene products. These products are of medical, agricultural or commercial value. This table shows a few of the examples of genetically engineered products that are already available.

Product	Use	Host Organism
Insulin	human hormone used to treat diabetes	bacteria /yeast
Factor VIII	human blood clotting factor, used to treat haemophiliacs	bacteria
AAT	enzyme used to treat cystic fibrosis and emphysema	sheep
rennin	enzyme used in manufacture of cheese	bacteria /yeast

The products are mostly proteins, which are produced directly when a gene is expressed, but they can also be non-protein products produced by genetically-engineered enzymes. The basic idea is to transfer a gene (often human) to another host organism (usually a microbe) so that it will make the gene product quickly, cheaply and ethically. It is also possible to make "designer proteins" by altering gene sequences, but while this is a useful research tool, there are no commercial applications yet.

Since the end-product is just a chemical, in principle any kind of organism could be used to produce it. By far the most common group of host organisms used to make gene products are the bacteria, since they can be grown quickly and the product can be purified from their cells. Unfortunately bacteria cannot not always make human proteins, and recently animals and even plants have also been used to make gene products. In neither case is it appropriate to extract the product from their cells, so in animals the product must be secreted in milk or urine, while in plants the product must be secreted from the roots. This table shows some of the advantages and disadvantages of using different organisms for the production of genetically-engineered gene products.

TYPE OF ORGANISM	ADVANTAGES	DISADVANTAGES

Prokaryotes (i.e.Bacteria)	no nucleus so DNA easy to modify; have plasmids; small genome; genetics well understood; asexual so can be cloned; small and fast growing; easy to grow commercially in fermenters; will use cheap carbohydrate; few ethical problems.	can't splice introns; no post-translational modification; small gene size
Eukaryotes	can do post-translational modifications; can accept large genes	Do not have plasmids (except yeast); often diploid so two copies of genes may need to be inserted; control of expression not well understood.
Fungi (yeast, mould)	asexual so can be cloned; haploid, so only one copy needed; can be grown in vats	can't always make animals gene products
Plants	photosynthetic so don't need much feeding; can be cloned from single cells; products can be secreted from roots or in sap.	cell walls difficult to penetrate by vector; slow growing; must be grown in fields; multicellular
Animals (pharming)	most likely to be able to make human proteins; products can be secreted in milk or urine	multicellular; slow growing

We'll look at some examples in detail.

Human Insulin

Insulin is a small protein hormone produced by the pancreas to regulate the blood sugar concentration. In the disease insulin-dependent diabetes the pancreas cells don't produce enough insulin, causing wasting symptoms and eventually death. The disease can be successfully treated by injection of insulin extracted from the pancreases of slaughtered cows and pigs. However the insulin from these species has a slightly different amino acid sequence from human insulin and this can lead to immune rejection and side effects.

The human insulin gene was isolated, cloned and sequenced in the 1970s, and so it became possible to insert this gene into bacteria, who could then produce human insulin in large amounts. Unfortunately it wasn't that simple. In humans, pancreatic cells first make pro-insulin, which then undergoes post-translational modification to make the final, functional insulin. Bacterial cells cannot do post-translational modification. Eventually a synthetic cDNA gene was made and inserted into the bacterium *E. coli,* which made pro-insulin, and the post-translational conversion to insulin was carried out chemically. This technique was developed by Eli Lilly and Company in 1982 and the product, "humulin" became the first genetically-engineered product approved for medical use.

In the 1990s the procedure was improved by using the yeast *Saccharomyces cerevisiae* instead of *E. coli*. Yeast, as a eukaryote, is capable of post-translational modification, so this simplifies the production of human insulin. However another company has developed a method of converting pig insulin into human insulin by chemically changing a few amino acids, and this turns out to be cheaper than the genetic engineering methods. This all goes to show that genetic engineers still have a lot to learn.

Bovine Somatotrophin (BST)

This is a growth hormone produced by cattle. The gene has been cloned in bacteria by the company Monsanto, who can produce large quantities of BST. in the USA cattle are often injected with BST every 2 weeks, resulting in a 10% increase in mass in beef cattle and a 25% increase in milk production in dairy cows. BST was tested in the UK in 1985, but it was not approved and its use is currently banned in the EU. This is partly due to public concerns and partly because there is already overproduction of milk and beef in the EU, so greater production is not necessary.

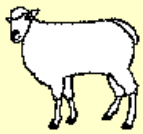
Rennin

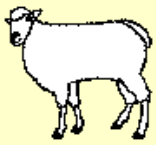
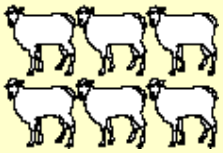
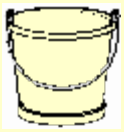
Rennin is an enzyme used in the production of cheese. It is produced in the stomach of juvenile mammals (including humans) and it helps the digestion of the milk protein caesin by solidifying it so that is remains longer in the stomach. The cheese industry used to obtain its rennin from the stomach of young calves when they were slaughtered for veal, but there are moral and practical objections to this source. Now an artificial cDNA gene for rennin has been made from mRNA extracted from calf stomach cells, and this gene has been inserted into a variety of microbes. The rennin extracted from these microbes has been very successful and 90% of all hard cheeses in the UK are made using microbial rennin. Sometimes (though not always) these products are labelled as "vegetarian cheese".

AAT (a-1-antitrypsin)

AAT is a human protein made in the liver and found in the blood. As the name suggests it is an inhibitor of protease enzymes like trypsin and elastase. There is a rare mutation of the AAT gene (a single base substitution) that causes AAT to be inactive, and so the protease enzymes to be uninhibited. The most noticeable effect of this in the lungs, where elastase digests the elastic tissue of the alveoli, leading to the lung disease emphysema. This condition can be treated by inhaling an aerosol spray containing AAT so that it reaches the alveoli and inhibits the elastase there.

AAT for this treatment can be extracted from blood donations, but only in very small amounts. The gene for AAT has been found and cloned, but AAT cannot be produced in bacteria because AAT is a glycoprotein, which means it needs to have sugars added by post translational modification. This kind of modification can only be carried out by animals (because they have a golgi body), and AAT is now produced by genetically-modified sheep. In order to make the AAT easy to extract, the gene was coupled to a promoter for the milk protein b-lactoglubulin. Since this promoter is only activated in mammary gland cells, the AAT gene will only be expressed in mammary gland cells, and so will be secreted into the sheep's milk. This makes it very easy to harvest and purify without harming the sheep. The first transgenic sheep to produce AAT was called Tracy, and she was produced in Edinburgh in 1993. This is how Tracy was made:

A female sheep is given a fertility drug to stimulate her egg production, and several mature eggs are collected from her ovaries.	
The eggs are fertilised *in vitro*.	
A plasmid is prepared containing the gene for human AAT and the promoter sequence for b-	

lactoglobulin. Hundreds of copies of this plasmid are microinjected into the nucleus of the fertilised zygotes. Only a few of the zygotes will be transformed, but at this stage you can't tell which.	
The zygotes divide *in vitro* until the embryos are at the 16-cell stage.	
The 16-cell embryos are implanted into the uterus of surrogate mother ewes. Only a few implantations result in a successful pregnancy.	
Test all the offspring from the surrogate mothers for AAT production in their milk. This is the only way to find if the zygote took up the AAT gene so that it can be expressed. About 1 in 20 eggs are successful.	
Collect milk from the transgenic sheep for the rest of their lives. Their milk contains about 35 g of AAT per litre of milk. Also breed from them in order to build up a herd of transgenic sheep.	
Purify the AAT, which is worth about £50 000 per mg.	

New Phenotypes

This means altering the characteristics of organisms by genetic engineering. The organisms are usually commercially-important crops or farm animals. It can be seen as a high-tech version of selective breeding, which has been used by humans to alter and improve their crops and animals for at least 10 000 years. Nevertheless GMOs have turned out to be a highly controversial development. We don't study any of these in detail, but this table gives an idea of what is being done.

ORGANISM	MODIFICATION
long life tomatoes	There are two well-known projects, both affecting the gene for the enzyme (PG) which softens the fruits as they ripen. Tomatoes that make less PG ripen more slowly and retain more flavour. The American "Flavr Savr" tomato used antisense technology to silence the gene, while the British Zeneca tomato disrupted the gene. Both were successful and were on sale for a few years, but neither is produced any more.
Insect-resistant crops	Genes for various powerful protein toxins have been transferred from the bacterium *Bacillus thuringiensis* to crop plants including maize, rice and potatoes. These Bt toxins are thousands of times more powerful than chemical insecticides, and since they are built-in to the crops, insecticide spraying (which is non-specific and damages the

	environment) is unnecessary.
Nitrogen-fixing crops	This is a huge project, which aims to transfer the 15-or-so genes required for nitrogen fixation from the nitrogen-fixing bacteria *Rhizobium* into cereals and other crop plants. These crops would then be able to fix their own atmospheric nitrogen and would not need any fertiliser. However, the process is extremely complex, and the project is nowhere near success.
tick-resistant sheep	The gene for the enzyme chitinase, which kills ticks by digesting their exoskeletons, has bee transferred from plants to sheep. These sheep should be immune to tick parasites, and may not need sheep dip.

Gene Therapy

This is perhaps the most significant, and most controversial kind of genetic engineering. It is also the least well-developed. The idea of gene therapy is to genetically alter humans in order to treat a disease. This could represent the first opportunity to cure incurable diseases. Note that this is quite different from using genetically-engineered microbes to produce a drug, vaccine or hormone to treat a disease by conventional means. Gene therapy means altering the genotype of a tissue or even a whole human.

Cystic Fibrosis (you must learn this one!)

Cystic fibrosis (CF) is the most common genetic disease in the UK, affecting about 1 in 2500. It is caused by a mutation in the gene for protein called CFTR (Cystic Fibrosis Transmembrane Regulator). The gene is located on chromosome 7, and there are actually over 300 different mutations known, although the most common mutation is a deletion of three bases, removing one amino acid out of 1480 amino acids in the protein. CFTR is a chloride ion channel protein found in the cell membrane of epithelial (lining) tissue cells, and the mutation stops the protein working, so chloride ions cannot cross the cell membrane.

Chloride ions build up inside these cells, which cause sodium ions to enter to balance the charge, and the increased concentration of the both these ions inside the epithelial cells decreases the osmotic potential. Water is therefore retained inside the cells, which means that the mucus secreted by these cells is drier and more sticky than normal. This sticky mucus block the tubes into which it is secreted, such as the small intestine, pancreatic duct, bile duct, sperm duct, bronchioles and alveoli.

These blockages lead to the symptoms of CF: breathlessness, lung infections such as bronchitis and pneumonia, poor digestion and absorption, and infertility. Of these symptoms the lung effects are the most serious causing 95% of deaths. CF is always fatal, though life expectancy has increased from 1 year to about 20 years due to modern treatments. These treatments include physiotherapy many times each day to dislodge mucus from the lungs, antibiotics to fight infections, DNAse drugs to loosen the mucus, enzymes to help food digestion and even a heart-lung transplant.

Given these complicated (and ultimately unsuccessful) treatments, CF is a good candidate for gene therapy, and was one of the first diseases to be tackled this way. The gene for CFTR was identified in 1989 and a cDNA clone was made soon after. The idea is to deliver copies of this good gene to the epithelial cells of the lung, where they can be incorporated into the nuclear DNA and make functional CFTR chloride channels. If about 10% of the cells could be corrected, this would cure the disease.

Two methods of delivery are being tried: liposomes and adenoviruses, both delivered with an aerosol inhaler, like those used by asthmatics. Clinical trials are currently underway, but as yet no therapy has been shown to be successful.

The Future of Gene Therapy

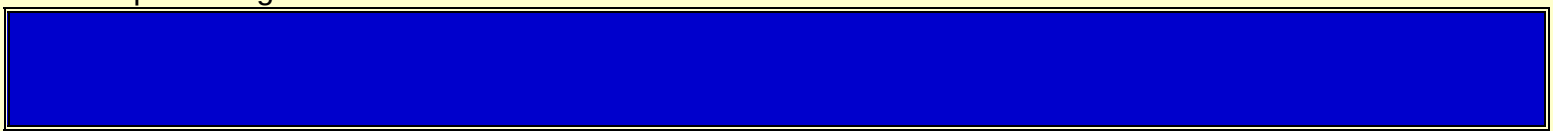
Gene therapy is in its infancy, and is still very much an area of research rather than application. No one has yet been cured by gene therapy, but the potential remains enticing. Gene therapy need not even be limited to treating genetic diseases, but could also help in treating infections and environmental diseases:

- White blood cells have be genetically modified to produce tumour necrosis factor (TNF), a protein that kills cancer cells, making these cells more effecting against tumours.
- Genes could be targeted directly at cancer cells, causing them to die, or to revert to normal cell division.
- White blood cells could be given antisense genes for HIV proteins, so that if the virus infected these cells it couldn't reproduce.

It is important to appreciate the different between somatic cell therapy and germ-line therapy.

- Somatic cell therapy means genetically altering specific body (or somatic) cells, such as bone marrow cells, pancreas cells, or whatever, in order to treat the disease. This therapy may treat or cure the disease, but any genetic changes will not be passed on their offspring.
- Germ-line therapy means genetically altering those cells (sperm cells, sperm precursor cell, ova, ova precursor cells, zygotes or early embryos) that will pass their genes down the "germ-line" to future generations. Alterations to any of these cells will affect every cell in the resulting human, and in all his or her descendants.

Germ-line therapy would be highly effective, but is also potentially dangerous (since the long-term effects of genetic alterations are not known), unethical (since it could easily lead to eugenics) and immoral (since it could involve altering and destroying human embryos). It is currently illegal in the UK and most other countries, and current research is focussing on somatic cell therapy only. All gene therapy trials in the UK must be approved by the Gene Therapy Advisory Committee (GTAC), a government body that reviews the medical and ethical grounds for a trial. Germ-line modification is allowed with animals, and indeed is the basis for producing GMOs.



AQA(B) AS Module 3:

Physiology And Transport

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AQA(B) AS Module 3: Physiology And Transport Specification

Mass Transport

Over large distances in organisms, efficient supply of materials is provided by mass transport (the bulk movement of substances through transport systems). The transport systems of larger organisms are intimately linked with specialised exchange systems, whose main function is to maintain concentration gradients.

Human Circulatory System

- The structure and function of the heart, including the atria and ventricles, atrioventricular and semilunar valves.
- The cardiac cycle related to the maintenance of blood flow through the heart. Candidates should be able to relate pressure and volume changes in the heart and aorta to events in the cardiac cycle. The role of the sinoatrial node, the atrioventricular node and the bundle of His in the maintenance of the heartbeat.
- The structure of arteries, arterioles, veins and capillaries related to their functions.
- The main substances transported by the blood system, and the sites at which exchange occurs. The relationship between blood, tissue fluid, lymph and plasma. The role of the lymph system in the return of tissue fluid to the blood system.
- The loading, transport and unloading of oxygen in relation to the oxygen haemoglobin dissociation curve, and the effects of pH and carbon dioxide concentration.

Energy and Exercise

- Glucose, glycogen and triglycerides as sources of energy for muscle contraction. ATP as the immediate energy source.
- Comparison of aerobic and anaerobic respiration as sources of ATP for muscle contraction, in terms of amounts of energy produced and products. (Biochemical details of pathways are not required.)
- Muscle fatigue in terms of increase in blood lactate and decrease in blood pH. The fate of lactate.
- The role of the medulla, pressure receptors and chemoreceptors in the walls of the aorta and carotid sinuses in the response of the heart to increased muscular activity.
- The role of the medulla in the brain and of the stretch receptors in the lungs in the maintenance of breathing. The role of the medulla in the brain and of the receptors in the lungs, aortic bodies and carotid bodies in the response of the breathing system to increased muscular activity.

Water Transport in Plants

- Structure of a primary root, to include root hairs, endodermis, xylem and phloem. The distribution of these tissues and their adaptations for function.
- Uptake of water and ions from the soil. Pathway of transport of water from root hairs to stomata, including apoplast and symplast pathways in the root.
- The roles of root pressure and cohesion–tension in moving water through the xylem.
- Transpiration, and the effects of light, temperature, humidity and air movement.
- Structural adaptations that reduce the rate of transpiration in xerophytic plants, related to survival in dry conditions.

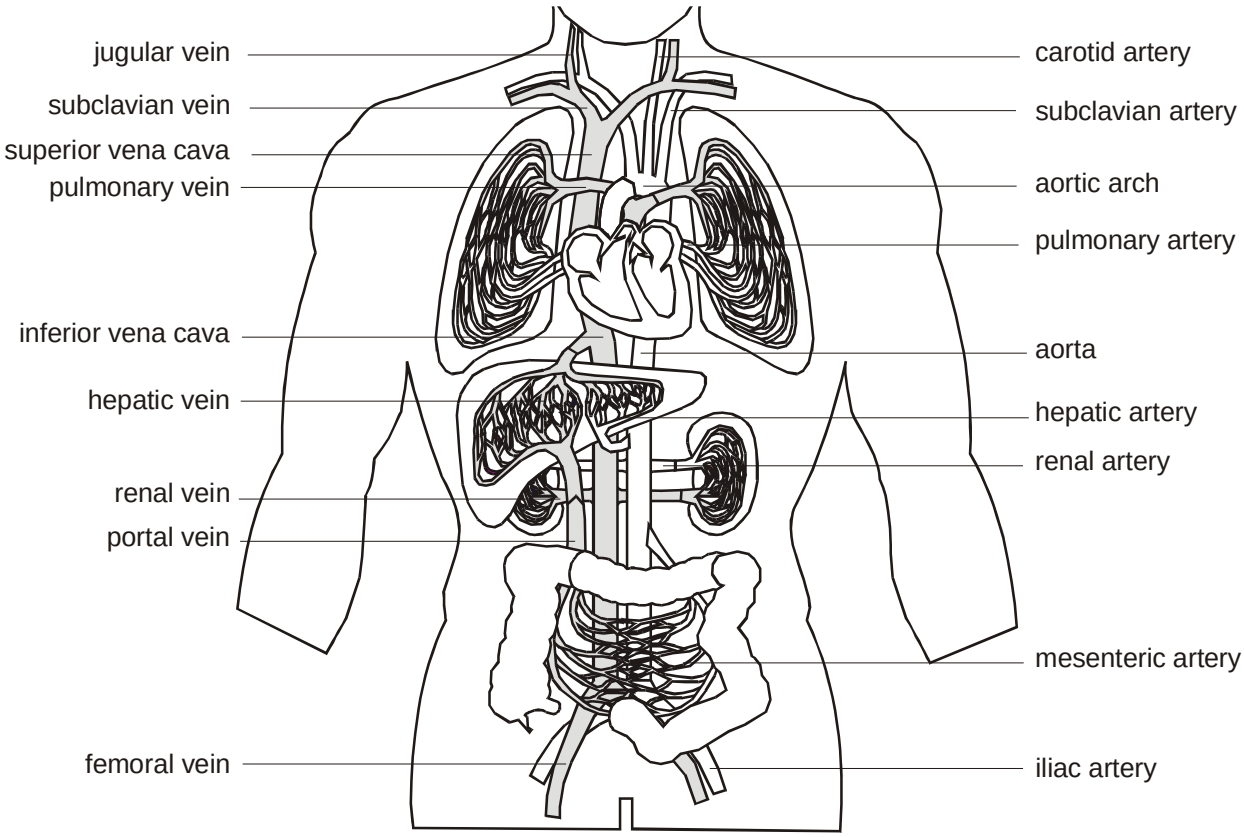
Solute Translocation in Plants

- Phloem as the tissue that transports organic substances.
- The mass flow hypothesis for the mechanism of translocation in plants. Evaluate the evidence for and against the mass flow hypothesis.
- The use of radioactive tracers and ringing experiments to determine the movement of ions and organic substances through plants. Candidates should be able to interpret evidence from tracer and ringing experiments.

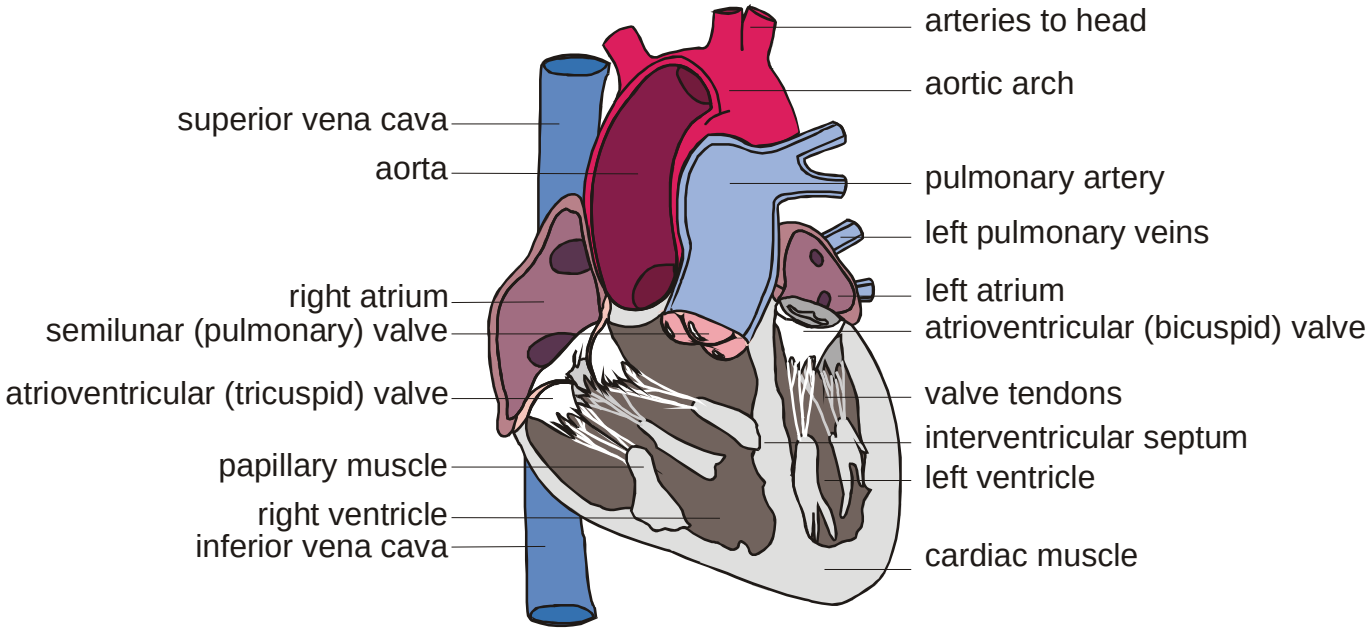
Human Circulatory System

Small organisms don't have a bloodstream, but instead rely on the simple diffusion of materials for transport around their cells. This is OK for single cells, but it would take days for molecules to diffuse through a large animal, so most animals have a circulatory system with a pump to transport materials quickly around their bodies. This is an example of a mass flow system, which means the transport of substances in the flow of a fluid (as opposed to diffusion, which is the random motion of molecules in a stationary fluid). The transport of materials in the xylem and phloem of plants is an other example of mass flow. Mass flow systems work together with the specialised exchange systems (such as lungs, gills and leaves), which we saw in module 1.

Humans have a double circulatory system with a 4-chambered heart. In humans the right side of the heart pumps blood to the lungs only and is called the pulmonary circulation, while the left side of the heart pumps blood to the rest of the body – the systemic circulation. The circulation of blood round the body was discovered by William Harvey in 1628. Until then people assumed that blood ebbed and flowed through the same tubes, because they hadn't seen capillaries.



The Heart



The human heart has four chambers: two thin-walled atria on top, which receive blood, and two thick-walled ventricles underneath, which pump blood. Veins carry blood into the atria and arteries carry blood away from the ventricles. Between the atria and the ventricles are atrioventricular valves, which prevent back-flow of blood from the ventricles to the atria. The left valve has two flaps and is called the bicuspid (or mitral) valve, while the right valve has 3 flaps and is called the tricuspid valve. The valves are held in place by valve tendons ("heart strings") attached to papillary muscles, which contract at the same time as the ventricles, holding the vales closed. There are also two semi-lunar valves in the arteries (the only examples of valves in arteries) called the pulmonary and aortic valves.

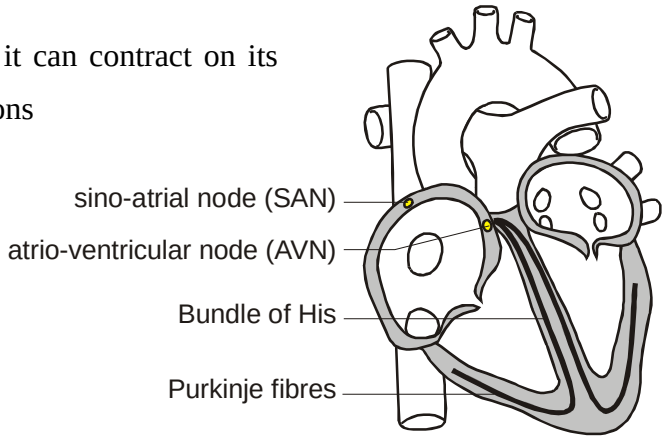
The left and right halves of the heart are separated by the inter-ventricular septum. The walls of the right ventricle are 3 times thinner than on the left and it produces less force and pressure in the blood. This is partly because the blood has less far to go (the lungs are right next to the heart), but also because a lower pressure in the pulmonary circulation means that less fluid passes from the capillaries to the alveoli.

The heart is made of cardiac muscle, composed of cells called myocytes. When myocytes receive an electrical impulse they contract together, causing a heartbeat. Since myocytes are constantly active, they have a great requirement for oxygen, so are fed by numerous capillaries from two coronary arteries. These arise from the aorta as it leaves the heart. Blood returns via the coronary sinus, which drains directly into the right atrium.

The Cardiac Cycle

When the cardiac muscle contracts the volume in the chamber decrease, so the pressure in the chamber increases, so the blood is forced out. Cardiac muscle contracts about 75 times per minute, pumping around 75 cm^3 of blood from each ventricle each beat (the stroke volume). It does this continuously for up to 100 years. There is a complicated sequence of events at each heartbeat called the cardiac cycle.

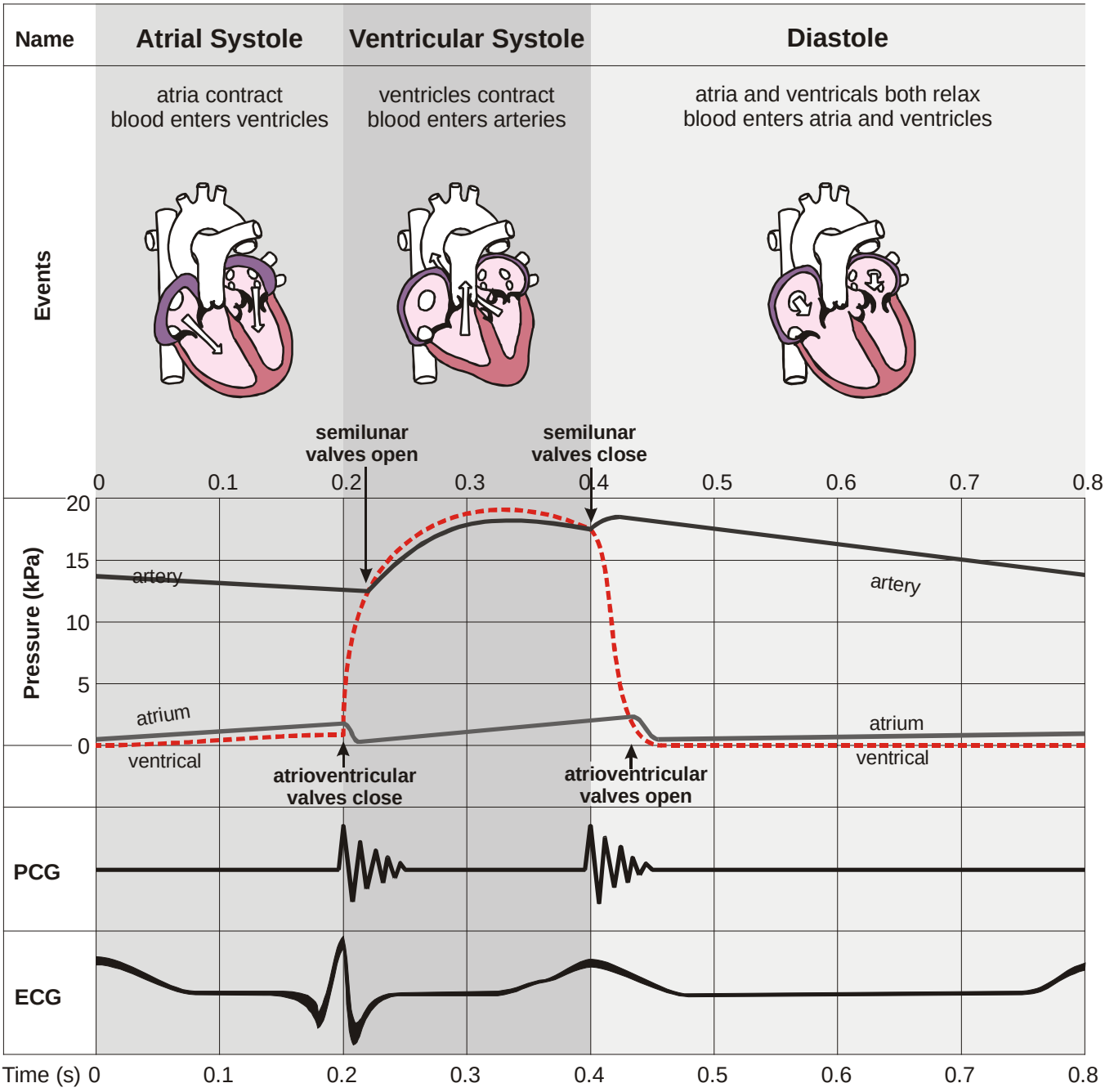
Cardiac muscle is myogenic, which means that it can contract on its own, without needing nerve impulses. Contractions are initiated within the heart by the sino-atrial node (SAN, or pacemaker) in the right atrium. This extraordinary tissue acts as a clock, and contracts spontaneously and rhythmically about once a second, even when surgically removed from the heart.



The cardiac cycle has three stages:

1. **Atrial Systole** (pronounced sis-toe-lay). The SAN contracts and transmits electrical impulses throughout the atria, which both contract, pumping blood into the ventricles. The ventricles are electrically insulated from the atria, so they do not contract at this time.
2. **Ventricular Systole**. The electrical impulse passes to the ventricles via the atrioventricular node (AVN), the bundle of His and the Purkinje fibres. These are specialised fibres that do not contract but pass the electrical impulse to the base of the ventricles, with a short but important delay of about 0.1s. The ventricles therefore contract shortly after the atria, from the bottom up, squeezing blood upwards into the arteries. The blood can't go into the atria because of the atrioventricular valves, which are forced shut with a loud "lub".
3. **Diastole.** The atria and the ventricles relax, while the atria fill with blood. The semilunar valves in the arteries close as the arterial blood pushes against them, making a "dup" sound.

The events of the three stages are shown in the diagram on the next page. The pressure changes show most clearly what is happening in each chamber. Blood flows because of pressure differences, and it always flows from a high pressure to a low pressure, if it can. So during atrial systole the atria contract, making the atrium pressure higher than the ventricle pressure, so blood flows from the atrium to the ventricle. The artery pressure is higher still, but blood can’t flow from the artery back into the heart due to the semi-lunar valves. The valves are largely passive: they open when blood flows through them the right way and close when blood tries to flow through them the wrong way.

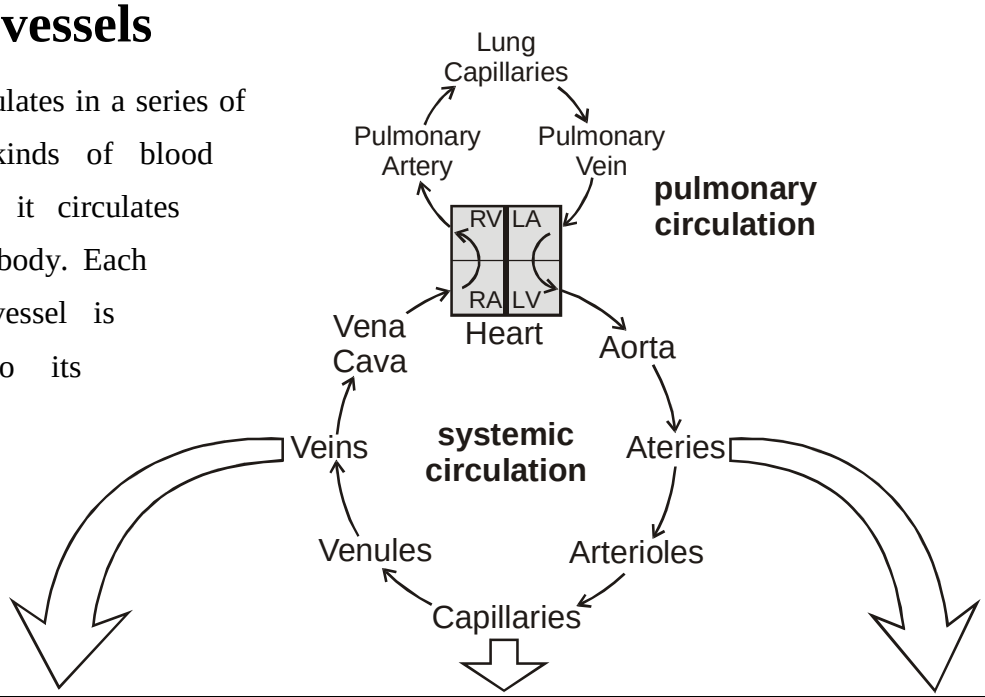


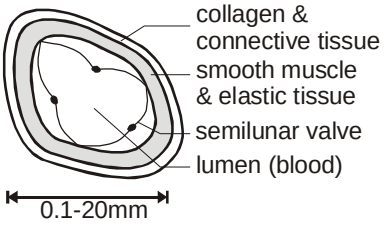
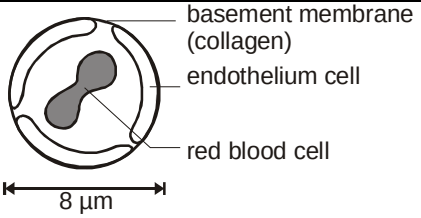
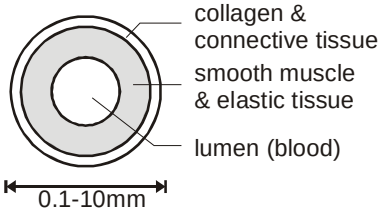
The PCG (or phonocardiogram) is a recording of the sounds the heart makes. The cardiac muscle itself is silent and the sounds are made by the valves closing. The first sound (lub) is the atrioventricular valves closing and the second (dub) is the semi-lunar valves closing.

The ECG (or electrocardiogram) is a recording of the electrical activity of the heart. There are characteristic waves of electrical activity marking each phase of the cardiac cycle. Changes in these ECG waves can be used to help diagnose problems with the heart.

Blood vessels

Blood circulates in a series of different kinds of blood vessels as it circulates round the body. Each kind of vessel is adapted to its function.

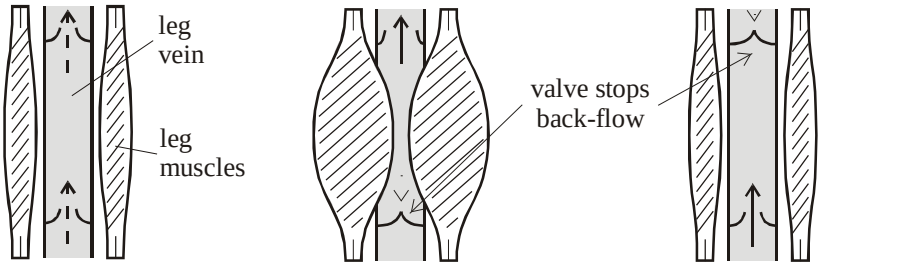


Veins and Venules	Capillaries	Arteries and Arterioles
		
Function is to carry blood from tissues to the heart	Function is to allow exchange of materials between the blood and the tissues	Function is to carry blood from the heart to the tissues
Thin walls, mainly collagen, since blood at low pressure	Very thin, permeable walls, only one cell thick to allow exchange of materials	Thick walls with smooth elastic layers to resist high pressure and muscle layer to aid pumping
Large lumen to reduce resistance to flow.	Very small lumen. Blood cells must distort to pass through.	Small lumen
Many valves to prevent back-flow	No valves	No valves (except in heart)
Blood at low pressure	Blood pressure falls in capillaries.	Blood at high pressure
Blood usually deoxygenated (except in pulmonary vein)	Blood changes from oxygenated to deoxygenated (except in lungs)	Blood usually oxygenated (except in pulmonary artery)

Arteries carry blood from the heart to every tissue in the body. They have thick, elastic walls to withstand the high pressure of blood from the heart. The arteries close to the heart are particularly elastic and expand during systole and recoil again during diastole, helping to even out the pulsating blood flow. The smaller arteries and arterioles are more muscular and can contract (vasoconstriction) to close off the capillary beds to which they lead; or relax (vasodilation) to open up the capillary bed. These changes are happening constantly under the involuntary control of the

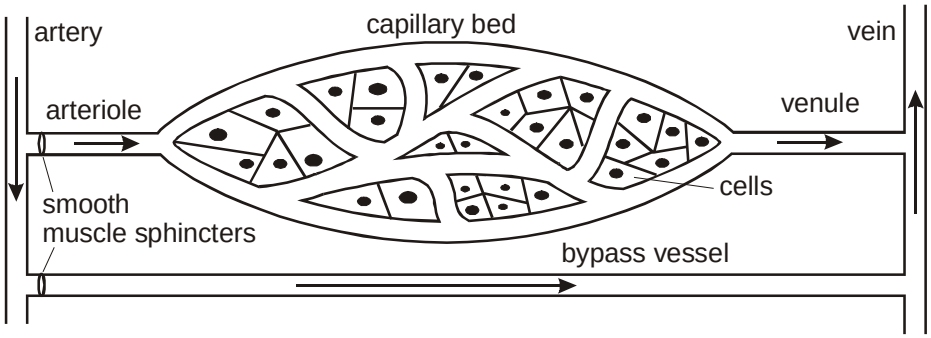
medulla in the brain, and are most obvious in the capillary beds of the skin, causing the skin to change colour from pink (skin arterioles dilated) to blue (skin arterioles constricted). There is not enough blood to fill all the body's capillaries, and at any given time up to 20% of the capillary beds are closed off.

Veins carry blood from every tissue in the body to the heart. The blood has lost almost all its pressure in the capillaries, so it is at low pressure inside veins and moving slowly. Veins therefore don't need thick walls and they have a larger lumen that arteries, to reduce the resistance to flow. They also have semi-lunar valves to stop the blood flowing backwards. It is particularly difficult for blood to flow upwards through the legs to heart, and the flow is helped by contractions of the leg and abdominal muscles:



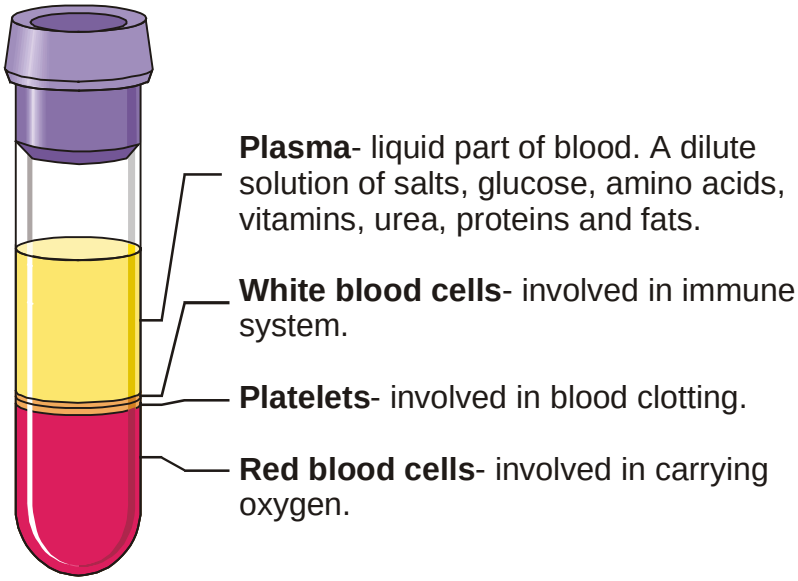
The body relies on constant contraction of these muscles to get the blood back to the heart, and this explains why soldiers standing still on parade for long periods can faint, and why sitting still on a long flight can cause swelling of the ankles and Deep Vein Thrombosis (DVT or "economy class syndrome"), where small blood clots collect in the legs.

Capillaries are where the transported substances actually enter and leave the blood. No exchange of materials takes place in the arteries and veins, whose walls are too thick and impermeable. Capillaries are very narrow and thin-walled, but there are a vast number of them (10^8 m in one adult!), so they have a huge surface area : volume ratio, helping rapid diffusion of substances between blood and cells. Capillaries are arranged in networks called capillary beds feeding a group of cells, and no cell in the body is more than 2 cells away from a capillary.



Blood

Blood is composed of 4 components, as shown in this diagram:

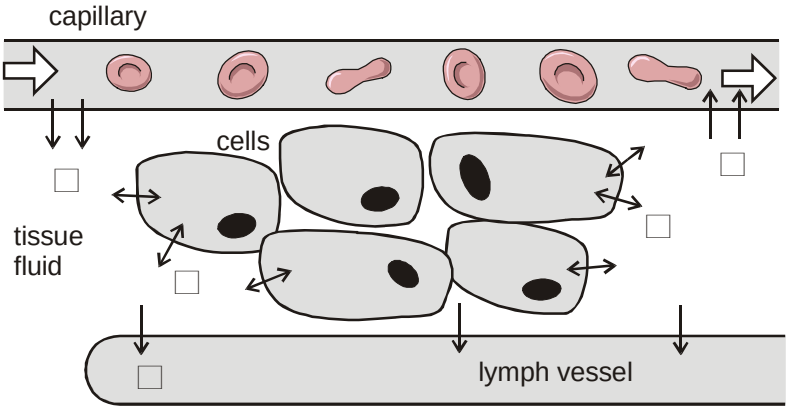


There are dozens of different substances in blood, all being transported from one part of the body to another. Some of the main ones are listed in this table:

Substance	Where	Reason
Oxygen	Red blood cells	Transported from lungs to all cells for respiration
Carbon dioxide	Plasma	Transported from all cells to lungs for excretion
Nutrients (e.g. glucose, amino acids, vitamins, lipids, nucleotides)	Plasma	Transported from small intestine to liver and from liver to all cells
Waste products (e.g. urea, lactic acid)	Plasma	Transported from cells to liver and from liver to kidneys for excretion
Ions (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, HPO_3^{2-}, SO_4^{2-})	Plasma	Transported from small intestine to cells, and help buffer the blood pH.
Hormones	Plasma	Transported from glands to target organs
Proteins (eg albumins)	Plasma	Amino acid reserve
Blood clotting factors	Plasma	At least 13 different substances (mainly proteins) required to make blood clot.
Antigens and antibodies	Plasma	Part of immune system
Water	Plasma	Transported from large intestine and cells to kidneys for excretion.
Bacteria and viruses	plasma	
Heat	Plasma	Transported from muscles to skin for heat exchange.

Tissue Fluid

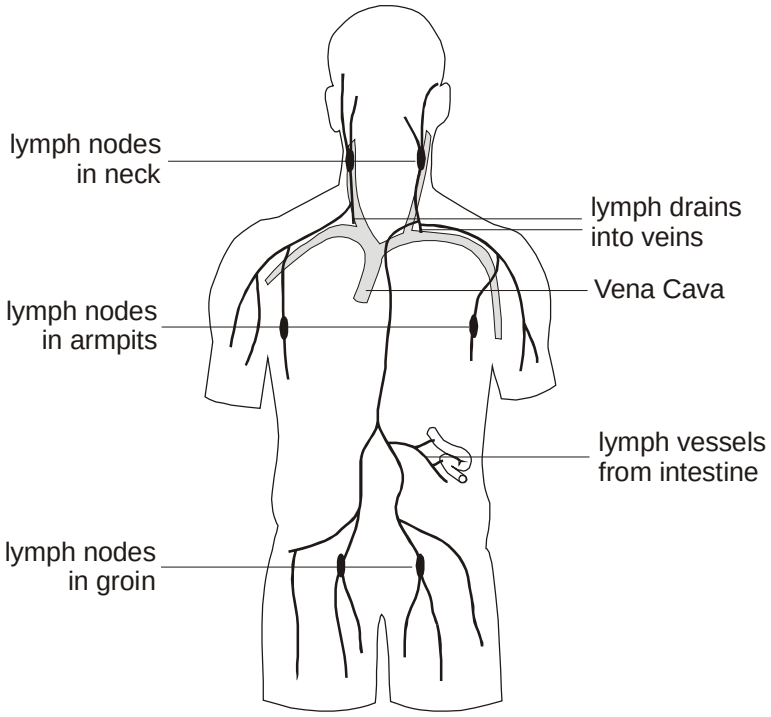
These substances are all exchanged between the blood and the cells in capillary beds. Substances do not actually move directly between the blood and the cell: they first diffuse into the tissue fluid that surrounds all cells, and then diffuse from there to the cells.



1. At the arterial end of the capillary bed the blood is still at high hydrostatic pressure, so blood plasma is squeezed out through the permeable walls of the capillary. Cells and proteins are too big to leave the capillary, so they remain in the blood.
2. This fluid now forms tissue fluid surrounding the cells. Materials are exchanged between the tissue fluid and the cells by all four methods of transport across a cell membrane. Gases and lipid-soluble substances (such as steroids) cross by lipid diffusion; water crosses by osmosis, ions cross by facilitated diffusion; and glucose and amino acids cross by active transport.
3. At the venous end of the capillary bed the blood is at low pressure, since it has lost so much plasma. Water returns to the blood by osmosis since the blood has a low water potential. Solutes (such as carbon dioxide, urea, salts, etc) enter the blood by diffusion, down their concentration gradients.
4. Not all the plasma that left the blood returns to it, so there is excess tissue fluid. This excess drains into lymph vessels, which are found in all capillary beds. Lymph vessels have very thin walls, like capillaries, and tissue fluid can easily diffuse inside, forming lymph.

The Lymphatic System

The lymphatic system consists of a network of lymph vessels flowing alongside the veins. The vessels lead towards the heart, where the lymph drains back into the blood system at the superior vena cava. There is no pump, but there are numerous semi-lunar valves, and lymph is helped along by contraction of muscles, just as in veins. Lymph vessels also absorb fats from the small intestine, where they form lacteals inside each villus. There are networks of lymph vessels at various places in the body (such as tonsils and armpits) called lymph nodes where white blood cells develop. These become swollen if more white blood cells are required to fight an infection.



Remember the difference between these four solutions:

Plasma The liquid part of blood. It contains dissolved glucose, amino acids, salts and vitamins; and suspended proteins and fats.

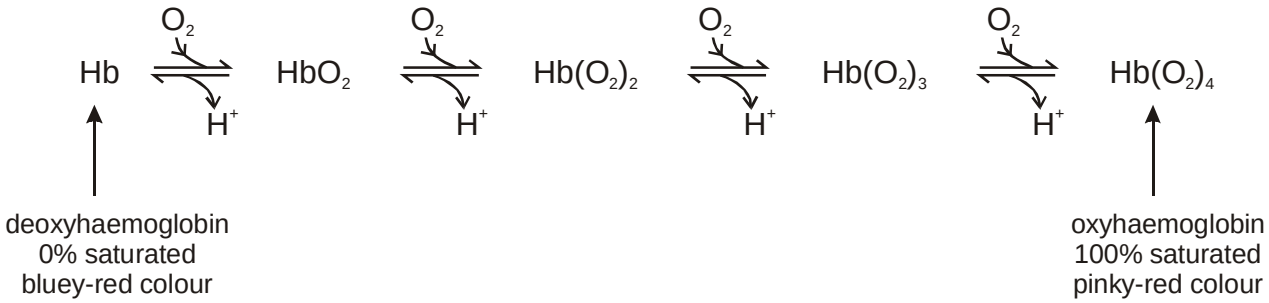
Serum Purified blood plasma used in hospitals for blood transfusions.

Tissue Fluid The solution surrounding cells. Its composition is similar to plasma, but without proteins (which stay in the blood capillaries).

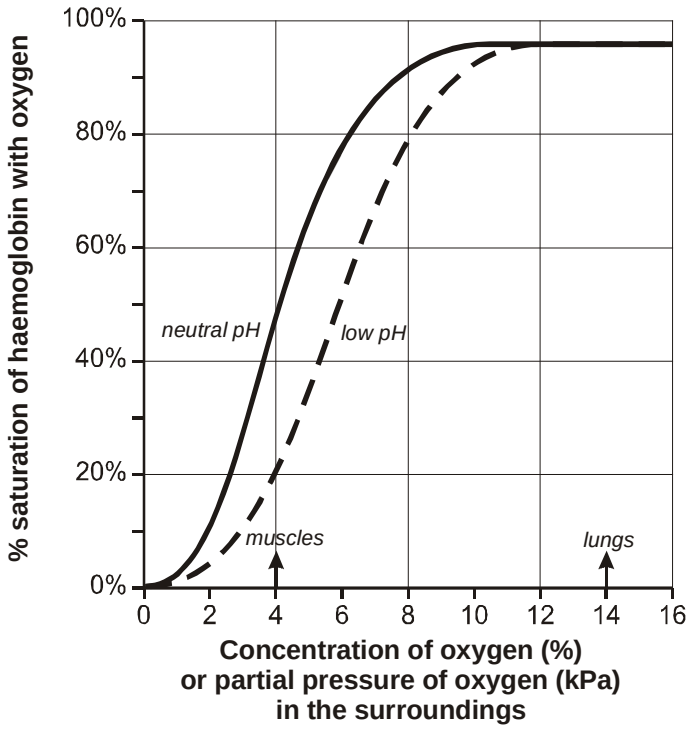
Lymph The solution inside lymph vessels. Its composition is similar to tissue fluid, but with more fats (from the digestive system).

Transport of Oxygen

Oxygen is carried in red blood cells bound to the protein haemoglobin. A red blood cell contains about 300 million haemoglobin molecules and there are 5 million red blood cells per cm^3 of blood. The result of this is that blood can carry up to 20% oxygen, whereas pure water can only carry 1%. The haemoglobin molecule consists of four polypeptide chains, with a haem prosthetic group at the centre of each chain. Each haem group contains one iron atom, and one oxygen molecule binds to each iron atom. So one haemoglobin molecule can bind up to four oxygen molecules. This means there are 4 binding steps as shown in this chemical equation:



A sample of blood can therefore be in any state from completely deoxygenated (0% saturated) to fully oxygenated (100% saturated). Since deoxyhaemoglobin and oxyhaemoglobin are different colours, it is easy to measure the % saturation of a sample of blood in a colorimeter. As the chemical equation shows, oxygen drives the reaction to the right, so the more oxygen there is in the surroundings, the more saturated the haemoglobin will be. This relation is shown in the oxygen dissociation curve:



The concentration of oxygen in the surroundings can be measured as a % (there's about 20% oxygen in air), but it's more correct to measure it as a partial pressure (PO_2, measured in kPa). Luckily, since the pressure of one atmosphere is about 100 kPa, the actual values for PO_2 and % O_2 are the same (e.g. 12% O_2 has a PO_2 of 12 kPa). The graph is read by starting with an oxygen concentration in the environment surrounding the blood capillaries on the horizontal axis, then reading off the state of the haemoglobin in the blood that results from the vertical axis.

This curve has an S (or sigmoid) shape, and shows several features that help in the transport of oxygen in the blood:

- In the alveoli of the lungs oxygen is constantly being brought in by ventilation, so its concentration is kept high, at around 14 kPa. As blood passes through the capillaries surrounding the alveoli the haemoglobin binds oxygen to become almost 100% saturated. Even if the alveolar oxygen concentration falls a little the haemoglobin stays saturated because the curve is flat here.
- In tissues, like muscle, liver or brain, oxygen is used by respiration, so is low, typically about 4 kPa. At this PO_2 the haemoglobin is only 50% saturated, so it unloads about half its oxygen (i.e. from about 100% saturated to about 50% saturated) to the cells, which use it for respiration.
- In tissues that are respiring quickly, such as contracting muscle cells, the PO_2 drops even lower, to about 2 kPa, so the haemoglobin saturation drops to about 10%, so almost 90% of the oxygen is unloaded, providing more oxygen for the muscle cells.
- Actively-respiring tissues also produce a lot of CO_2, which dissolves in tissue fluid to make carbonic acid and so lowers the pH. The chemical equation above shows that hydrogen ions drive the reaction to the left, so low pH reduces the % saturation of haemoglobin at any PO_2. This is shown on the graph by the dotted line, which is lower than the normal dissociation curve. This downward shift is called the Bohr effect, after the Danish scientist who first discovered it. So at a PO_2 of 2%, the actual saturation is nearer 5%, so almost all the oxygen loaded in the lungs is unloaded in respiring tissues.

It is important to remember that oxygen can only diffuse in and out of the blood from capillaries, which are permeable. Blood in arteries and veins is "sealed in", so no oxygen can enter or leave the blood whatever the external conditions. So as haemoglobin travels from the lungs to a capillary bed in a body tissue and back to the lungs, it "switches" from one position on the dissociation curve to another position, without experiencing the intermediate stages of the curve.

Transport of Carbon Dioxide

Carbon dioxide is carried between respiring tissues and the lungs by 3 different methods:

1. **As dissolved gas in blood plasma (2%)**

   Very little travels this way as CO_2 is not very soluble in water (about 0.02%)

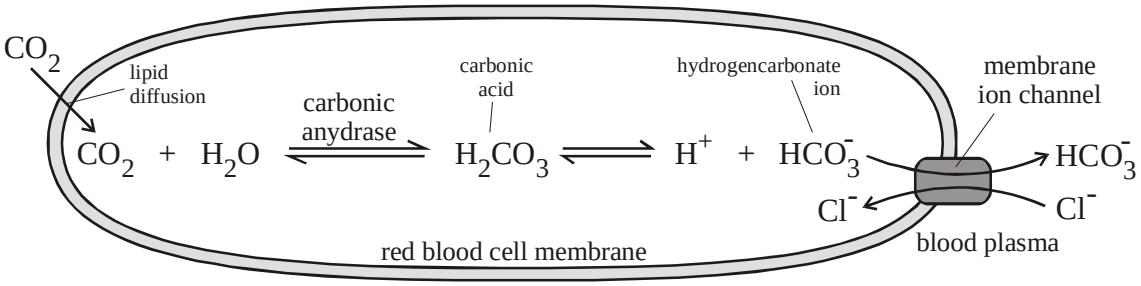
2. **As Carbamino Haemoglobin (13%)**

   Carbon dioxide can bind to amino groups in haemoglobin molecules, forming carbamate ions:

   $$Hb—NH_2 + CO_2 \equiv Hb—NHCOO^- + H^+$$

   Since there are so many haemoglobin molecules in re blood cells, and each one has many amino groups, quite a lot of CO_2 can be carried this way.

3. **As Hydrogen Carbonate ions (85%)**



   Carbon dioxide reacts with water to form carbonic acid, which immediately dissociates to form a hydrogen carbonate (or bicarbonate) ion and a proton. This protons binds to haemoglobin, as in the cause of the Bohr effect. Hydrogen carbonate is very soluble, so most CO_2 is carried this way. The reaction in water is very slow, but red blood cells contain the enzyme carbonic anhydrase, which catalyses the reaction with water by a factor of 10^8 times.

   In respiring tissues CO_2 produced by respiration diffuses into the red blood cells and forms hydrogen carbonate, which diffuses out of the cell into the blood plasma through an ion channel in the red blood cell membrane. This channel carries one chloride ion into the cell for every hydrogen carbonate ion it carries out, and this helps to keep the charge in the cell constant. In the lungs the reverse happens: hydrogen carbonate diffuses back into the red blood cell through the channel (and chloride goes out) and CO_2 is formed by carbonic anhydrase (remember enzymes will catalyse reactions in either direction), which diffuses into the plasma and into the alveoli.

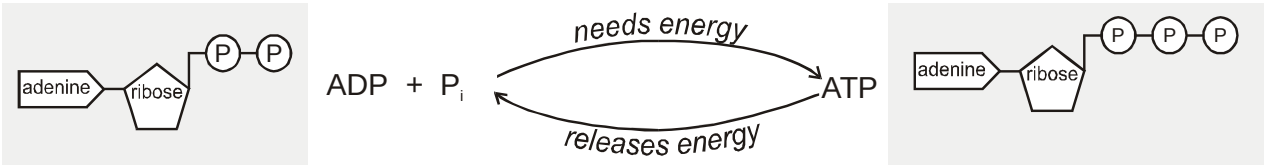
In all three cases the direction of the reactions is governed by the CO_2 concentration. So in the tissues, where CO_2 is high, the reactions go to the right, while in the lungs, where CO_2 is low, the reactions go to the left.

Energy and Respiration

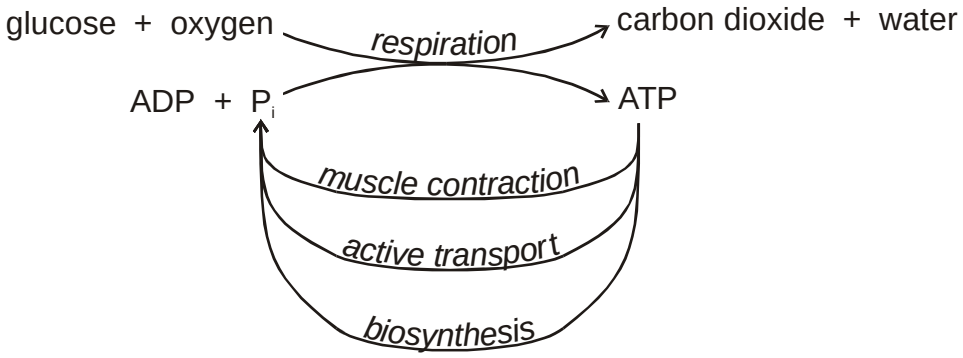
All living cells require energy, and this energy is provided by respiration.

glucose + oxygen ✶ carbon dioxide + water (+ energy)

What form is this energy in? It's in the form of chemical energy stored in a compound called ATP (adenosine triphosphate). So all respiration really does is convert chemical energy stored in glucose into chemical energy stored in ATP. ATP is a nucleotide, one of the four found in DNA (see module 2 p4), but it also has this other function as an energy storage molecule. ATP is built up from ADP and phosphate (PO_4^{3-} , abbreviated to P_i):

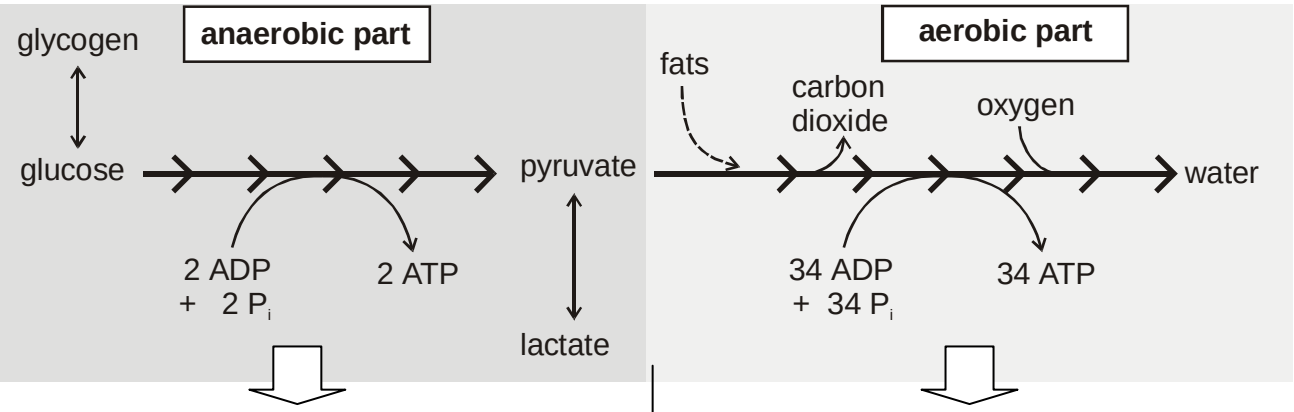


All the processes in a cell that require energy (such as muscle contraction, active transport and biosynthesis) use ATP to provide that energy. So these processes all involve ATPase enzymes, which catalyse the breakdown of ATP to ADP + P_i, and make use of the energy released. So the ATP molecules in a cell are constantly being cycled between ATP and ADP + P_i:



Aerobic and Anaerobic Respiration

Respiration is not a single reaction, but consists of about 30 individual reaction steps. For now we can usefully break respiration into just two parts: anaerobic and aerobic.



The first part of respiration is simply the breakdown of glucose to a compound called pyruvate. This doesn't require oxygen, so is described as anaerobic respiration (without air). It is also called glycolysis and it takes place in the cytoplasm of cells. It only produces 2 molecules of ATP per molecule of glucose.

Normally pyruvate goes straight on to the aerobic part, but if there is no oxygen it is converted to lactate (or lactic acid) instead. Lactate stores a lot of energy, but it isn't wasted: when oxygen is available it is converted back to pyruvate, which is then used in the aerobic part of respiration.

The second part of respiration is the complete oxidation of pyruvate to carbon dioxide and water. Oxygen is needed for this, so it is described as aerobic respiration (with air). It takes place in the mitochondria of cells and produces far more ATP: 34 molecules of ATP per molecule of glucose.

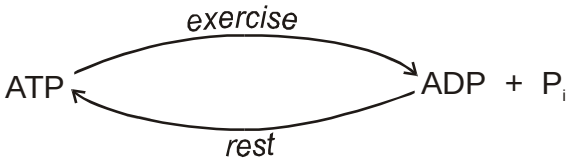
Fats (mainly triglycerides) can also be used in aerobic respiration (but not anaerobic) to produce ATP.

Energy for Exercise

More energy is used for muscle contraction in animals than for any other process. The proteins in muscle use ATP to provide the energy for contraction, but the exact way in which the ATP is made varies depending on the length of the contraction. There are five sources of ATP:

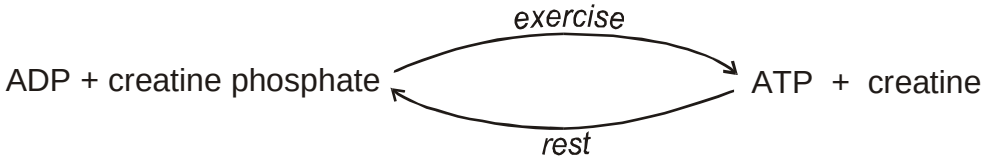
1. **ATP stored in muscles**

   A muscle cell stores only enough ATP for a few seconds of contraction. This ATP was made by respiration while the muscle was relaxed, and is available for immediate use.



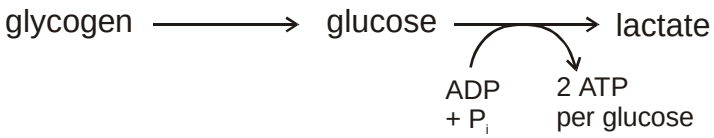
2. **ATP from creatine phosphate**

   Creatine phosphate is a short-term energy store in muscle cells, and there is about ten times more creatine phosphate than ATP. It is made from ATP while the muscle is relaxed and can very quickly be used to make ATP when the muscle is contracting. This allows about 30 seconds of muscle contraction, enough for short bursts of intense activity such as a 100 metre sprint.



3. **ATP from anaerobic respiration of glucose**

   Anaerobic respiration doesn't provide much ATP (2 ATP molecules for each glucose molecule), but it is quick, since it doesn't require oxygen to be provided by the blood. It is used for muscle activities lasting a few minutes. There is not much glucose as such in muscle cells, but there is plenty of glycogen, which can be broken down quickly to make quite large amounts of glucose.



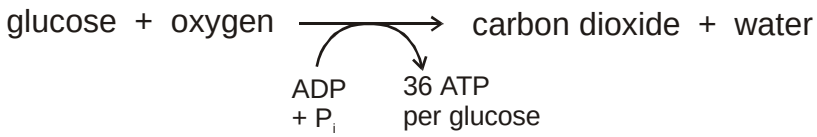
   The end product of anaerobic respiration is lactate, which gradually diffuses out of muscle cells into the blood and is carried to the liver. Here it is converted back to pyruvate.

   Some muscles are specially adapted for anaerobic respiration and can therefore only sustain short bursts of activity. These are the white (or fast twitch) muscles (such as birds' breast muscle and

frogs legs) and they are white because they contain few mitochondria and little myoglobin. Mitochondria are not needed for anaerobic respiration.

4. ATP from aerobic respiration of glucose

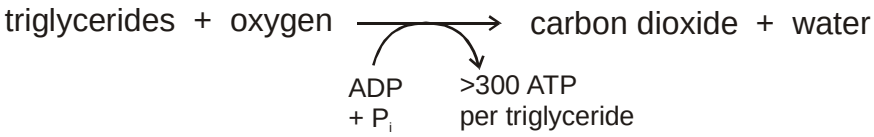
For longer periods of exercise muscle cells need oxygen supplied by the blood for aerobic respiration. This provides far more energy (36 molecules of ATP from each molecule of glucose), but the rate at which it can be produced is limited by how quickly oxygen can be provided. This is why you can't run a marathon at the same speed as a sprint.



Muscles that are specially adapted for aerobic respiration are called red (or slow twitch) muscles (such as heart, leg and back muscles). They are red because they contain many mitochondria (which are red) and a lot of the red protein myoglobin, which is similar to haemoglobin, but is used as an oxygen store in these muscles. Myoglobin helps to provide the oxygen needed for aerobic respiration.

5. ATP from aerobic respiration of fats

The biggest energy store in the body is in the form of triglycerides, which store more energy per gram than glucose or glycogen. They are first broken down to fatty acids and glycerol, and then fully oxidised to carbon dioxide and water by aerobic respiration. Since fats are insoluble it takes time to "mobilise" them (i.e. dissolve and digest them), so fats are mainly used for extended periods of exercise, and for the countless small contractions that are constantly needed to maintain muscle tone and body posture.



Muscle Fatigue

Most muscles can't keep contracting for ever, but need to have a rest. This is called muscle fatigue. It starts after 30s to 5 mins of continuous contraction (depending on muscle type) and can be quite painful. It is caused by the build-up of two chemicals inside muscle cells.

- Phosphate from ATP splitting. This tends to drive the muscle ATPase reaction backwards and so reduces muscle force.
- Lactate from anaerobic respiration. This lowers the pH and so slows the enzymes involved in muscle contraction.

Exercise and Heart Rate

The rate at which the heart beats and the volume of blood pumped at each beat (the stroke volume) can both be controlled. The product of these two is called the cardiac output – the amount of blood flowing in a given time:

heart rate x stroke volume = cardiac output

Controlled via sino-atrial node.

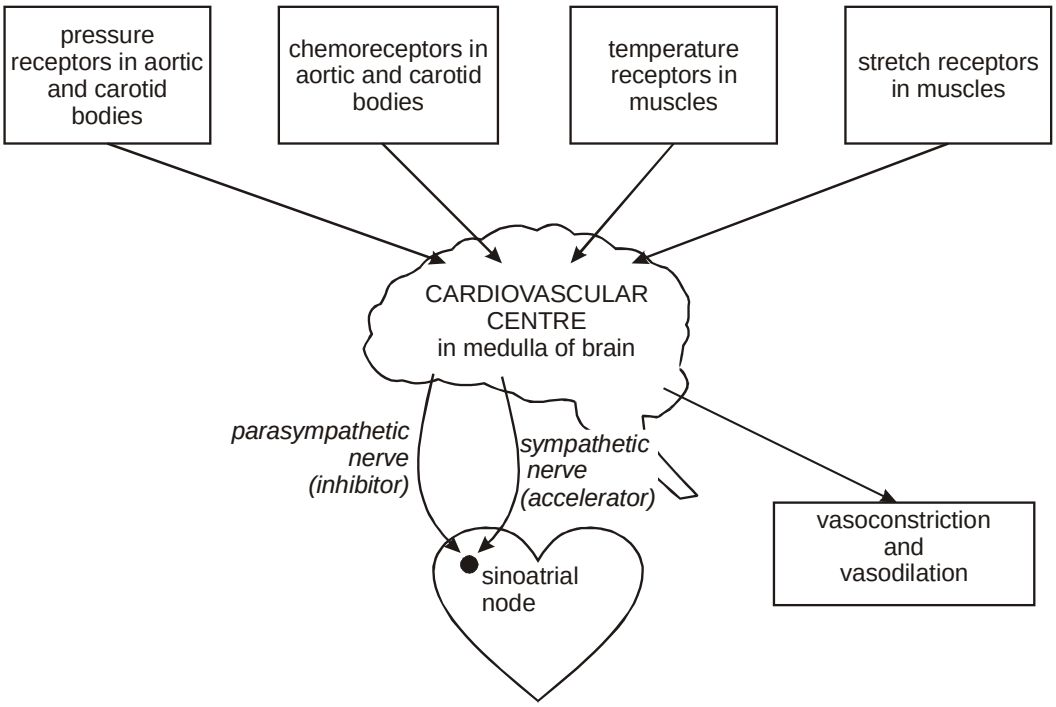
Controlled via blood pressure. If pressure is high, more blood fills the heart at diastole, so stroke volume increases.

	heart rate (beats / min)	stroke volume (cm^3 / beat)	cardiac output (cm^3 / min)
at rest	75	75	5 600
at exercise	180	120	22 000

As the table shows, the cardiac output can increase dramatically when the body exercises. There are several benefits from this:

- to get oxygen to the muscles faster
- to get glucose to the muscles faster
- to get carbon dioxide away from the muscles faster
- to get lactate away from the muscles faster
- to get heat away from the muscles faster

But what makes the heart beat faster? It is clearly an involuntary process (you don't have to think about it), and like many involuntary processes (such as breathing, coughing and sneezing) it is controlled by a region of the brain called the medulla. The medulla and its nerves are part of the autonomic nervous system (i.e. involuntary). The part of the medulla that controls the heart is called the cardiovascular centre. It receives inputs from various receptors around the body and sends output through two nerves to the sino-atrial node in the heart.



How does the cardiovascular centre control the heart?

The cardiovascular centre can control both the heart rate and the stroke volume. Since the heart is myogenic, it does not need nerve impulses to initiate each contraction. But the nerves from the cardiovascular centre can change the heart rate. There are two separate nerves from the cardiovascular centre to the sino-atrial node: the sympathetic nerve to slow the heart rate down and the parasympathetic nerve to speed it up.

The cardiovascular centre can also change the stroke volume by controlling blood pressure. It can increase the stroke volume by sending nerve impulses to the arterioles to cause vasoconstriction, which increases blood pressure so more blood fills the heart at diastole. Alternatively it can decrease the stroke volume by causing vasodilation and reducing the blood pressure.

How does the cardiovascular centre respond to exercise?

When the muscles are active they respire more quickly and cause several changes to the blood, such as decreased oxygen concentration, increased carbon dioxide concentration, decreased pH (since the carbon dioxide dissolves to form carbonic acid, see p xx) and increased temperature. All of these changes are detected by various receptor cells around the body, but the pH changes are the most sensitive and therefore the most important. The main chemoreceptors (receptor cells that can detect chemical changes) are found in:

- The walls of the aorta (the aortic body), monitoring the blood as it leaves the heart
- The walls of the carotid arteries (the carotid bodies), monitoring the blood to the head and brain
- The medulla, monitoring the tissue fluid in the brain

The chemoreceptors send nerve impulses to the cardiovascular centre indicating that more respiration is taking place, and the cardiovascular centre responds by increasing the heart rate.

exercise → more cellular respiration in mucles → more CO_2 in blood → low blood pH → detected by chemoreceptors in aortic and carotid bodies → impulses to cardiovascular centre → impulses to sino-atrial node → increased heart rate

A similar job is performed by temperature receptors and stretch receptors in the muscles, which also detect increased muscle activity.

Exercise and Breathing

Both the rate and depth (volume) of breathing can be varied. The product of these two is called the ventilation rate – the volume air ventilating the lungs each minute:

breathing rate x tidal volume = ventilation rate

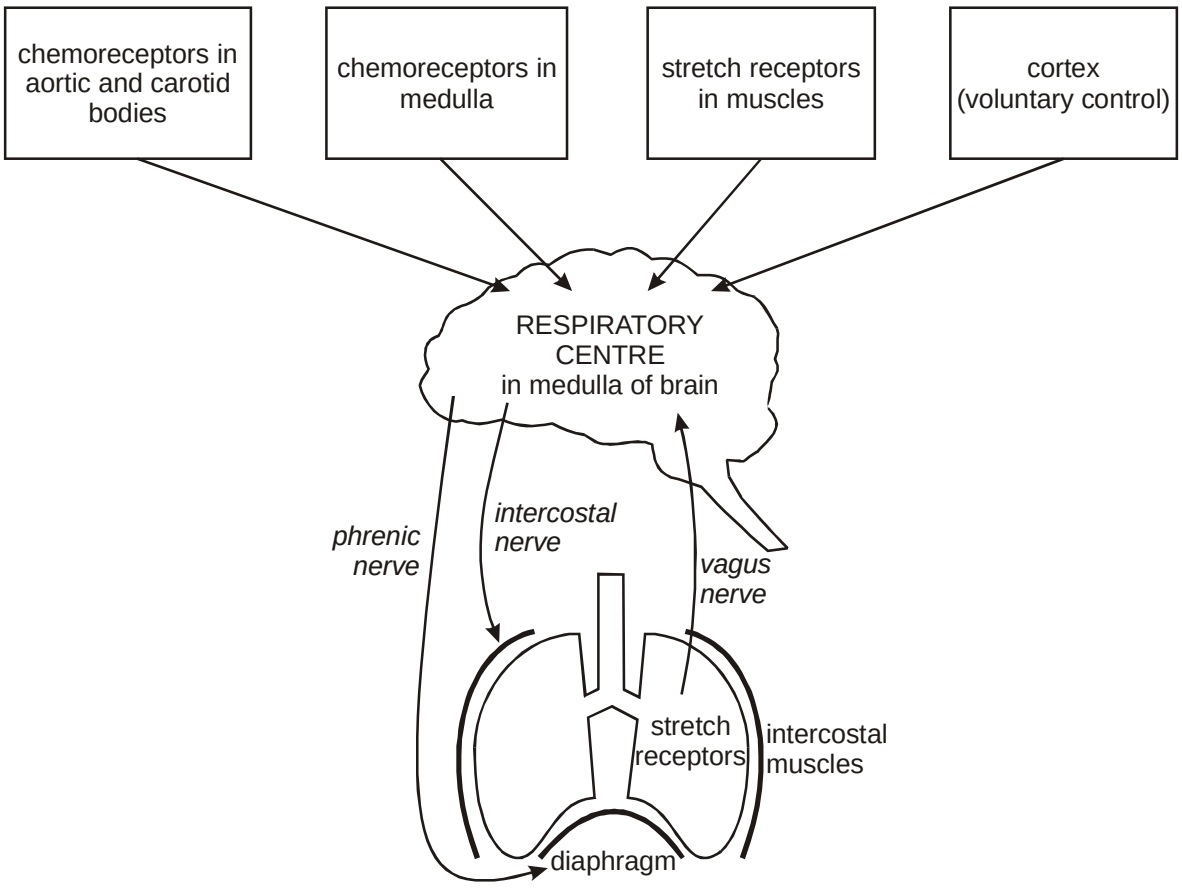
Both controlled via the nerves from the respiratory centre.

	breathing rate (breaths/min)	tidal volume (cm^3 / breath)	ventilation rate (cm^3 / min)
at rest	12	500	6 000
at exercise	18	1000	18 000

When the body exercises the ventilation rate and depth increases so that

- Oxygen can diffuse from the air to the blood faster
- Carbon dioxide can diffuse from the blood to the air faster

Again, this is an involuntary process and is controlled by a region of the medulla called the respiratory centre, which plays a similar role to the cardiovascular centre. The respiratory centre receives inputs from various receptors around the body and sends output through two nerves to the muscles around the lungs.



How does the respiratory centre control ventilation?

Unlike the heart, the muscles that cause breathing cannot contract on their own, but need nerve impulses from the brain for each breath. The respiratory centre transmits regular nerve impulses to the diaphragm and intercostal muscles to cause inhalation. Stretch receptors in the alveoli and bronchioles detect inhalation and send inhibitory signals to the respiratory centre to cause exhalation. This negative feedback system in continuous and prevents damage to the lungs.

How does respiratory centre respond to exercise?

The process is the same as for heart rate, with the chemoreceptors in the aortic and carotid bodies detecting an increase in respiration.

exercise → more cellular respiration in mucles → more CO_2 in blood → low blood pH → detected by chemoreceptors in aortic and carotid bodies → impulses to respiratory centre → faster impulses to intercostal muscles and diaphragm → increased ventilation rate

Again, the stretch receptors in the muscles give a more rapid indication of muscular activity, allowing an anticipatory increase in breathing rate even before the carbon dioxide concentration the blood has changed.

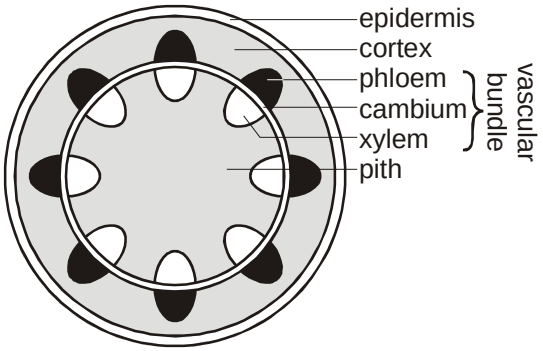
One difference between ventilation and heartbeat is that ventilation is also under voluntary control from the cortex, the voluntary part of the brain. This allows you to hold your breath or blow out candles, but it can be overruled by the autonomic system in the event of danger. For example if you hold your breath for a long time, the carbon dioxide concentration in the blood increases so much that the respiratory centre forces you to gasp and take a breath. Pearl divers hyperventilate before diving to lower the carbon dioxide concentration in their blood, so that it takes longer to build up.

During sleep there is so little cellular respiration taking place that it is possible to stop breathing for a while, but the respiratory centre starts it up again as the carbon dioxide concentration increases. It is possible that one cause of cot deaths may be an underdeveloped respiratory centre in young babies, which allows breathing to slow down or stop for too long.

Transport Systems in Plants

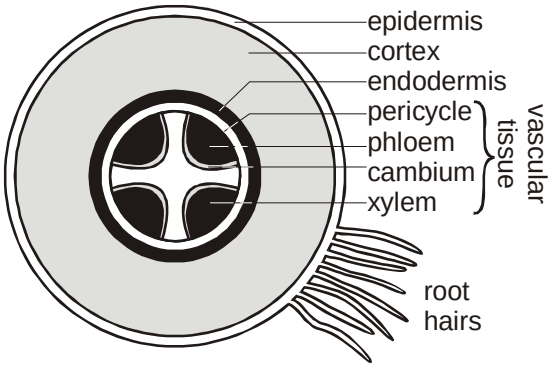
Plants don't have a circulatory system like animals, but they do have a sophisticated transport system for carrying water and dissolved solutes to different parts of the plant, often over large distances.

Stem Structure

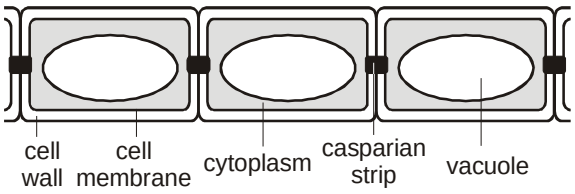


- Epidermis. One cell thick. In young plants the epidermis cells may secrete a waterproof cuticle, and in older plants the epidermis may be absent, replaced by bark.
- Cortex. Composed of various "packing" cells, to give young plants strength and flexibility, and are the source of plant fibres such as sisal and hemp.
- Vascular Tissue. This contains the phloem and xylem tissue, which grow out from the cambium. In dicot plants (the broad-leafed plants), the vascular tissue is arranged in vascular bundles, with phloem on the outside and xylem on the inside. In older plants the xylem bundles fuse together to form the bulk of the stem.
- Pith. The central region of a stem, used for food storage in young plants. It may be absent in older plants (i.e. they're hollow).

Root Structure



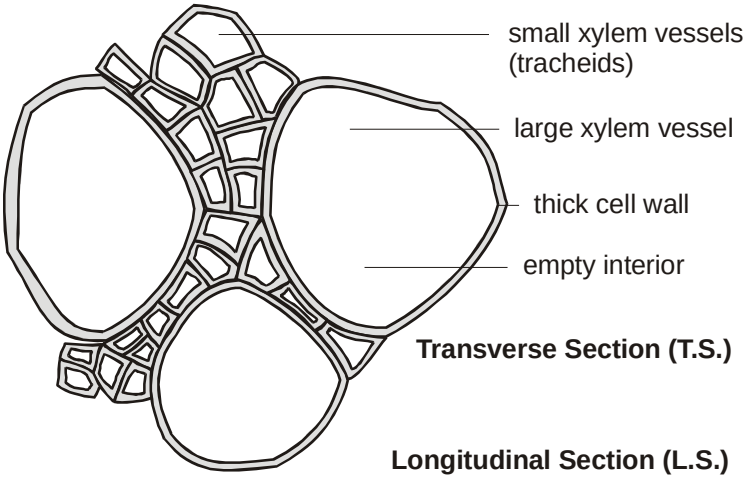
- Epidermis. A single layer of cells often with long extensions called root hairs, which increase the surface area enormously. A single plant may have 10^{10} root hairs.
- Cortex. A thick layer of packing cells often containing stored starch.
- Endodermis. A single layer of tightly-packed cells containing a waterproof layer called the casparian strip. This prevents the movement of water between the cells.

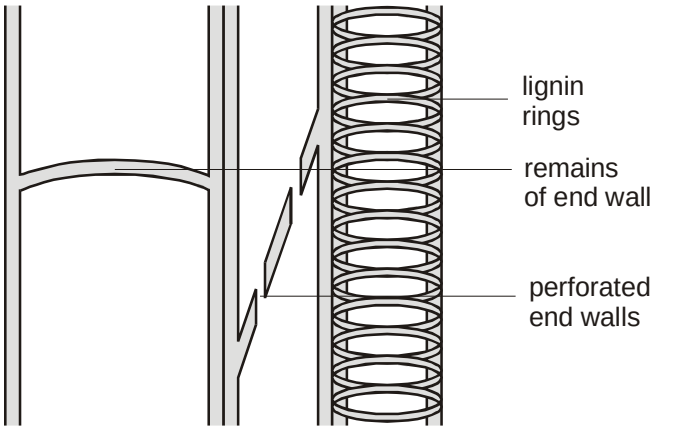


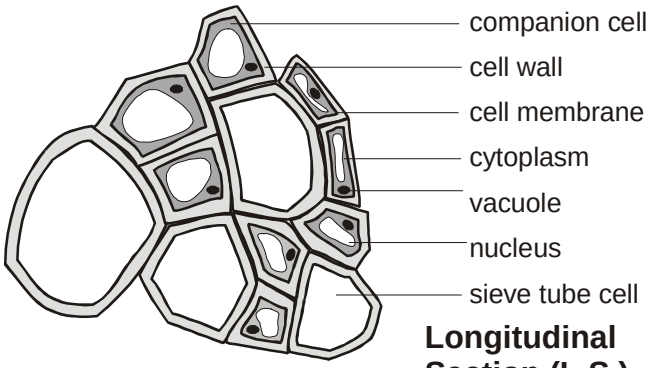
- Pericycle. A layer of undifferentiated meristematic (growing) cells.
- Vascular Tissue. This contains xylem and phloem cells, which are continuous with the stem vascular bundles. The arrangement is different, and the xylem usually forms a star shape with 2-6 arms.

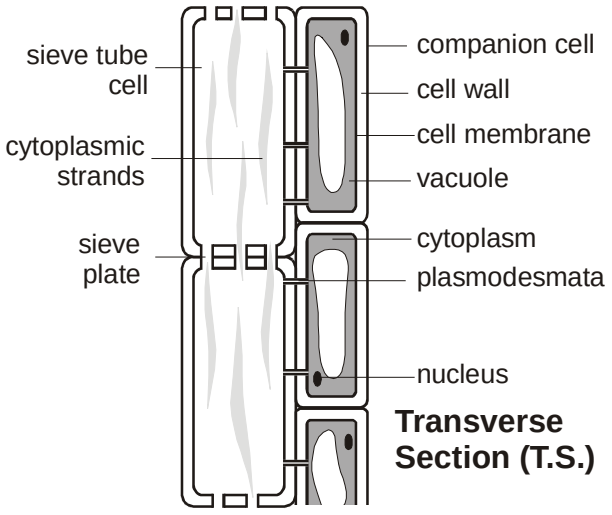
Xylem Tissue

Xylem tissue is composed of dead cells joined together to form long empty tubes. Different kinds of cells form wide and narrow tubes, and the end cells walls are either full of holes, or are absent completely. Before death the cells form thick cell walls containing lignin, which is often laid down in rings or helices, giving these cells a very characteristic appearance under the microscope. Lignin makes the xylem vessels very strong, so that they don't collapse under pressure, and they also make woody stems strong.









Phloem Tissue

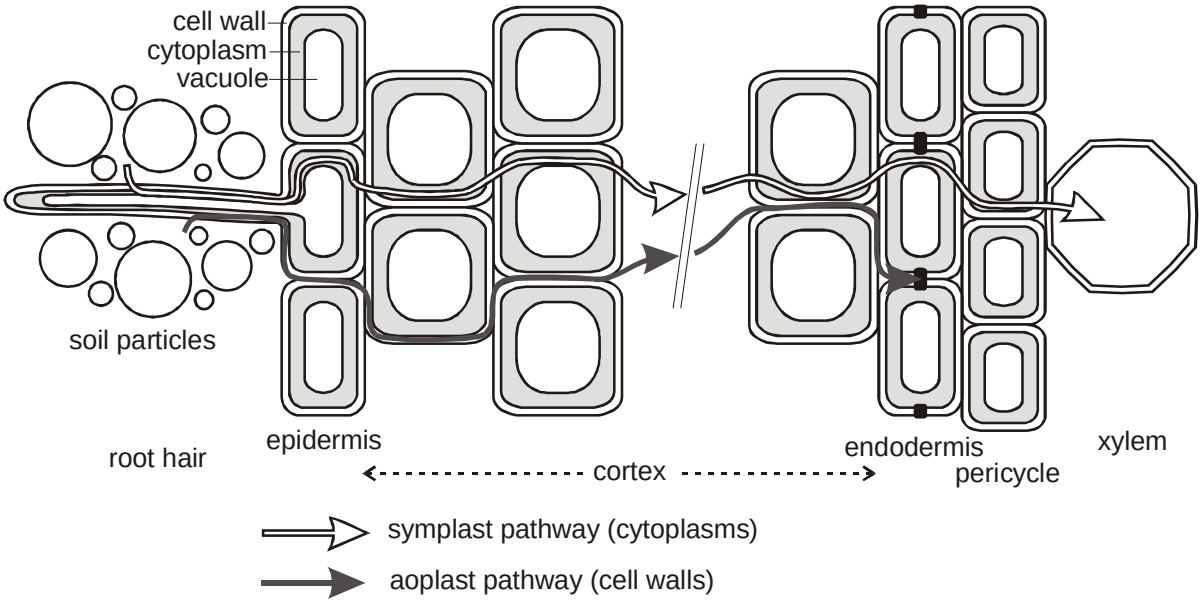
Phloem tissue is composed of sieve tube cells, which form long columns with holes in their end walls called sieve plates. These cells are alive, but they lose their nuclei and other organelles, and their cytoplasm is reduced to strands around the edge of the cells. These cytoplasmic strands pass through the holes in the sieve plates, so forming continuous filaments. The centre of these tubes is empty. Each sieve tube cell is associated with one or more companion cells, normal cells with nuclei and organelles. These companion cells are connected to the sieve tube cells by plasmodesmata, and provide them with proteins, ATP and other nutrients.

Water Transport in Plants

Vast amounts of water pass through plants. A large tree can use water at a rate of 1 dm^3 min^{-1}. Only 1% of this water is used by the plant cells for photosynthesis and turgor, and the remaining 99% evaporates from the leaves and is lost to the atmosphere. This evaporation from leaves is called transpiration.

The movement of water through a plant can be split into three sections: through the roots, stem and leaves:

1. **Movement through the Roots**



Water moves through the root by two paths:

- The Symplast pathway consist of the living cytoplasms of the cells in the root (10%). Water is absorbed into the root hair cells by osmosis, since the cells have a lower water potential that the water in the soil. Water then diffuses from the epidermis through the root to the xylem down a water potential gradient. The cytoplasms of all the cells in the root are connected by plasmodesmata through holes in the cell walls, so there are no further membranes to cross until the water reaches the xylem, and so no further osmosis.
- The Apoplast pathway consists of the cell walls between cells (90%). The cell walls are quite thick and very open, so water can easily diffuse through cell walls without having to cross any cell membranes by osmosis. However the apoplast pathway stops at the endodermis because of the waterproof casparian strip, which seals the cell walls. At this point water has to cross the cell membrane by osmosis and enter the symplast. This allows the plant to have some control over the uptake of water into the xylem.

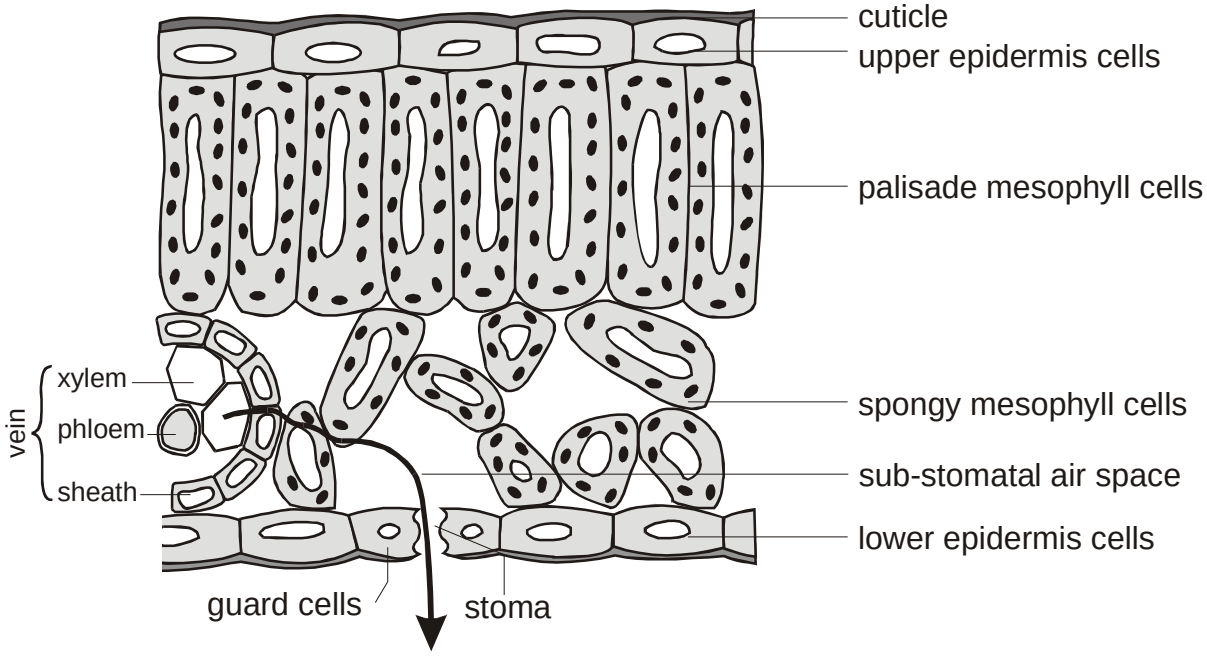
The uptake of water by osmosis actually produces a force that pushes water up the xylem. This force is called root pressure, which can be measured by placing a manometer over a cut stem, and is of the order of 100 kPa (about 1 atmosphere). This helps to push the water a few centimetres up short and young stems, but is nowhere near enough pressure to force water up a long stem or a tree. Root pressure is the cause of guttation, sometimes seen on wet mornings, when drops of water are forced out of the ends of leaves.

2. Movement through the Stem

The xylem vessels form continuous pipes from the roots to the leaves. Water can move up through these pipes at a rate of 8m h^{-1}, and can reach a height of over 100m. Since the xylem vessels are dead, open tubes, no osmosis can occur within them. The driving force for the movement is transpiration in the leaves. This causes low pressure in the leaves, so water is sucked up the stem to replace the lost water. The column of water in the xylem vessels is therefore under tension (a stretching force). Fortunately water has a high tensile strength due to the tendency of water molecules to stick together by hydrogen bonding (cohesion), so the water column does not break under the tension force. This mechanism of pulling water up a stem is sometimes called the cohesion-tension mechanism.

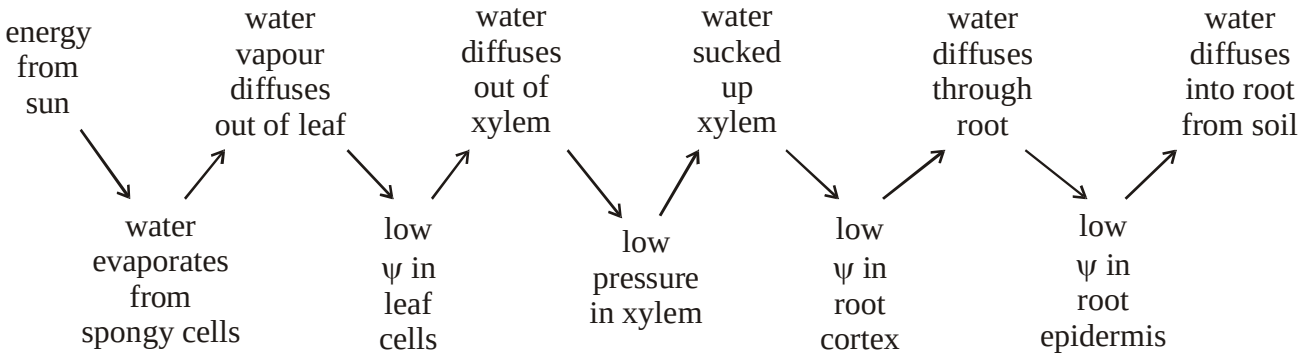
The very strong lignin walls of the xylem vessels stops them collapsing under the suction pressure, but in fact the xylem vessels (and even whole stems and trunks) do shrink slightly during the day when transpiration is maximum.

3. Movement through the Leaves



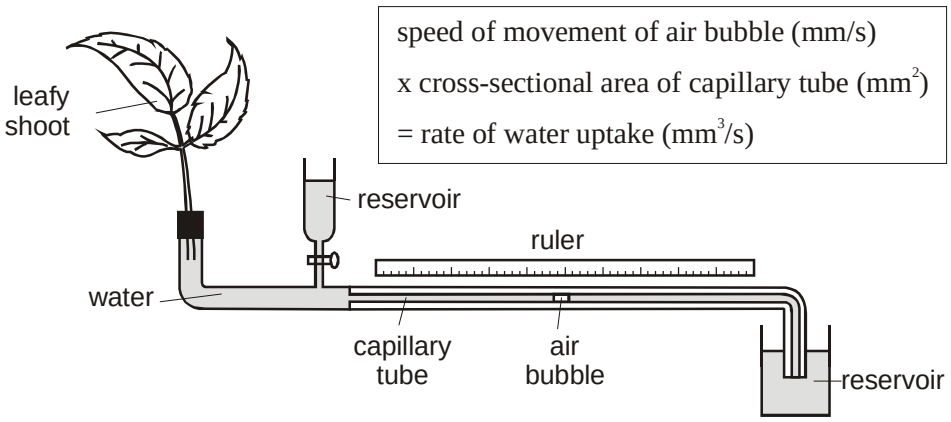
The xylem vessels ramify in the leaves to form a branching system of fine vessels called leaf veins. Water diffuses from the xylem vessels in the veins through the adjacent cells down its water potential gradient. As in the roots, it uses the symplast pathway through the living cytoplasm and the apoplast pathway through the non-living cell walls. Water evaporates from the spongy cells into the sub-stomatal air space, and diffuses out through the stomata.

Evaporation is endothermic and is driven by solar energy, which is therefore the ultimate source of energy for all the water movements in plants:



Factors affecting Transpiration

The rate of transpiration can be measured in the lab using a potometer ("drinking meter"):



A potometer actually measures the rate of water uptake by the cut stem, not the rate of transpiration; and these two are not always the same. During the day plants often transpire more water than they take up (i.e. they lose water and may wilt), and during the night plants may take up more water than they transpire (i.e. they store water and become turgid). The difference can be important for a large tree, but for a small shoot in a potometer the difference is usually trivial and can be ignored.

The potometer can be used to investigate how various environmental factors affect the rate of transpiration.

- Light. Light stimulates the stomata to open allowing gas exchange for photosynthesis, and as a side effect this also increases transpiration. This is a problem for some plants as they may lose water during the day and wilt.
- Temperature. High temperature increases the rate of evaporation of water from the spongy cells, and reduces air humidity, so transpiration increases.
- Humidity. High humidity means a higher water potential in the air, so a lower water potential gradient between the leaf and the air, so less evaporation.
- Air movements. Wind blows away saturated air from around stomata, replacing it with drier air, so increasing the water potential gradient and increasing transpiration.

Many plants are able to control their stomata, and if they are losing too much water and their cells are wilting, they can close their stomata, reducing transpiration and water loss. So long periods of light, heat, or dry air could result in a decrease in transpiration when the stomata close.

Adaptations to dry habitats

Plants in different habitats are adapted to cope with different problems of water availability.

Mesophytes plants adapted to a habitat with adequate water

Xerophytes plants adapted to a dry habitat

Halophytes plants adapted to a salty habitat

Hydrophytes plants adapted to a freshwater habitat

Some adaptations of xerophytes are:

Adaptation	How it works	Example
thick cuticle	stops uncontrolled evaporation through leaf cells	most dicots
small leaf surface area	less area for evaporation	conifer needles, cactus spines
low stomata density	fewer gaps in leaves	
stomata on lower surface of leaf only	more humid air on lower surface, so less evaporation	most dicots
shedding leaves in dry/cold season	reduce water loss at certain times of year	deciduous plants
sunken stomata	maintains humid air around stomata	marram grass, pine
stomatal hairs	maintains humid air around stomata	marram grass, couch grass
folded leaves	maintains humid air around stomata	marram grass,
succulent leaves and stem	stores water	cacti
extensive roots	maximise water uptake	cacti

Mineral Ion transport in Plants

Ions are absorbed from the soil by both passive and active transport. Specific ion pumps in the membranes of root hair cells pump ions from the soil into the cytoplasms of the epidermis cells. Two lines of evidence indicate that active transport is being used:

- The concentrations of ions inside root cells are up to 100 time greater than in the soil, so they are being transported up their concentration gradient.
- If respiratory inhibitors such as cyanide are applied to living roots, ion uptake is greatly reduced, since there is no ATP being made to drive the membrane pumps. Any remaining uptake must be passive.

The active uptake of ions is partly responsible for the water potential gradient in roots, and therefore for the uptake of water by osmosis.

Ions diffuse down their concentration gradient from the epidermis to the xylem. They travel up the xylem by mass flow as the water is pulled up the stem (in other words they are simply carried up in the flow of the xylem solution). In the leaves they are selectively absorbed into the surrounding cells by membrane pumps.

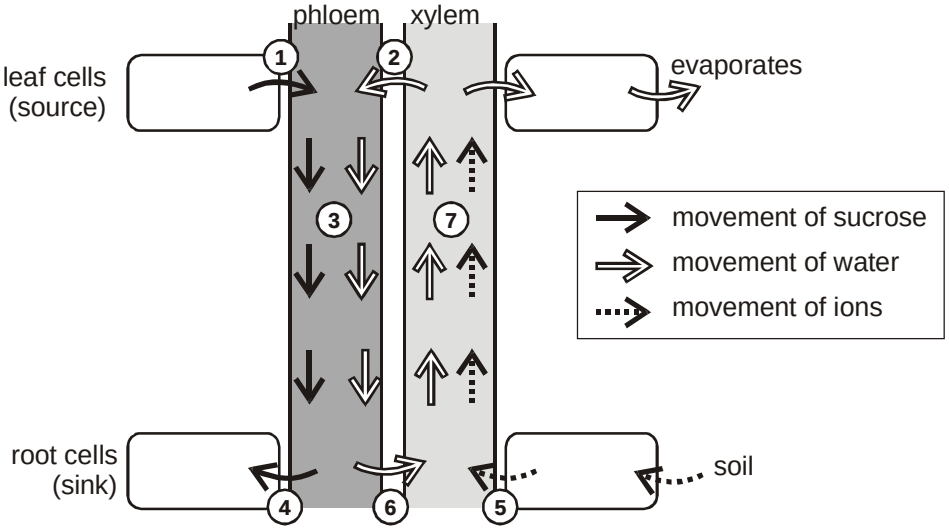
Solute Transport in Plants

The phloem contains a very concentrated solution of dissolved solutes, mainly sucrose, but also other sugars, amino acids, and other metabolites. This solution is called the sap, and the transport of solutes in the phloem is called translocation.

Unlike the water in the xylem, the contents of the phloem can move both up or down a plant stem, often simultaneously. It helps to identify where the sugar is being transported from (the source), and where to (the sink).

- During the summer sugar is mostly transported from the leaves, where it is made by photosynthesis (the source) to the roots, where it is stored (the sink).
- During the spring, sugar is often transported from the underground root store (the source) to the growing leaf buds (the sink).
- Flowers and young buds are not photosynthetic, so sugars can also be transported from leaves or roots (the source) to flowers or buds (sinks).

Surprisingly, the exact mechanism of sugar transport in the phloem is not known, but it is certainly far too fast to be simple diffusion. The main mechanism is thought to be the mass flow of fluid up the xylem and down the phloem, carrying dissolved solutes with it. Plants don't have hearts, so the mass flow is driven by a combination of active transport (energy from ATP) and evaporation (energy from the sun). This is called the mass flow theory, and it works like this:



1. Sucrose produced by photosynthesis is actively pumped into the phloem vessels by the companion cells.
2. This decreases the water potential in the leaf phloem, so water diffuses from the neighbouring xylem vessels by osmosis.

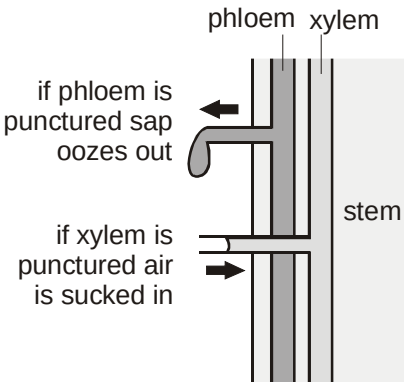
3. This is increases the hydrostatic pressure in the phloem, so water and dissolved solutes are forced downwards to relieve the pressure. This is mass flow: the flow of water together with its dissolved solutes due to a force.
4. In the roots the solutes are removed from the phloem by active transport into the cells of the root.
5. At the same time, ions are being pumped into the xylem from the soil by active transport, reducing the water potential in the xylem.
6. The xylem now has a lower water potential than the phloem, so water diffuses by osmosis from the phloem to the xylem.
7. Water and its dissolved ions are pulled up the xylem by tension from the leaves. This is also mass flow.

This mass-flow certainly occurs, and it explains the fast speed of solute translocation. However there must be additional processes, since mass flow does not explain how different solutes can move at different speeds or even in different directions in the phloem. One significant process is cytoplasmic streaming: the active transport of molecules and small organelles around cells on the cytoskeleton.

Translocation Experiments

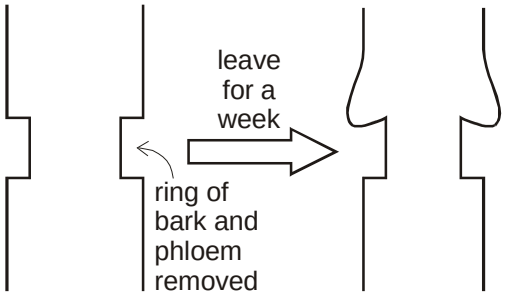
1. Puncture Experiments

If the phloem is punctured with a hollow tube then the sap oozes out, showing that there is high pressure (compression) inside the phloem (this is how maple syrup is tapped). If the xylem is punctured then air is sucked in, showing that there is low pressure (tension) inside the xylem. This illustrates the main difference between transport in xylem and phloem: Water is pulled up in the xylem, sap is pushed down in the phloem.



2. Ringing Experiments

Since the phloem vessels are outside the xylem vessels, they can be selectively removed by cutting a ring in a stem just deep enough to cut the phloem but not the xylem. After a week there is a swelling above the ring, reduced growth below the ring and the leaves are unaffected. This was early evidence that

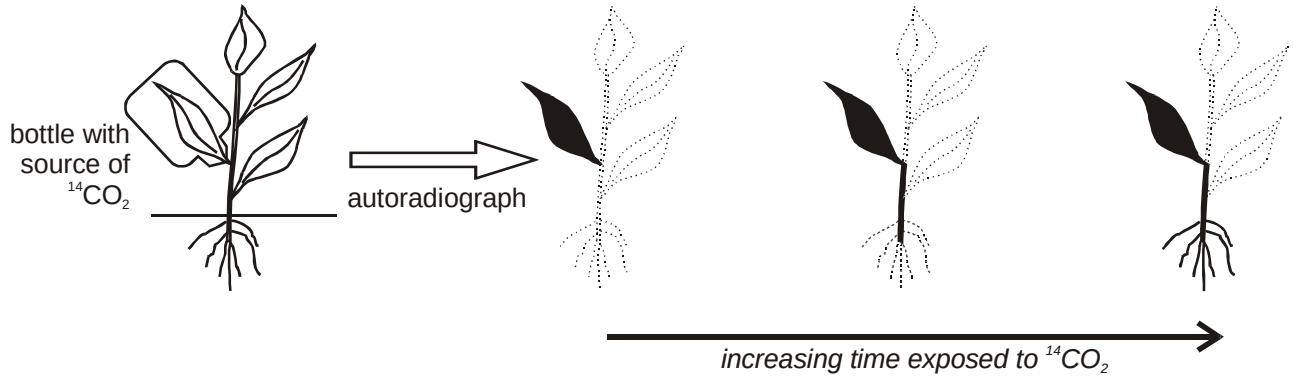


sugars were transported downwards in the phloem.

3. Radioactive Tracer Experiments

Radioactive isotopes can be used trace precisely where different compounds are being transported from and to, as well as measuring the rate of transport. The radioactivity can be traced using photographic film (an autoradiograph) or a GM tube. This techniques can be used to trace sugars, ions or even water.

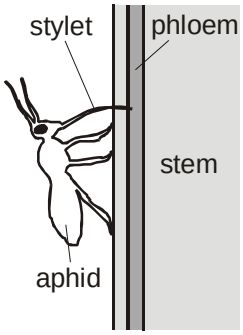
In a typical experiment a plant is grown in the lab and one leaf is exposed for a short time to carbon dioxide containing the radioactive isotope ^{14}C. This $^{14}CO_2$ will be taken up by photosynthesis and the ^{14}C incorporated into glucose and then sucrose. The plant is then frozen in liquid nitrogen to kill and fix it quickly, and placed onto photographic film in the dark. The resulting autoradiograph shows the location of compounds containing ^{14}C.



This experiment shows that organic compounds (presumably sugars) are transported downwards from the leaf to the roots. More sophisticated experiments using fluorescently labelled compounds can locate the compound specifically to the phloem cells.

4. Aphid Stylet Experiments

Aphids, such as greenfly, have specialised mouthparts called stylets, which they use to penetrate phloem tubes and sup of the sugary sap therein. If the aphids are anaesthetised with carbon dioxide and cut off, the stylet remains in the phloem so pure phloem sap can be collected through the stylet for analysis. This surprising technique is more accurate than a human with a syringe and the aphid's enzymes ensure that the stylet doesn't get blocked.



AQA(B) A2 Module 4

Energy, Control And Continuity

Contents

Human Nervous system	Hormones
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	Temperature Homeostasis
	Blood Sugar Homeostasis
	Blood water Homeostasis
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	Monohybrid cross
	Sex Determination and Sex-Linkage
	Codominance
	Multiple Alleles
	Dihybrid Cross
	Polygenes
	Epistasis
	Meiosis
Population Genetics	Variation
	Natural Selection
	Speciation
	Classification

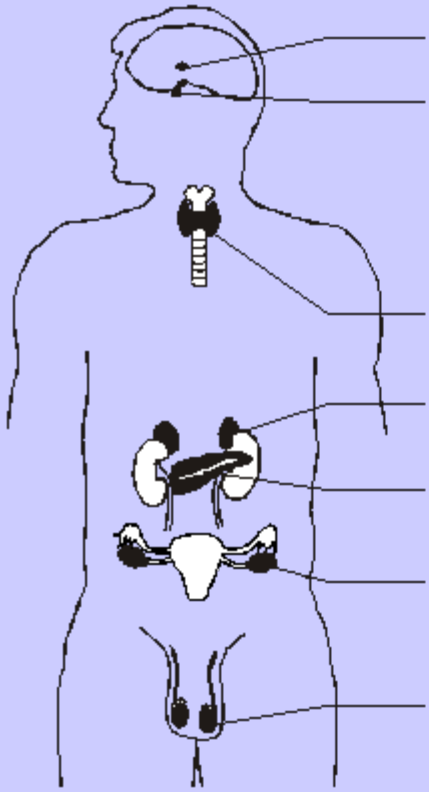
The Hormone System

Humans have two complementary control systems that they can use to respond to their environment: the nervous system and the endocrine (hormonal) system. We'll now look briefly at the hormone system.

Hormones are secreted by glands into the blood stream. There are two kinds of glands:

- Exocrine glands secrete chemicals to the outside, or to body cavities, usually through ducts (tubes). E.g. sweat glands, mammary glands, digestive glands.
- Endocrine glands do not have ducts but secrete chemicals directly into the tissue fluid whence they diffuse into the blood stream. E.g. thyroid gland, pituitary gland, adrenal gland. The hormone-secreting glands are all endocrine glands.

This table shows some of the main endocrine glands and their hormones. The hormones marked with a * are ones that we shall look at in detail later.

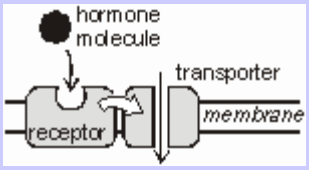


Gland	Hormone	Target organ	Function
Pineal gland	melatonin	many	biological clock
Pituitary gland	FSH	ovaries	menstrual cycle
	LH	ovaries	menstrual cycle
	ADH*	kidneys	water homeostasis
	growth hormone	many	stimulates cell division
	oxytocin	uterus	birth contractions
	prolactin	mammary glands	milk production
Thyroid gland	thyroxine*	liver	metabolic rate
Adrenal glands	adrenaline*	many	fight or flight
	cortisol	many	anti-stress
Pancreas	insulin*	liver	glucose homeostasis
	glucagon*	liver	glucose homeostasis
Ovaries	oestrogen	uterus	menstrual cycle
	progesterone	uterus	menstrual cycle
Testes	testosterone	many	male characteristics

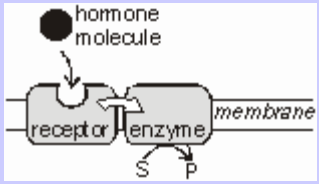
Once a hormone has diffused into the blood stream it is carried all round the body to all organs. However, it only affects certain target organs, which can respond to it. These target organs have specific receptor molecules in their cells to which the hormone binds. These receptors are protein molecules, and they form specific hormone-receptor complexes, very much like enzyme-substrate complexes. Cells without the specific receptor will just ignore a hormone. The hormone-receptor complex can affect almost any aspect of a cell's function, including metabolism, transport, protein synthesis, cell division or cell death.

There are three different ways in which a hormone can affect cell function:

Some hormones affect the permeability of the cell membrane. They bind to a receptor on the membrane, which then activates a transporter, so substances can enter or leave the cell. (E.g. insulin stimulates glucose uptake.)

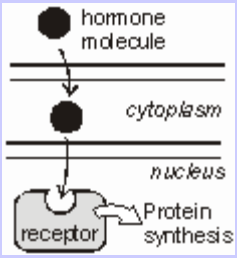


Some hormones release a "second messenger" inside the cell. They bind to a receptor on the membrane, which then activates an enzyme in the membrane, which catalyses the production of a chemical in the cytoplasm, which affects various aspects of the cell. (E.g. adrenaline stimulates glycogen breakdown.)



The steroid hormones are lipid-soluble so can easily pass through membranes by lipid diffusion. They diffuse to the nucleus, where the bind to a receptor, which activates protein synthesis.

(E.g. testosterone stimulates spermatogenesis.)



So in most cases, the hormone does not enter the cell. The effect of a hormone is determined not by the hormone itself, but by the receptor in the target cell. So the same hormone can have different effects in different target cells.

Comparison of Nervous and Hormone Systems

Nervous System	Hormone System
Transmitted by specific neurone cells	Transmitted by the circulatory system
Effect localised by neurone anatomy	Effect localised by target cell receptors
Fast-acting (ms–s)	Slow-acting (mins–days)
Short-lived response	Long-lived response

The two systems work closely together: endocrine glands are usually controlled by the nervous system, and a response to a stimulus often involves both systems.

Excretion and Homeostasis

Excretion means the removal of waste products from cells. There are five important excretory organs in humans:

- Skin excretes sweat, containing water, ions and urea
- Lungs excrete carbon dioxide and water

- Liver excretes bile, containing bile pigments, cholesterol and mineral ions
- Gut excretes mucosa cells, water and bile in faeces. (The bulk of faeces comprises plant fibre and bacterial cells, which have never been absorbed into the body, so are not excreted but egested.)
- Kidneys excrete urine, containing urea, mineral ions, water and other "foreign" chemicals from the blood.

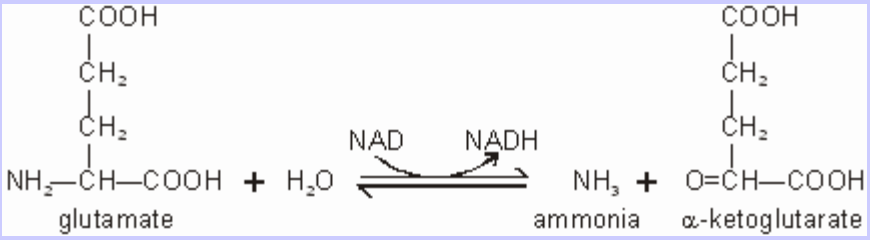
This section is mainly concerned with the excretion of nitrogenous waste as urea. The body cannot store protein in the way it can store carbohydrate and fat, so it cannot keep excess amino acids. The "carbon skeleton" of the amino acids can be used in respiration, but the nitrogenous amino group must be excreted.

Amino Acid Metabolism

Amino acid metabolism takes place in the liver, this module focuses on two main stages:

1. Deamination

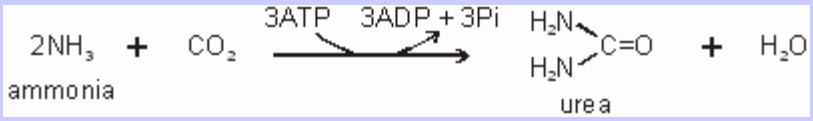
In this reaction an amino group is removed from an amino acid to form ammonia and an organic acid. The most common example is glutamate deamination:



This reaction is catalysed by the enzyme glutamate dehydrogenase. The NADH produced is used in the respiratory chain; the a-ketoglutarate enters the Krebs cycle; and the ammonia is converted to urea in the urea cycle.

2. Urea Synthesis

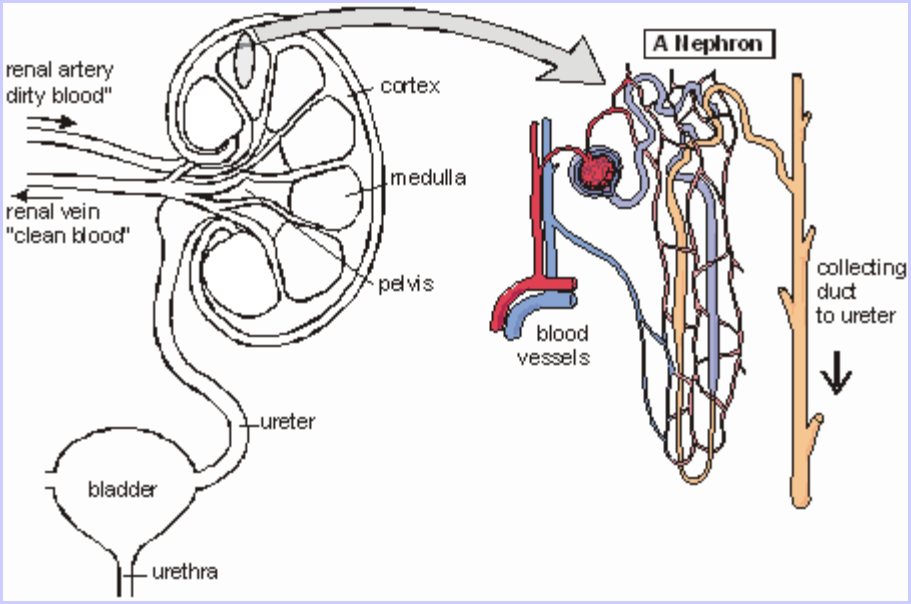
In this reaction ammonia is converted to urea, ready for excretion by the kidney.



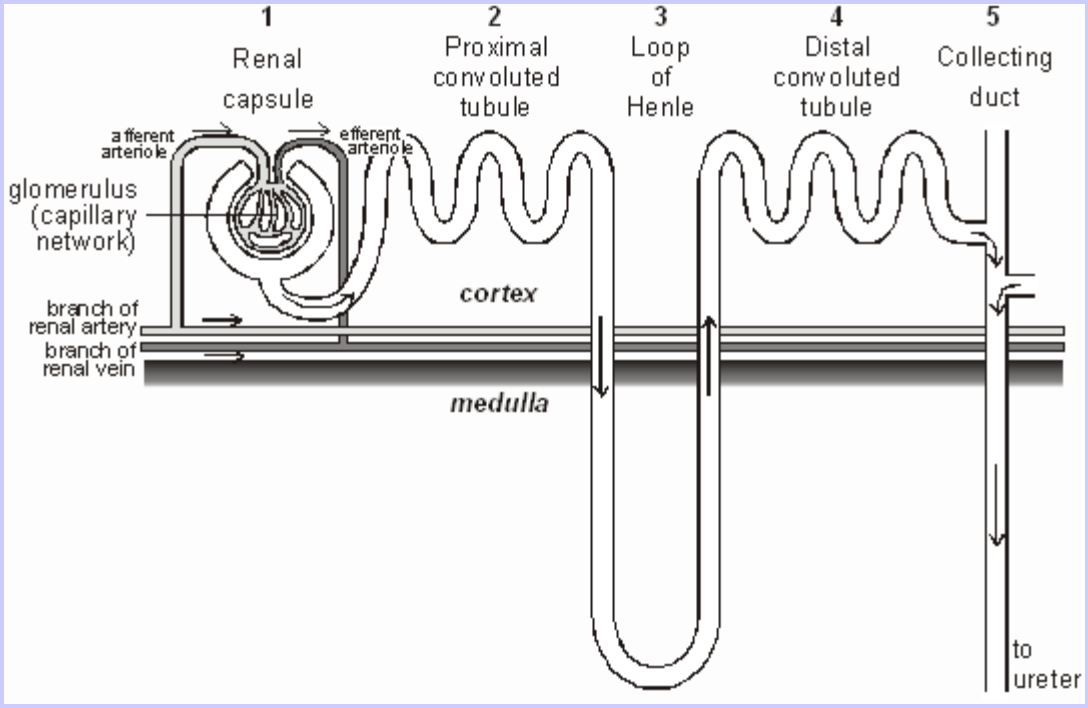
Ammonia is highly toxic. Urea is less toxic than ammonia, so it is safer to have in the bloodstream. The disadvantage is that it "costs" 3 ATP molecules to make one urea molecule. This process of converting ammonia into urea shown above is not a single reaction, but is a summary of another cyclic pathway, called the ornithine cycle.

The Kidney

The kidneys remove urea and other toxic wastes from the blood, forming a dilute solution called urine in the process. The two kidneys have a very extensive blood supply and the whole blood supply passes through the kidneys every 5 minutes, ensuring that waste materials do not build up. The renal artery carries blood to the kidney, while the renal vein carries blood, now with far lower concentrations of urea and mineral ions, away from the kidney. The urine formed passes down the ureter to the bladder.



The important part of the kidney is a folded tube called a nephron. There are a million nephrons in each kidney. There are five steps in producing urine in a nephron:



1. Renal capsule – Ultrafiltration

The renal artery splits into numerous arterioles, each feeding a nephron. The arteriole splits into numerous capillaries, which form a knot called a glomerulus. The glomerulus is enclosed by the renal capsule (or Bowman's capsule)- the first part of the nephron. The blood pressure in the capillaries of the glomerulus forces plasma out of the blood by ultrafiltration. Both the capillary walls and the capsule walls are formed

from a single layer of flattened cells with gaps between them, so that all molecules with a molecular mass of <70k are squeezed out of the blood to form a filtrate in the renal capsule. Only blood cells and large plasma proteins remain in the blood.

2. Proximal Convoluted Tubule – Reabsorption.

The proximal convoluted tubule is the longest (14mm) and widest (60µm) part of the nephron. It is lined with epithelial cells containing microvilli and numerous mitochondria. In this part of the nephron over 80% of the filtrate is reabsorbed into the tissue fluid and then to the blood. This ensures that all the "useful" materials that were filtered out of the blood (such as glucose and amino acids) are now returned to the blood.

- All glucose, all amino acids and 85% of mineral ions are reabsorbed by active transport from the filtrate to the tissue fluid. They then diffuse into the blood capillaries.
- Small proteins are reabsorbed by pinocytosis, digested, and the amino acids diffuse into the blood.
- 80% of the water is reabsorbed to the blood by osmosis.
- Surprisingly, some urea is reabsorbed to the blood by diffusion. Urea is a small, uncharged molecule, so it can pass through membranes by lipid diffusion and there isn't much the kidney can do about it. Since this is a passive process, urea diffuses down its concentration gradient until the concentrations of urea in the filtrate and blood are equal. So in each pass through the kidneys half the urea is removed from the blood and half remains in the blood.

3. Loop of Henle – Formation of a Salt Bath.

The job of the loop of Henle is to make the tissue fluid in the medulla hypertonic compared to the filtrate in the nephron. The purpose of this "salt bath" is to reabsorb water as explained in step 5. The loop of Henle does this by pumping sodium and chloride ions out of the filtrate into the tissue fluid. The first part of the loop (the descending limb) is impermeable to ions, but some water leaves by osmosis. This makes the filtrate more concentrated as it descends. The second part of the loop (the ascending limb) contains an Na^+ and a Cl^- pump, so these ions are actively transported out of the filtrate into the surrounding tissue fluid. Water would follow by osmosis, but it can't, because the ascending limb is impermeable to water. So the tissue fluid becomes more salty (hypertonic) and the filtrate becomes less salty (hypotonic). Since the filtrate is most concentrated at the base of the loop, the tissue fluid is also more concentrated at the base of the medulla, where it is three times more concentrated than seawater.

4. Distal Convoluted tubule – Homeostasis and Secretion

The distal convoluted tubule is relatively short and has a brush border (i.e. microvilli) with numerous membrane pumps for active transport. Final Na^+ reabsorption occurs and the process of water reabsorption explained next in step 5 also takes place to a degree in the distal convoluted tubule

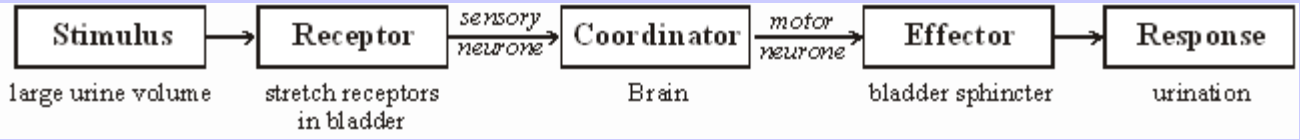
5. Collecting Duct – Concentration

As the collecting duct passes through the hypertonic salt bath in the medulla, water leaves the filtrate by osmosis, so concentrating the urine and conserving water. The water leaves through special water channels in the cell membrane called aquaporins. These aquaporin channels can be controlled by the hormone ADH, so allowing the amount of water in the urine to be controlled. More ADH opens the channels, so more water is conserved in the body, and more concentrated urine is produced. This is described in more detail in water

homeostasis later.

The Bladder

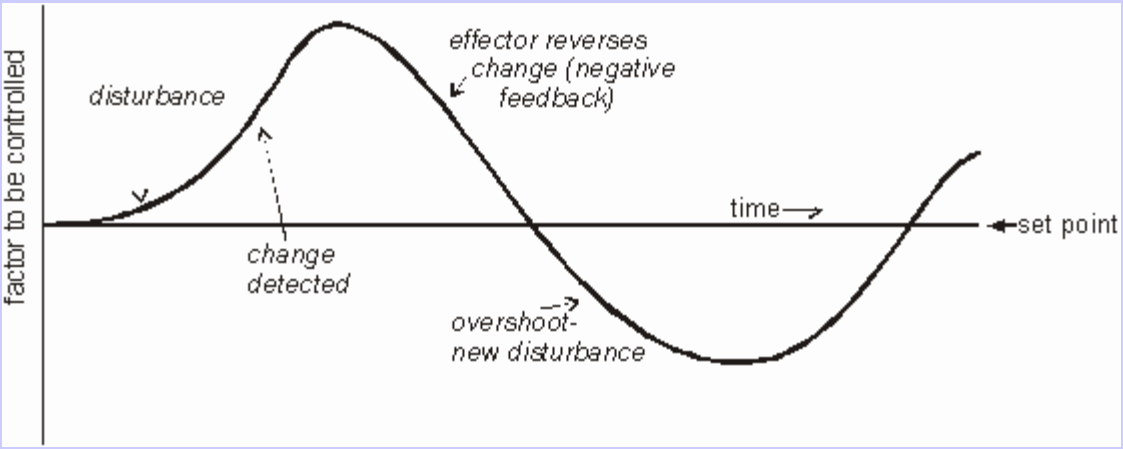
The collecting ducts all join together in the pelvis of the kidney to form the ureter, which leads to the bladder. The filtrate, now called urine, is produced continually by each kidney and drips into the bladder for storage. The bladder is an expandable bag, and when it is full, stretch receptors in the elastic walls send impulses to the medulla, which causes the sphincter muscles to relax, causing urination. This is an involuntary reflex response that we can learn to control to a certain extent when we are young.



Homeostasis

Homeostasis literally means "same state" and it refers to the process of keeping the internal body environment in a steady state. The importance of this cannot be over-stressed, and a great deal of the hormone system and autonomic nervous system is dedicated to homeostasis. In module 3 we saw how the breathing and heart rates were maintained. Here we shall look at three more examples of homeostasis in detail: temperature, blood glucose and blood water.

All homeostatic mechanisms use negative feedback to maintain a constant value (called the set point). Negative feedback means that whenever a change occurs in a system, the change automatically causes a corrective mechanism to start, which reverses the original change and brings the system back to normal. It also means that the bigger then change the bigger the corrective mechanism. Negative feedback applies to electronic circuits and central heating systems as well as to biological systems.

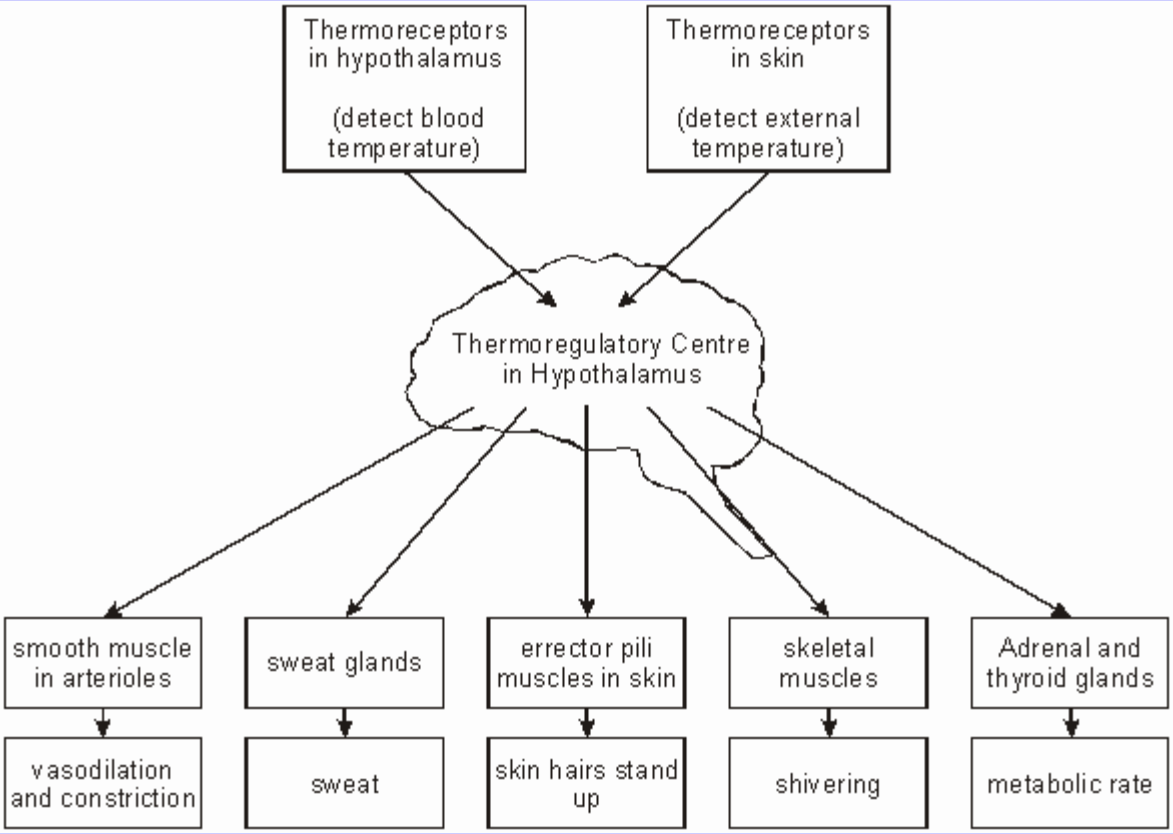


So in a system controlled by negative feedback the level is never maintained perfectly, but constantly oscillates about the set point. An efficient homeostatic system minimises the size of the oscillations.

Temperature Homeostasis (thermoregulation)

One of the most important examples of homeostasis is the regulation of body temperature. Not all animals can do this. Animals that maintain a fairly constant body temperature are called homeotherms, while those that have a variable body temperature are called poikilotherms. The homeotherms maintain their body temperatures at around 37°C, so are sometimes called warm-blooded animals, but in fact piokilothermic animals can also have very warm blood during the day by basking in the sun.

In humans temperature homeostasis is controlled by the thermoregulatory centre in the hypothalamus. It receives input from two sets of thermoreceptors: receptors in the hypothalamus itself monitor the temperature of the blood as it passes through the brain (the core temperature), and receptors in the skin monitor the external temperature. Both pieces of information are needed so that the body can make appropriate adjustments. The thermoregulatory centre sends impulses to several different effectors to adjust body temperature:



The thermoregulatory centre is part of the autonomic nervous system, so the various responses are all involuntary. The exact responses to high and low temperatures are described in the table below. Note that some of the responses to low temperature actually generate heat (thermogenesis), while others just conserve heat. Similarly some of the responses to heat actively cool the body down, while others just reduce heat production or transfer heat to the surface. The body thus has a range of responses available, depending on the internal and external temperatures.

Effector	Response to low temperature	Response to high temperature
Smooth muscles in	Muscles contract causing vasoconstriction. Less heat is carried from the core to the surface	Muscles relax causing vasodilation.

peripheral arterioles in the skin.	of the body, maintaining core temperature. Extremities can turn blue and feel cold and can even be damaged (frostbite).	More heat is carried from the core to the surface, where it is lost by radiation. Skin turns red.
Sweat glands	No sweat produced.	Glands secrete sweat onto surface of skin, where it evaporates. Water has a high latent heat of evaporation, so it takes heat from the body.
Erector pili muscles in skin (attached to skin hairs)	Muscles contract, raising skin hairs and trapping an insulating layer of still, warm air next to the skin. Not very effective in humans, just causing "goosebumps".	Muscles relax, lowering the skin hairs and allowing air to circulate over the skin, encouraging convection and evaporation.
Skeletal muscles	Muscles contract and relax repeatedly, generating heat by friction and from metabolic reactions.	No shivering.
Adrenal and thyroid glands	Glands secrete adrenaline and thyroxine respectively, which increase the metabolic rate in different tissues, especially the liver, so generating heat.	Glands stop releasing adrenaline and thyroxine.
Behaviour	Curling up, huddling, finding shelter, putting on more clothes.	Stretching out, finding shade, swimming, removing clothes.

The thermoregulatory centre normally maintains a set point of 37.5 ± 0.5 °C in most mammals. However the set point can be altered is special circumstances:

- Fever. Chemicals called pyrogens released by white blood cells raise the set point of the thermoregulatory centre causing the whole body temperature to increase by 2-3 °C. This helps to kill bacteria (+ white blood cells work best at this temperature) and explains why you shiver even though you are hot.
- Hibernation. Some mammals release hormones that reduce their set point to around 5°C while they hibernate. This drastically reduces their metabolic rate and so conserves their food reserves.
- Torpor. Bats and hummingbirds reduce their set point every day while they are inactive. They have a high surface area:volume ratio, so this reduces heat loss.

Blood Glucose Homeostasis

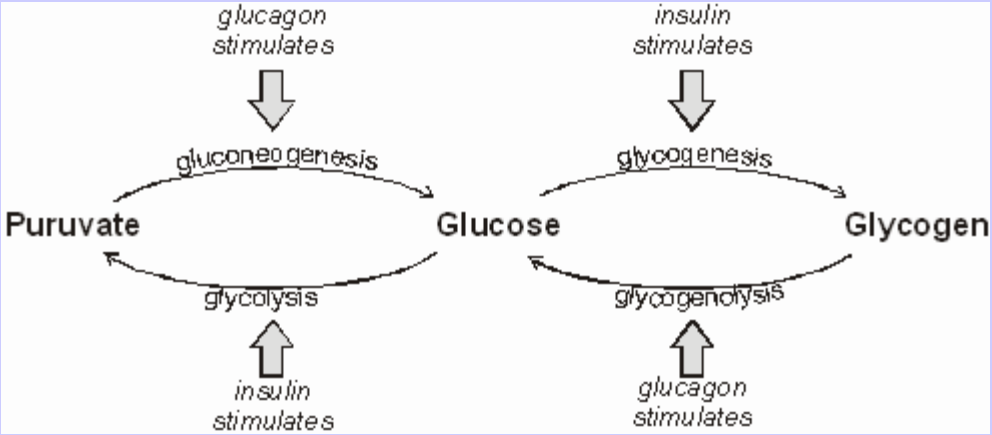
Glucose is the transport carbohydrate in animals, and its concentration in the blood affects every cell in the body. Its concentration is therefore strictly controlled within the range 80-100 mg $100cm^{-3}$, and very low level (hypoglycaemia) or very high levels (hyperglycaemia) are both serious and can lead to death.

Blood glucose concentration is controlled by the pancreas. The pancreas has glucose receptor cells, which

monitor the concentration of glucose in the blood, and it also has endocrine cells (called the islets of Langerhans), which secrete the hormones glucagon, and insulin. These two hormones are antagonistic, and have opposite effects on blood glucose:

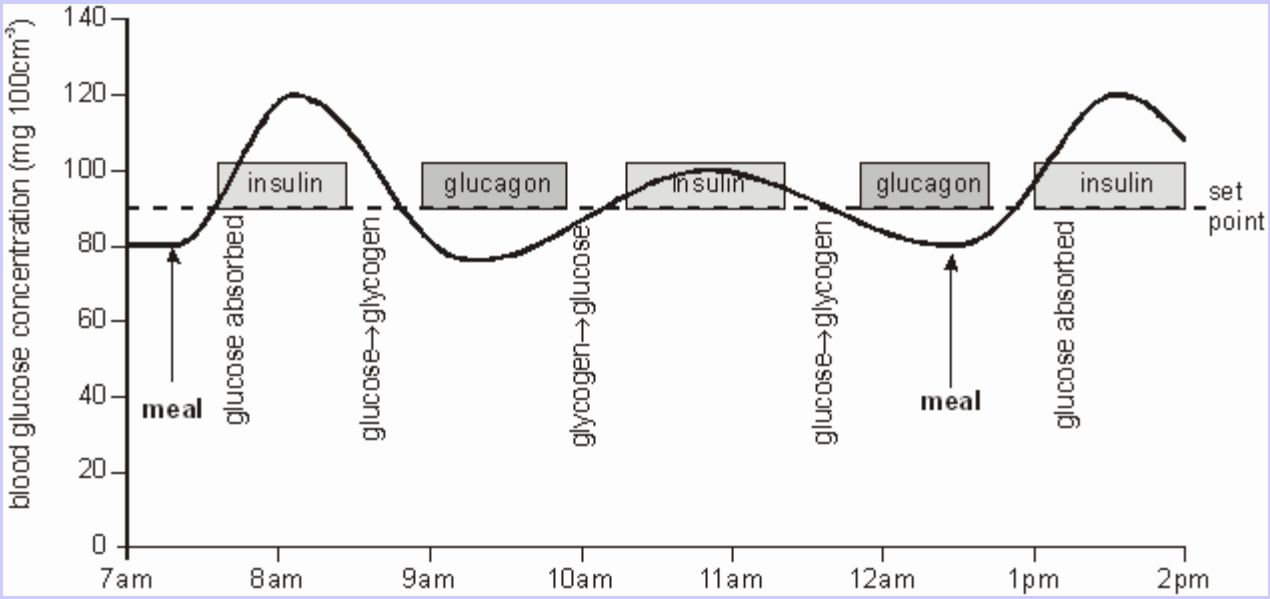
- insulin stimulates the uptake of glucose by liver cells by activating glucose carrier proteins so glucose is transported into liver cells by facilitated diffusion. Glycogen also stimulates the conversion of glucose to glycogen (glycogenesis). It therefore decreases blood glucose.

- glucagon stimulates the breakdown of glycogen to glucose in the liver (glycogenolysis), and in extreme cases it can also stimulate the synthesis of glucose from pyruvate. It therefore increases blood glucose.



After a meal, glucose is absorbed from the gut into the hepatic portal vein, increasing the blood glucose concentration. This is detected by the pancreas, which secretes insulin in response. Insulin causes glucose to be taken up by the liver and converted to glycogen. This reduces blood glucose, which causes the pancreas to stop secreting insulin. If the glucose level falls too far, the pancreas detects this and releases glucagon. Glucagon causes the liver to break down some of its glycogen store to glucose, which diffuses into the blood. This increases blood glucose, which causes the pancreas to stop producing glucagon.

These negative feedback loops continue all day, as shown in this graph:



Diabetes Mellitus

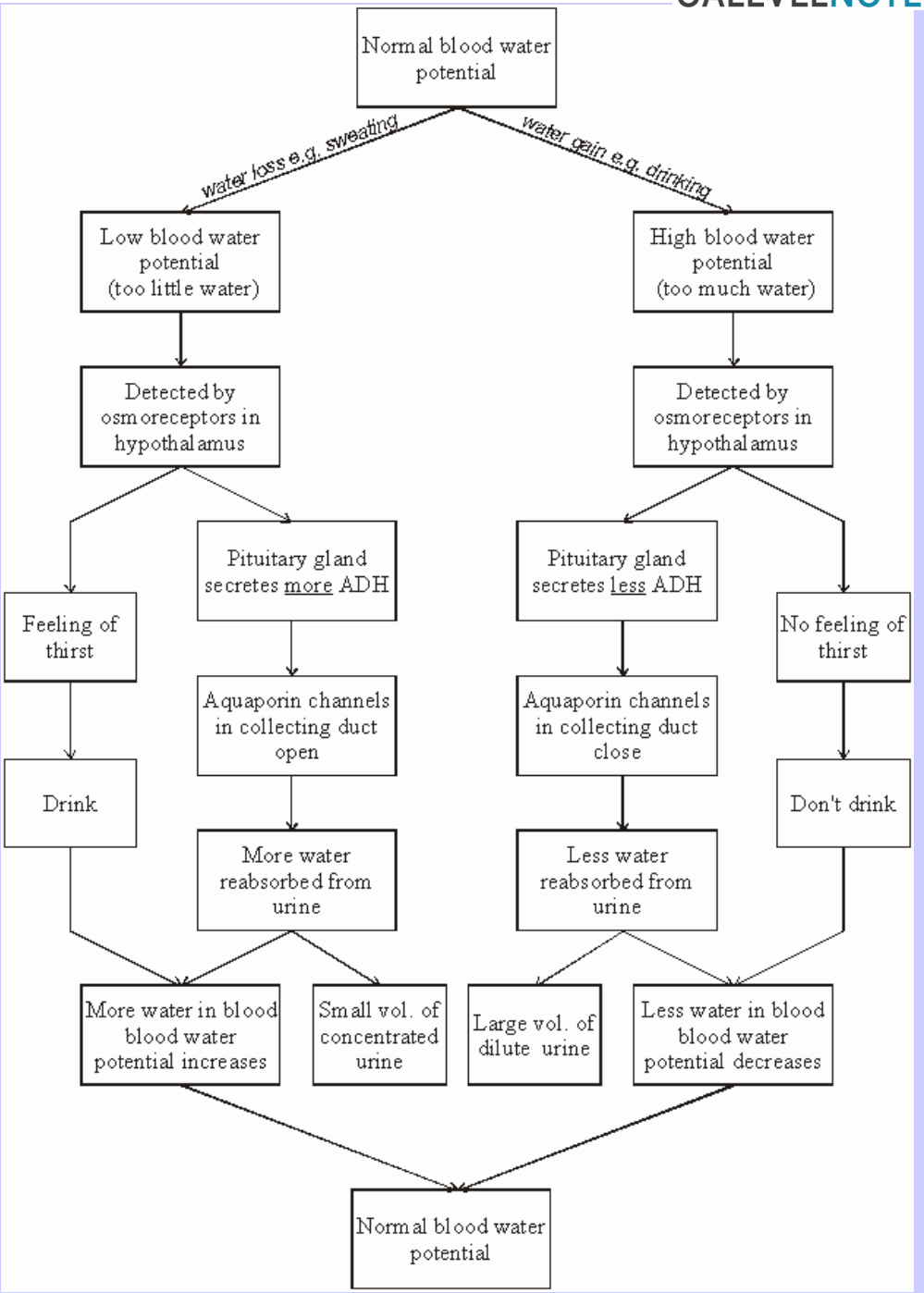
Diabetes is a disease caused by a failure of glucose homeostasis. There are two forms of the disease. In insulin-dependent diabetes (also known as type 1 or early-onset diabetes) there is a severe insulin deficiency due to autoimmune killing of b cells. In non insulin-dependent diabetes (also known as type 2 or late-onset diabetes) insulin is produced, but the insulin receptors in the target cells don't work, so insulin has no effect. In both cases there is a very high blood glucose concentration after a meal, so the active transport pumps in the proximal convoluted tubule of the kidney can't reabsorb it all from the kidney filtrate, so much of the glucose is excreted in urine (diabetes mellitus means "sweet fountain"). This leads to the symptoms of diabetes:

- high thirst due to osmosis of water from cells to the blood.
- copious urine production due to excess water in blood.
- poor vision due to osmotic effects in the eye.
- tiredness due to loss of glucose in urine and poor uptake of glucose by liver and muscle cells.

Diabetes can be treated by injections with insulin or by careful diet.

Blood Water Homeostasis (Osmoregulation)

The water potential of the blood must be regulated to prevent loss or gain of water from cells. Blood water homeostasis is controlled by the hypothalamus. It contains osmosreceptor cells, which can detect changes in the water potential of the blood passing through the brain. In response, the hypothalamus controls the sensation of thirst, and it also secretes the hormone ADH (antidiuretic hormone). ADH is stored in the pituitary gland, and its target cells are the endothelial cells of the collecting ducts of the kidney nephrons. These cells are unusual in that water molecules can only cross their membranes via water channels called aquaporins, rather than through the lipid bilayer. ADH causes these water channels to open. The effects of ADH are shown in this diagram:



Classical Genetics

In module 2 we studied molecular genetics. Here we are concerned with classical genetics, which is the study of inheritance of characteristics at the whole organism level. It is also known as transmission genetics or Mendelian genetics, since it was pioneered by Gregor Mendel.

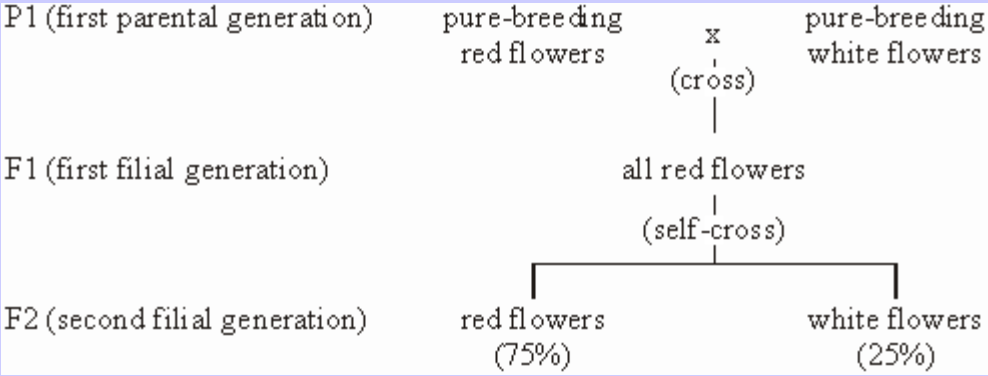
Gregor Mendel

Mendel (1822-1884) was an Austrian monk at Brno monastery. He was a keen gardener and scientist, and studied at Vienna university, where he learnt statistics. He investigated inheritance in pea plants and

published his results in 1866. They were ignored at the time, but were rediscovered in 1900, and Mendel is now recognised as the "Father of Genetics". His experiments succeeded where other had failed because:

- Mendel investigated simple well-defined characteristics (or traits), such as flower colour or seed shape, and he varied one trait at a time. Previous investigators had tried to study many complex traits, such as human height or intelligence.
- Mendel use an organism whose sexual reproduction he could easily control by carefully pollinating stigmas with pollen using a brush. Peas can also be self pollinated, allowing self crosses to be performed. This is not possible with animals.
- Mendel repeated his crosses hundreds of times and applied statistical tests to his results.
- Mendel studied two generations of peas at a time.

A typical experiment looked like this:



Mendel made several conclusions from these experiments:

1. There are no mixed colours (e.g. pink), so this disproved the widely-held blending theories of inheritance that characteristics gradually mixed over time.
2. A characteristic can disappear for a generation, but then reappear the following generation, looking exactly the same. So a characteristic can be present but hidden.
3. The outward appearance (the phenotype) is not necessarily the same as the inherited factors (the genotype) For example the P1 red plants are not the same as the F1 red plants.
4. One form of a characteristic can mask the other. The two forms are called dominant and recessive respectively.
5. The F2 ratio is always close to 3:1. Mendel was able to explain this by supposing that each individual has two versions of each inherited factor, one received from each parent. We'll look at his logic in a minute.

Mendel's factors are now called genes and the two alternative forms are called alleles. So in the example above we would say that there is a gene for flower colour and its two alleles are "red" and "white". One allele comes from each parent, and the two alleles are found on the same position (or locus) on the homologous chromosomes. With two alleles there are three possible combinations of alleles (or genotypes) and two possible appearances (or phenotypes):

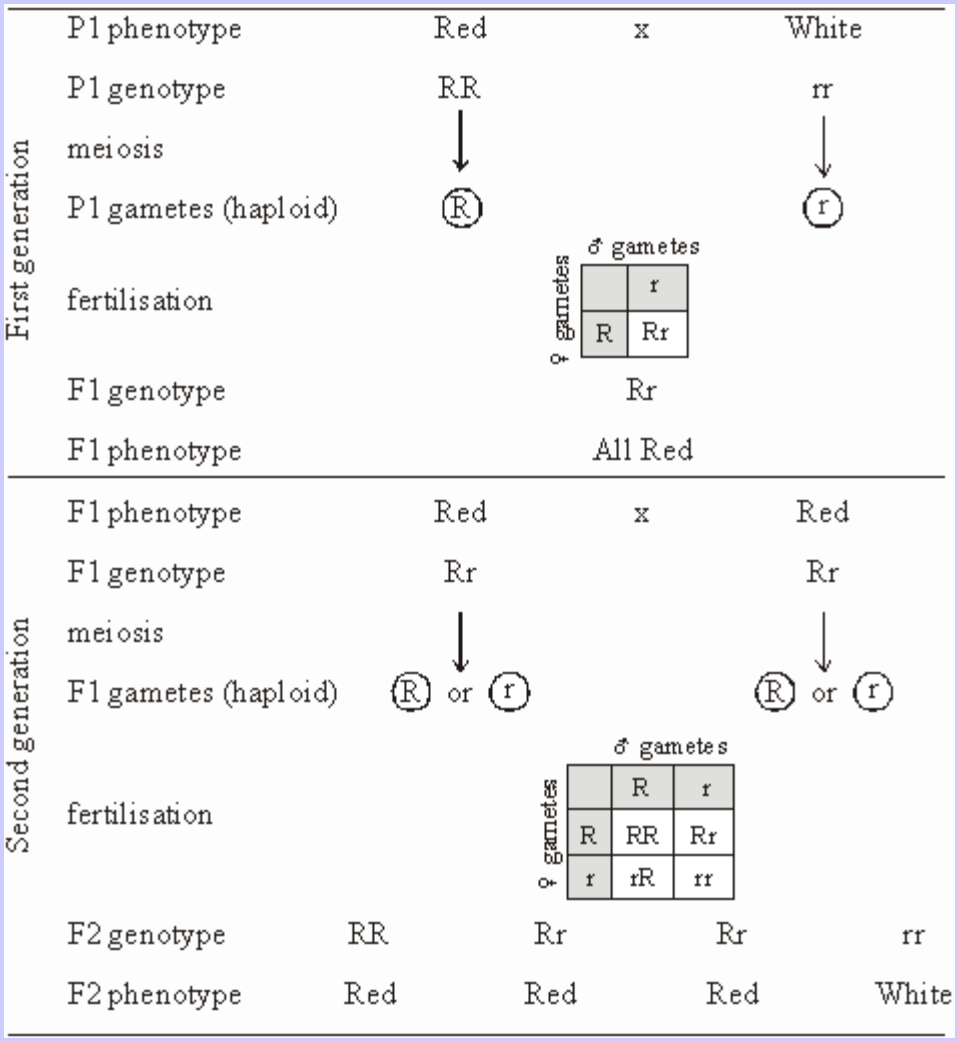
Genotype	Name	Phenotype

RR	homozygous dominant	red
rr	homozygous recessive	white
Rr, rR	heterozygous	red

The Monohybrid Cross

A simple breeding experiment involving just a single characteristic, like Mendel's experiment, is called a monohybrid cross. We can now explain Mendel's monohybrid cross in detail.

At fertilisation any male gamete can fertilise any female gamete at random. The possible results of a fertilisation can most easily be worked out using a Punnett Square as shown in the diagram. Each of the possible outcomes has an equal chance of happening, so this explains the 3:1 ratio observed by Mendel.



This is summarised in Mendel's First Law, which states that individuals carry two discrete hereditary factors (alleles) controlling each characteristic. The two alleles segregate (or separate) during meiosis, so each

gamete carries only one of the two alleles.

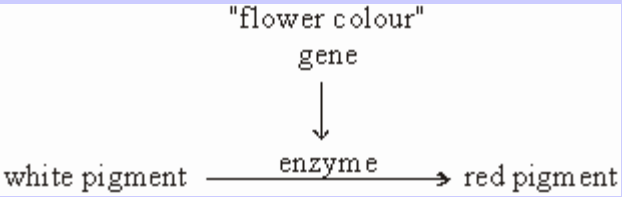
The Test Cross

You can see an individual's phenotype, but you can't see its genotype. If an individual shows the recessive trait (white flowers in the above example) then they must be homozygous recessive as it's the only genotype that will give that phenotype. If they show the dominant trait then they could be homozygous dominant or heterozygous. You can find out which by performing a test cross with a pure-breeding homozygous recessive. This give two possible results:

- If the offspring all show the dominant trait then the parent must be homozygous dominant.
- If the offspring are a mixture of phenotypes in a 1:1 ratio, then the parent must be heterozygous.

How does Genotype control Phenotype?

Mendel never knew this, but we can explain in detail the relation between an individual's genes and its appearance. A gene was originally defined as an inherited factor that controls a characteristic, but we now know that a gene is also a length of DNA that codes for a protein. It is the proteins that actually control phenotype in their many roles as enzymes, pumps, transporters, motors, hormones, or structural elements. For example the flower colour gene actually codes for an enzyme that converts a white pigment into a red pigment:



- The dominant allele is the normal (or "wild-type") form of the gene that codes for functioning enzyme, which therefore makes red-coloured flowers.
- The recessive allele is a mutation of the gene. This mutated gene codes for non-functional enzyme, so the red pigment can't be made, and the flower remains white. Almost any mutation in a gene will result in an inactive gene product (usually an enzyme), since there are far more ways of making an inactive protein than a working one.

Sometimes the gene actually codes for a protein apparently unrelated to the phenotype. For example the gene for seed shape in peas (round or wrinkled) actually codes for an enzyme that synthesises starch! The functional enzyme makes lots of starch and the seeds are full and rounded, while the non-functional enzyme makes less starch so the seeds wrinkle up.

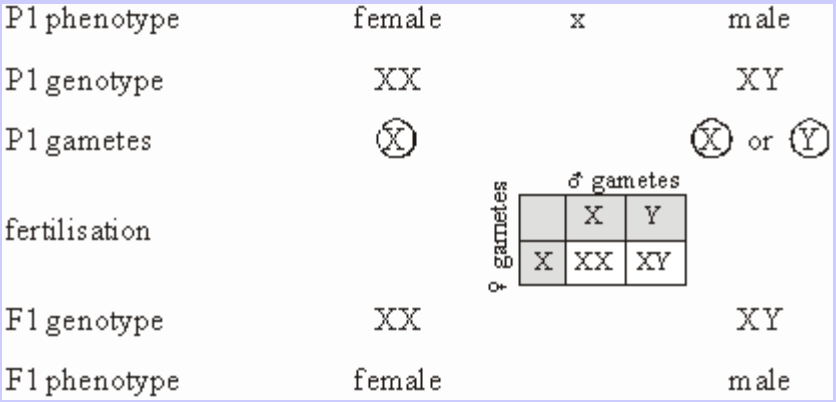
This table shows why the allele that codes for a functional protein is usually dominant over an allele that codes for a non-function protein. In a heterozygous cell, some functional protein will be made, and this is usually enough to have the desired effect. In particular, enzyme reactions are not usually limited by the amount of enzyme, so a smaller amount will have little effect.

Genotype	Gene product	Phenotype
homozygous dominant	all functional enzyme	red

(RR)		
homozygous recessive (rr)	no functional enzyme	white
heterozygous (Rr)	some functional enzyme	red

Sex Determination

In module 2 we saw that sex is determined by the sex chromosomes (X and Y). Since these are non-homologous they are called heterosomes, while the other 22 pairs are called autosomes. In humans the sex chromosomes are homologous in females (XX) and non-homologous in males (XY), though in other species it is the other way round. The inheritance of the X and Y chromosomes can be demonstrated using a monohybrid cross:

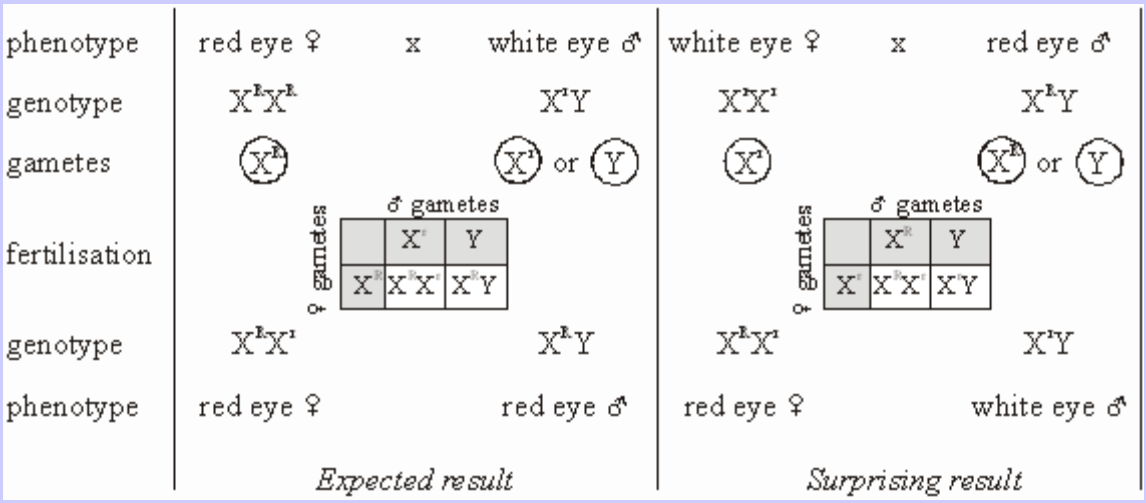


This shows that there will always be a 1:1 ratio of males to females. Note that female gametes (eggs) always contain a single X chromosome, while the male gametes (sperm) can contain a single X or a single Y chromosome. Sex is therefore determined solely by the sperm. There are techniques for separating X and Y sperm, and this is used for planned sex determination in farm animals using IVF.

Sex Linkage

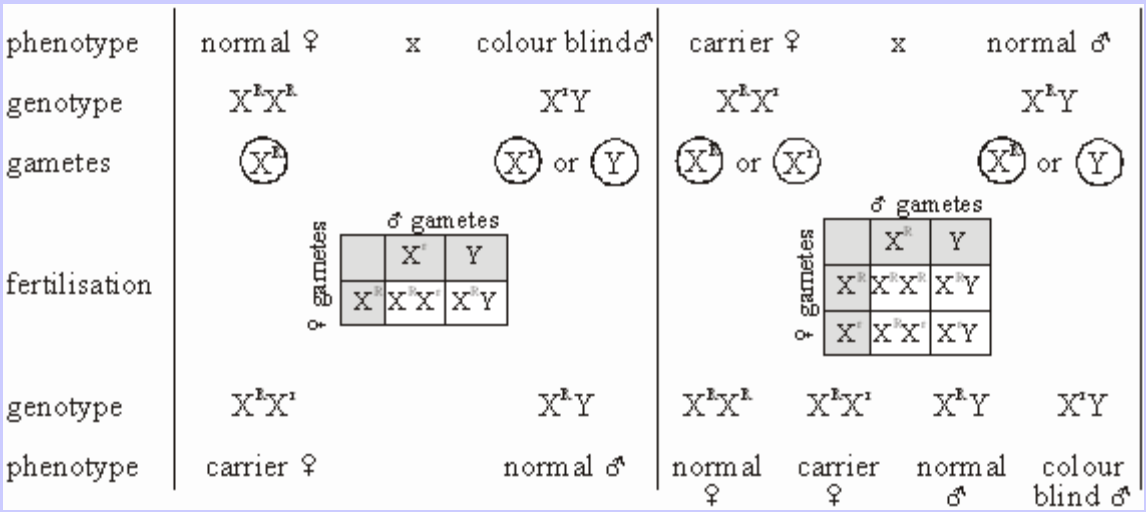
The X and Y chromosomes don't just determine sex, but also contain many other genes that have nothing to do with sex determination. The Y chromosome is very small and seems to contain very few genes, but the X chromosome is large and contains thousands of genes for important products such as rhodopsin, blood clotting proteins and muscle proteins. Females have two copies of each gene on the X chromosome (i.e. they're diploid), but males only have one copy of each gene on the X chromosome (i.e. they're haploid). This means that the inheritance of these genes is different for males and females, so they are called sex linked characteristics.

The first example of sex linked genes discovered was eye colour in *Drosophila* fruit flies. Red eyes (R) are dominant to white eyes (r) and when a red-eyed female is crossed with a white-eyed male, the offspring all have red eyes, as expected for a dominant characteristic (left cross below). However, when the opposite cross was done (a white-eye male with a red-eyed female) all the male offspring had white eyes (right cross below). This surprising result was not expected for a simple dominant characteristic, but it could be explained if the gene for eye colour was located on the X chromosome. Note that in these crosses the alleles are written in the form X^R (red eyes) and X^r (white eyes) to show that they are on the X chromosome.



Males always inherit their X chromosome from their mothers, and always pass on their X chromosome to their daughters.

Another well-known example of a sex linked characteristic is colour blindness in humans. 8% of males are colour blind, but only 0.7% of females. As explained on p31, the genes for green-sensitive and red-sensitive rhodopsin are on the X chromosome, and mutations in either of these lead to colour blindness. The diagram below shows two crosses involving colour blindness, using the symbols X^R for the dominant allele (normal rhodopsin, normal vision) and X^r for the recessive allele (non-functional rhodopsin, colour blind vision).



Other examples of sex linkage include haemophilia, premature balding and muscular dystrophy.

Codominance

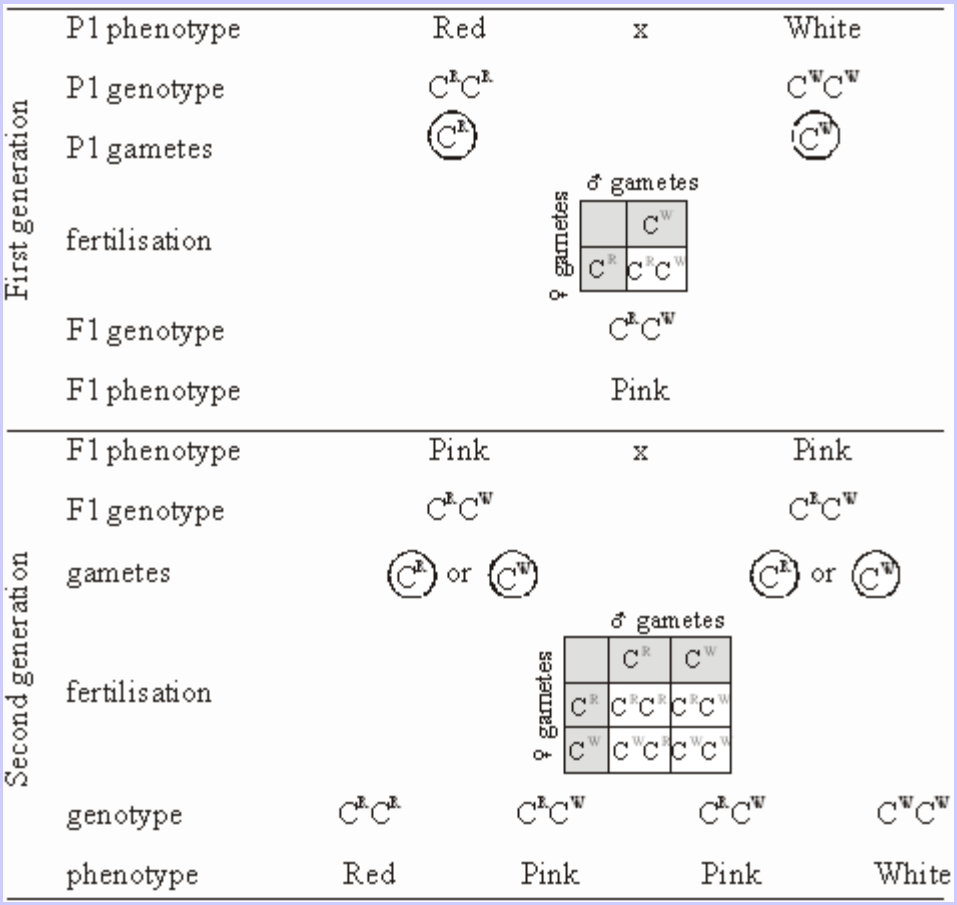
In most situations (and all of Mendel's experiments) one allele is completely dominant over the other, so there are just two phenotypes. But in some cases there are three phenotypes, because neither allele is dominant over the other, so the heterozygous genotype has its own phenotype. This situation is called codominance or incomplete dominance. Since there is no dominance we can no longer use capital and small letters to indicate the alleles, so a more formal system is used. The gene is represented by a letter, and the different alleles by superscripts to the gene letter.

A good example of codominance is flower colour in snapdragon (*Antirrhinum*) plants. The flower colour gene
R W

C has two alleles: C (red) and C (white). The three genotypes and their phenotypes are:

Genotype	Gene product	Phenotype
homozygous RR	all functional enzyme	red
homozygous WW	no functional enzyme	white
heterozygous (RW)	some functional enzyme	pink

In this case the enzyme is probably less active, so a smaller amount of enzyme will make significantly less product, and this leads to the third phenotype. The monohybrid cross looks like this:



Note that codominance is not an example of "blending inheritance" since the original phenotypes reappear in the second generation. The genotypes are not blended and they still obey Mendel's law of segregation. It is only the phenotype that appears to blend in the heterozygotes.

Another example of codominance is sickle cell haemoglobin in humans. The gene for haemoglobin Hb has two codominant alleles: Hb^A (the normal gene) and Hb^S (the mutated gene). There are three phenotypes:

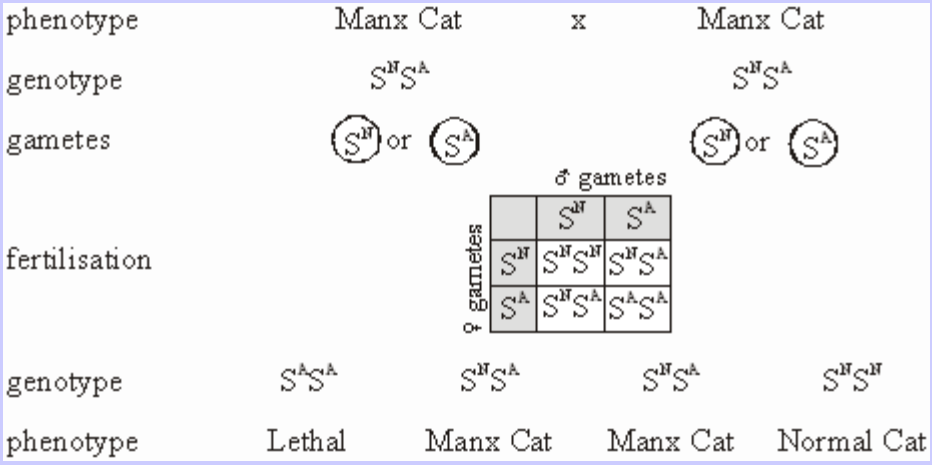
Hb^AHb^A	Normal. All haemoglobin is normal, with normal red blood cells.
Hb^AHb^S	Sickle cell trait. 50% of the haemoglobin in every red blood cell is normal, and 50% is abnormal. The red blood cells are slightly distorted, but can carry oxygen, so this condition is viable. However these red blood cells cannot support the malaria parasite, so this phenotype confers immunity to malaria.

Hb^SHb^S	Sickle cell anaemia. All haemoglobin is abnormal, and molecules stick together to form chains, distorting the red blood cells into sickle shapes. These sickle red blood cells are destroyed by the spleen, so this phenotype is fatal.

Other examples of codominance include coat colour in cattle (red/white/roan), and coat colour in cats (black/orange/tortoiseshell).

Lethal Alleles

An unusual effect of codominance is found in Manx cats, which have no tails. If two Manx cats are crossed the litter has ratio of 2 Manx kittens to 1 normal (long-tailed) kitten. The explanation for this unexpected ratio is explained in this genetic diagram:



The gene S actually controls the development of the embryo cat's spine. It has two codominant alleles: S^N (normal spine) and S^A (abnormal, short spine). The three phenotypes are:

S^NS^N	Normal. Normal spine, long tail
S^NS^A	Manx Cat. Last few vertebrae absent, so no tail.
S^AS^A	Lethal. Spine doesn't develop, so this genotype is fatal early in development. The embryo doesn't develop and is absorbed by the mother, so there is no evidence for its existence.

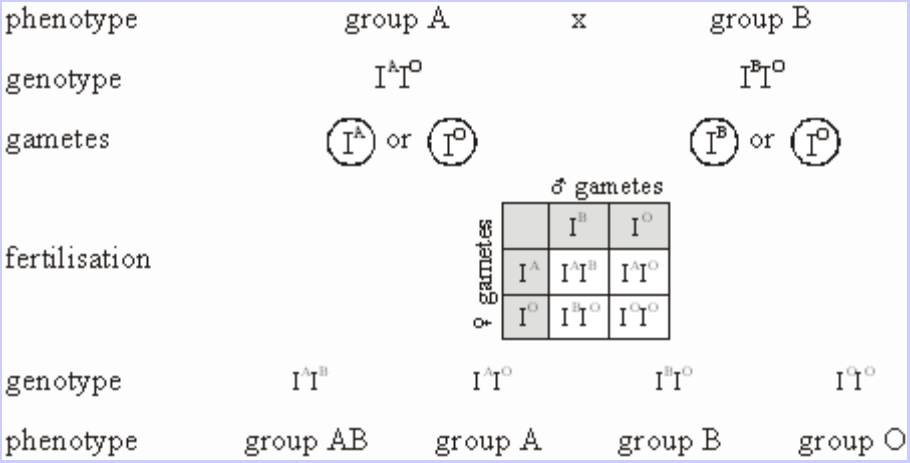
Many human genes also have lethal alleles, because many genes are so essential for life that a mutation in these genes is fatal. If the lethal allele is expressed early in embryo development then the fertilised egg may not develop enough to start a pregnancy, or the embryo may miscarry. If the lethal allele is expressed later in life, then we call it a genetic disease, such as muscular dystrophy or cystic fibrosis.

Multiple Alleles

An individual has two copies of each gene, so can only have two alleles of any gene, but there can be more than two alleles of a gene in a population. An example of this is blood group in humans. The red blood cell antigen is coded for by the gene I (for isohaemaglutinogen), which has three alleles I^A, I^B and I^O. (They are written this way to show that they are alleles of the same gene.) I^A and I^B are codominant, while I^O is recessive. The possible genotypes and phenotypes are:

Phenotype (blood group)	Genotypes	antigens on red blood cells	plasma antibodies
A	I^AI^A, I^AI^O	A	anti-B
B	I^BI^B, I^BI^O	B	anti-A
AB	I^AI^B	A and B	none
O	I^OI^O	none	anti-A and anti-B

The cross below shows how all four blood groups can arise from a cross between a group A and a group B parent.



Other examples of multiple alleles are: eye colour in fruit flies, with over 100 alleles; human leukocyte antigen (HLA) genes, with 47 known alleles.

Multiple Genes

So far we have looked at the inheritance of a single gene controlling a single characteristic. This simplification allows us to understand the basic rules of heredity, but inheritance is normally much more complicated than that. We'll now turn to the inheritance of characteristics involving two genes. This gets more complicated, partly because there are now two genes to consider, but also because the two genes can interact with each other. We'll look at three situations:

- 2 independent genes, controlling 2 characteristics (the dihybrid cross).
- 2 independent genes controlling 1 characteristic (polygenes)

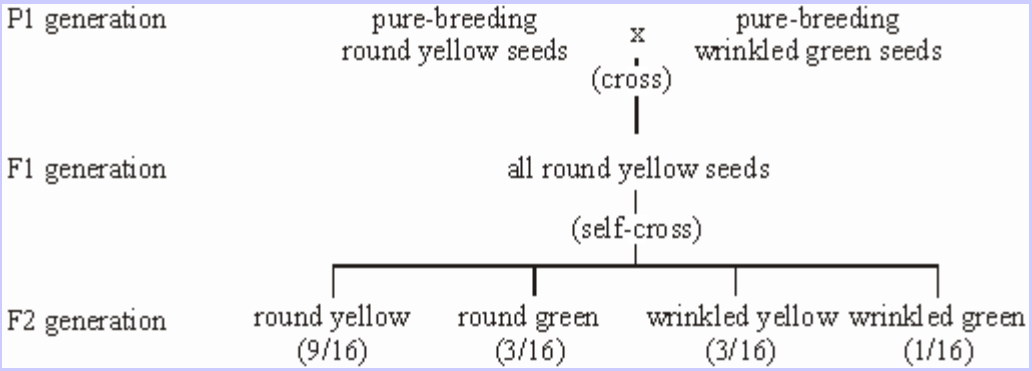
- 2 interacting genes controlling 1 characteristic (epistasis)

The Dihybrid Cross

Mendel also studied the inheritance of two different characteristics at a time in pea plants, so we'll look at one of his dihybrid crosses. The two traits are seed shape and seed colour. Round seeds (R) are dominant to wrinkled seeds (r), and yellow seeds (Y) are dominant to green seeds (y). With these two genes there are 4 possible phenotypes:

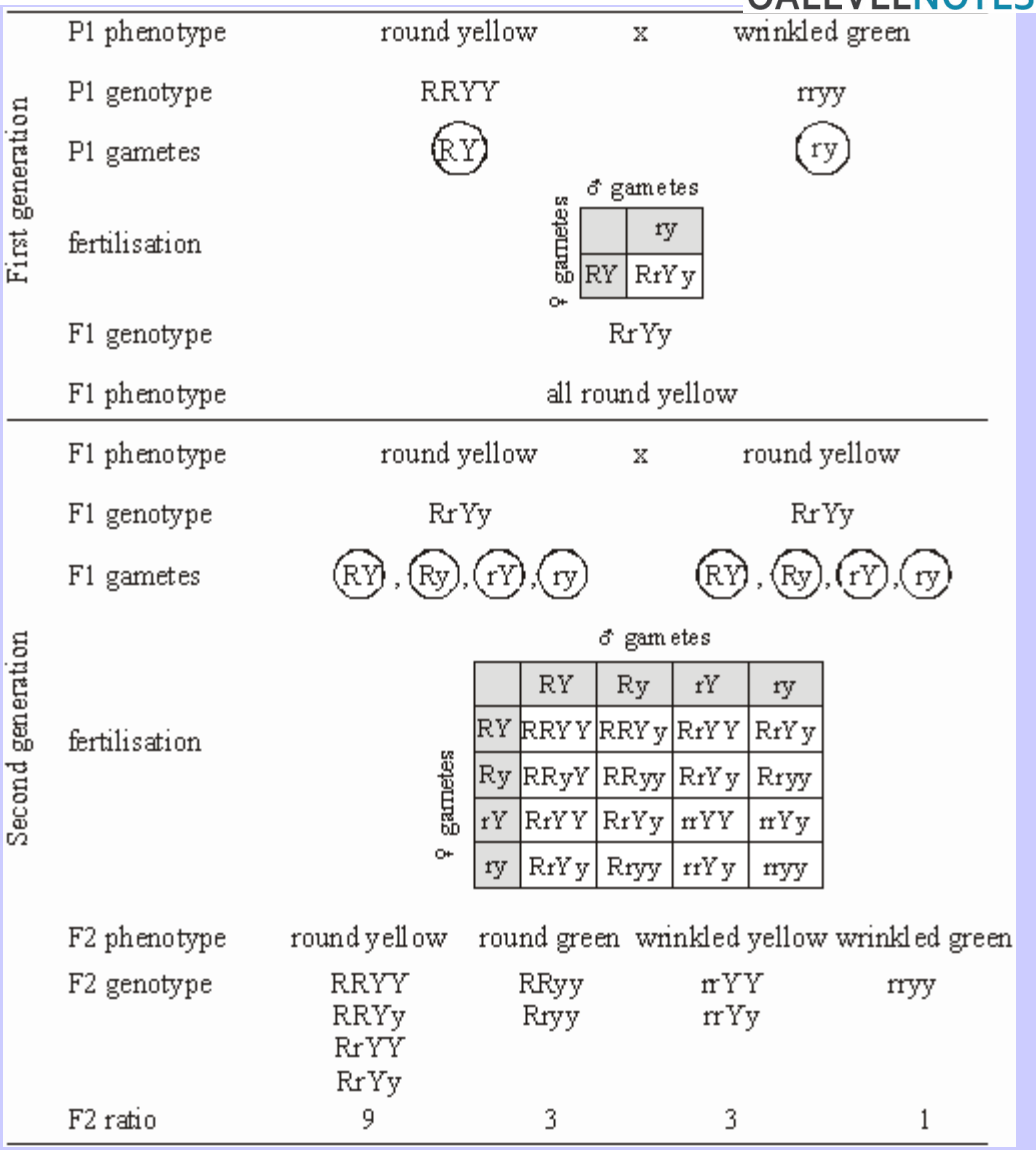
Genotypes	Phenotype
RRYY, RRYy, RrYY, RrYy	round yellow
RRyy, Rryy	round green
rrYY, rrYy	wrinkled yellow
rryy	wrinkled green

Mendel's dihybrid cross looked like this:



All 4 possible phenotypes are produced, but always in the ratio 9:3:3:1. Mendel was able to explain this ratio if the factors (genes) that control the two characteristics are inherited independently; in other words one gene does not affect the other. This is summarised in Mendel's second law (or the law of independent assortment), what states that alleles of different genes are inherited independently.

We can now explain the dihybrid cross in detail:



The gametes have one allele of each gene, and that allele can end up with either allele of the other gene. This gives 4 different gametes for the second generation, and 16 possible genotype outcomes.

Dihybrid Test Cross

There are 4 genotypes that all give the same round yellow phenotype. Just like we saw with the monohybrid cross, these four genotypes can be distinguished by crossing with a double recessive phenotype. This gives 4 different results:

Original genotype	result of test cross
RRYY	all round yellow
RRYy	1 round yellow : 1 round green
RrYY	1 round yellow : 1 wrinkled yellow
RrYy	1 round yellow : 1 round green: 1 wrinkled yellow: 1 wrinkled green

Polygenes

Sometimes two genes at different loci (i.e. separate genes) can combine to affect one single characteristic. An example of this is coat colour in Siamese cats. One gene controls the colour of the pigment, and black hair (B) is dominant to brown hair (b). The other gene controls the dilution of the pigment in the hairs, with dense pigment (D) being dominant to dilute pigment (d). This gives 4 possible phenotypes:

Genotypes	Phenotype	F_2 ratio
BBDD, BBDd, BbDD, BbDd	"seal" (black dense)	9
BBdd, Bbdd	"blue" (black dilute)	3
bbDD, bbDd	"chocolate" (brown dense)	3
bbdd	"lilac" (brown dilute)	1

The alleles are inherited in exactly the same way as in the dihybrid cross above, so the same 9:3:3:1 ratio in the F2 generation is produced. The only difference is that here, we are looking at a single characteristic, but with a more complicated phenotype ratio than that found in a monohybrid cross.

A more complex example of a polygenic character is skin colour in humans. There are 5 main categories of skin colour (phenotypes) controlled by two genes at different loci. The amount of skin pigment (melanin) is proportional to the number of dominant alleles of either gene:

Phenotype (skin colour)	Genotypes	No. of dominant alleles	F_2 ratio
Black	AABB	4	1
Dark	AaBB, AABb	3	4
Medium	AAbb, AaBb, aaBB	2	6
Light	Aabb, aaBb	1	4
White (albino)	aabb	0	1

Some other examples of polygenic characteristics are: eye colour, hair colour, and height. The important

point about a polygenic character is that it can have a number of different phenotypes, and almost any phenotypic ratio.

Epistasis

In epistasis, two genes control a single character, but one of the genes can mask the effect of the other gene. A gene that can mask the effect of another gene is called an epistatic gene (from the Greek meaning "to stand on"). This is a little bit like dominant and recessive alleles, but epistasis applies to two genes at different loci. Epistasis reduces the number of different phenotypes for the character, so instead of having 4 phenotypes for 2 genes, there will be 3 or 2. We'll look at three examples of epistasis.

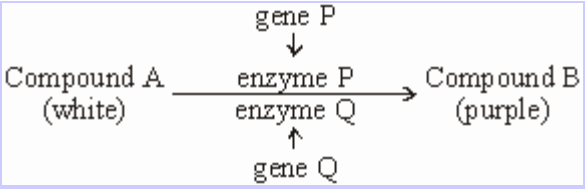
1. Dependent genes. In mice one gene controls the production of coat pigment, and black pigment (B) is dominant to no pigment (b). Another gene controls the dilution of the pigment in the hairs, with dense pigment (D) being dominant to dilute pigment (d). This is very much like the Siamese cat example above, but with one important difference: the pigment gene (B) is epistatic over the dilution gene (D) because the recessive allele of the pigment gene is a mutation that produces no pigment at all, so there is nothing for the dilution gene to affect. This gives 3 possible phenotypes:

Genotypes	Phenotype	F_2 ratio
BBDD, BBDd, BbDD, BbDd	Black (black dense)	9
BBdd, Bbdd	Brown (black dilute)	3
bbDD, bbDd, bbdd	White (no pigment)	4

2. Enzymes in a pathway. In a certain variety of sweet pea there are two flower colours (white and purple), but the F2 ratio is 9:7. This is explained if the production of the purple pigment is controlled by two enzymes in a pathway, coded by genes at different loci.

Gene P is epistatic over gene Q because the recessive allele of gene P is a mutation that produces inactive enzyme, so there is no compound B for enzyme Q to react with. This gives just two possible phenotypes:

Genotypes	Phenotype	F_2 ratio
PPQQ, PPQq, PpQQ, PpQq	Purple	9
PPqq, Ppqq, ppQQ, ppQq, ppqq	White	7



3. Duplicate Genes. This occurs when genes at two different loci make enzyme that can catalyse the same reaction (this can happen by gene duplication). In this case the coloured pigment is always made unless both genes are present as homozygous recessive (ppqq), so the F2 ratio is 15:1.

Genotypes	Phenotype	F_2 ratio
PPQQ, PPQq, PpQQ, PpQq, PPqq, Ppqq, ppQQ, ppQq	Purple	15
ppqq	White	1

So epistasis leads to a variety of different phenotype ratios.

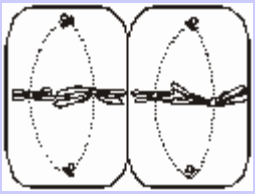
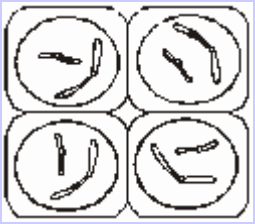
Meiosis

Meiosis is the special form of cell division used to produce gametes. It has two important functions:

- To form haploid cells with half the normal chromosome number
- To re-arrange the chromosomes with a novel combination of genes (genetic recombination)

Meiosis comprises two successive divisions, without DNA replication in between. The second division is a bit like mitosis, but the first division is different in many important respects. The details are shown in this diagram for a hypothetical cell with 2 pairs of homologous chromosomes (n=2):

First Division		Second Division	
Interphase I • chromatin not visible • DNA & proteins replicated		Interphase II • Short • no DNA replication • chromosomes remain visible.	
Prophase I • chromosomes visible • homologous chromosomes join together to form a bivalent		Prophase II • centrioles replicate and move to new poles.	

Metaphase I • bivalents line up on equator		Metaphase II • chromosomes line up on equator.	
Anaphase I • chromosomes separate (not chromatids- centromere doesn't split)		Anaphase II • centromeres split • chromatids separate.	
Telophase I • nuclei form • cell divides • cells have 2 chromosomes, not 4 chromatids.		Telophase II • 4 haploid cells, each with 2 chromatids • cells often stay together to form a tetrad.	

Genetic Variation in Sexual Reproduction

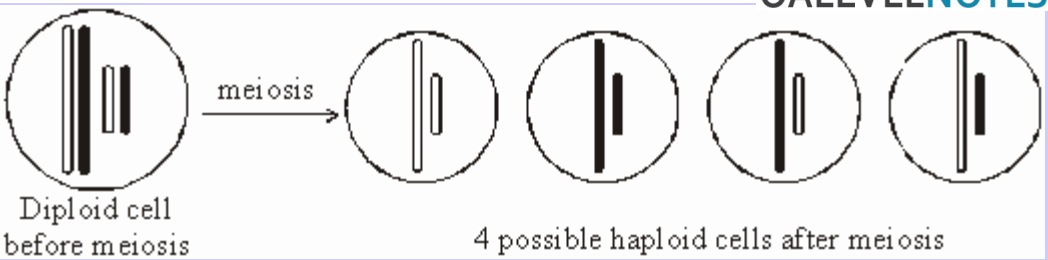
As mentioned in module 2, the whole point of meiosis and sex is to introduce genetic variation, which allows species to adapt to their environment and so to evolve. There are three sources of genetic variation in sexual reproduction:

- Independent assortment in meiosis
- Crossing over in meiosis
- Random fertilisation

We'll look at each of these in turn.

1. Independent Assortment

This happens at metaphase I in meiosis, when the bivalents line up on the equator. Each bivalent is made up of two homologous chromosomes, which originally came from two different parents (they're often called maternal and paternal chromosomes). Since they can line up in any orientation on the equator, the maternal and paternal versions of the different chromosomes can be mixed up in the final gametes.



In this simple example with 2 homologous chromosomes (n=2) there are 4 possible different gametes (2^2). In humans with n=23 there are over 8 million possible different gametes (2^{23}). Although this is an impressively large number, there is a limit to the mixing in that genes on the same chromosome must always stay together. This limitation is solved by crossing over.

2. Crossing Over

This happens at prophase I in meiosis, when the bivalents first form. While the two homologous chromosomes are joined in a bivalent, bits of one chromosome are swapped (crossed over) with the corresponding bits of the other chromosome.

The points at which the chromosomes actually cross over are called chiasmata (singular chiasma), and they involve large, multi-enzyme complexes that cut and join the DNA. There is always at least one chiasma in a bivalent, but there are usually many, and it is the chiasmata that actually hold the bivalent together. The chiasmata can be seen under the microscope and they can give the bivalents some strange shapes at prophase I. There are always equal amounts crossed over, so the chromosomes stay the same length.

Crossing over means that maternal and paternal alleles can be mixed, even though they are on the same chromosome.

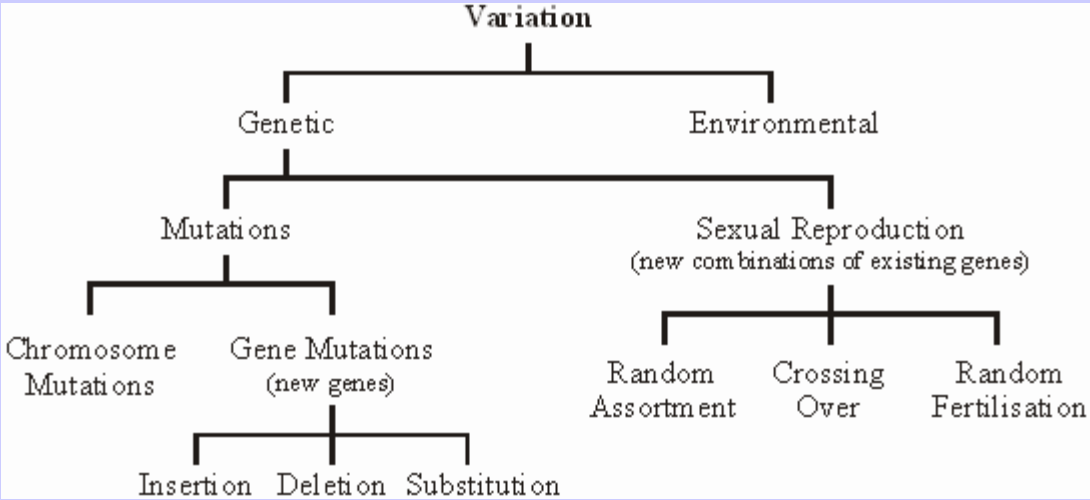
3. Random Fertilisation

This takes place when two gametes fuse to form a zygote. Each gamete has a unique combination of genes, and any of the numerous male gametes can fertilise any of the numerous female gametes. So every zygote is unique.

These three kinds of genetic recombination explain Mendel's laws of genetics.

Variation

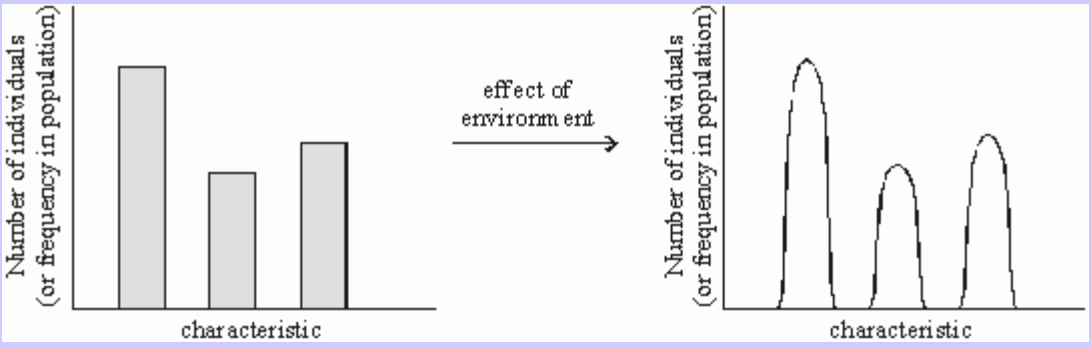
Variation means the differences in characteristics (phenotype) within a species. There are many causes of variation as this chart shows:



Variation in a population can be studied by measuring the characteristic (height, eye colour, seed shape, or whatever) in a large number of different individuals and then plotting a frequency histogram. This graph has the values of the characteristic on the X axis (grouped into bins if necessary) and the number of individuals showing that characteristic on the Y axis. These histograms show that there are two major types of variation: discontinuous and continuous.

Discontinuous Variation

Sometimes the characteristic has just a few discrete categories (like blood group). The frequency histogram has separate bars (or sometimes peaks).



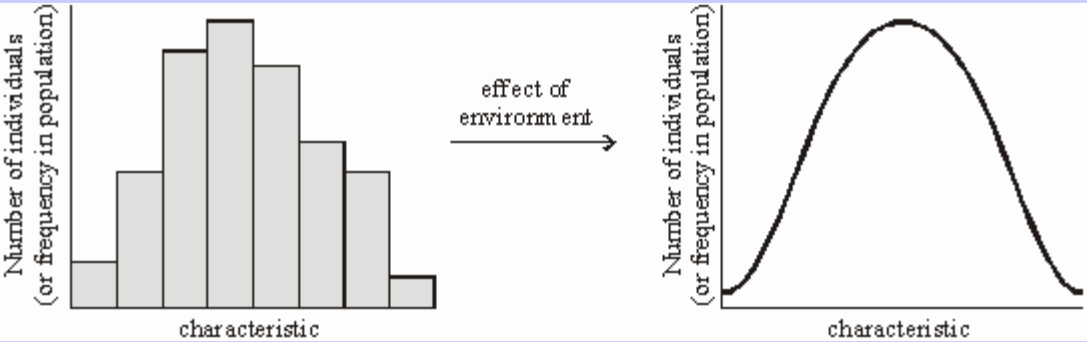
This is discontinuous variation. The characteristics:

- have distinct categories into which individuals can be placed
- tend to be qualitative, with no overlap between categories
- are controlled by one gene, or a small number of genes
- are largely unaffected by the environment

Discontinuous characteristics are rare in humans and other animals, but are more common in plants. Some examples are human blood group, detached ear lobes, flower colour, seed colour, etc. these characteristics are very useful for geneticists because they give clear-cut results.

Continuous Variation

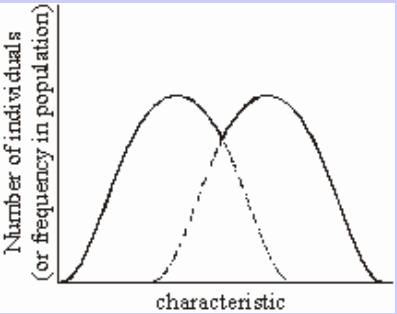
Sometimes the character has a continuous range of values (like height). The frequency histogram is a smooth curve (usually the bell-shaped normal distribution curve).



This is continuous variation. The characteristics:

- have no distinct categories into which individuals can be placed
- tend to be quantitative, with overlaps between categories
- are controlled by a large number of genes (polygenic)
- are significantly affected by the environment

Continuous characteristics are very common in humans and other animals. Some examples are height, hair colour, heart rate, muscle efficiency, intelligence, growth rate, rate of photosynthesis, etc.



Sometimes you can see the effect of both variations. For example the histogram of height of humans can be bimodal (i.e. it's got two peaks). This is because the two sexes (a discontinuous characteristic) each have their own normal distribution of height (a continuous characteristic).

Evolution and Natural Selection

History of ideas of Life on Earth

17th Century	Most people believed in Creationism, which considered that all life was created just as it is now. This was not based on any evidence, but was instead a belief.
18th Century	Naturalists began systematic classification systems (especially Linnaeus 1707-1778) and noticed that groups of living things had similar characteristics and appeared to be related. So their classifications looked a bit like a family tree. European naturalists travelled more widely and discovered more fossils, which clearly showed that living things had changed over time, so were not always the same. Extinctions were also observed (e.g. dodo), so species were not fixed.
19th Century	Lamark (1809) proposed a theory that living things changed by inheriting acquired characteristics. e.g. giraffes stretched their necks to reach food, and their offspring inherited stretched necks. This is now known to be wrong, since many experiments (and experience) have shown that acquired characteristics are not inherited, but nevertheless Lamark's theory was the first to admit that species changed, and to try to explain it. Charles Darwin (1859) published "*On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*", which has been recognised as one of the most important books ever written. A very similar theory was also proposed by Alfred Wallace, and Darwin and Wallace agreed to publish at the same time.

Darwin's Theory of Evolution by Natural Selection

Darwin's theory was based on four observations:

- Individuals within a species differ from each other - there is variation.
- Offspring resemble their parents- characteristics are inherited.
- Far more offspring are generally produced than survive to maturity - they suffer from predation, disease and competition.
- Populations are usually fairly constant is size.

Darwin's concluded that individuals that were better adapted to their environment compete better than the others, survive longer and reproduce more, so passing on more of their successful characteristics to the next

generation. Darwin used the memorable phrases *survival of the fittest*, *struggle for existence* and *natural selection*.

Darwin explained the giraffe's long neck as follows. In a population of horse-like animals there would be random genetic variation in neck length. In an environment where there were trees and bushes, the longer-necked animals were better adapted and so competed well compared to their shorter-necked relatives. These animals lived longer, through more breeding seasons, and so had more offspring. So in the next generation there were more long-neck genes than short-neck genes in the population. If this continued over very many generations, then in time the average neck length would increase. [Today it is thought more likely that the selection was for long legs to run away from predators faster, and if you have long legs you need a long neck to be able to drink. But the process of selection is just the same.]

Darwin wasn't the first to suggest evolution of species, but he was the first to suggest a plausible mechanism for the evolution - natural selection, and to provide a wealth of evidence for it.

Darwin used the analogy of selective breeding (or artificial selection) to explain natural selection. In selective breeding, desirable characteristics are chosen by humans, and only those individuals with the best characteristics are used for breeding. In this way species can be changed over a long period of time. All domesticated species of animal and plant have been selectively bred like this, often for thousands of years, so that most of the animals and plants we are most familiar with are not really natural and are nothing like their wild relatives (if any exist). The analogy between artificial and natural selection is a very good one, but there is one important different - Humans have a goal in mind, nature does not.

Types of Natural Selection

There are three kinds of Natural Selection.

1. Directional Selection

This occurs whenever the environment changes in a particular way. There is therefore selective pressure for species to change in response to the environmental change.

- The peppered moth (studied by Kettlewell). These light coloured moths are well camouflaged from bird predators against the pale bark of birch trees, while rare mutant dark moths are easily picked off. During the industrial revolution in the 19th century, birch woods near industrial centres became black with pollution. In this changed environment the black moths had a selective advantage and became the most common colour, while the pale moths were easily predated and became rare.

- Bacterial resistance to antibiotics. Antibiotics kill bacteria, but occasionally a chance mutant appears that is resistant to that antibiotic. In an environment where the antibiotic is often present, this mutant has an enormous selective advantage since all the normal (*wild type*) bacteria are killed leaving the mutant cell free to reproduce and colonise the whole environment without any competition. Some farmers routinely feed antibiotics to their animals to prevent infection, but this is a perfect environment for resistant bacteria to thrive. The best solution is to stop using the antibiotic so that the resistant strain has no selective advantage, and may die out.

  "Environment" includes biotic as well as abiotic, so organisms evolve in response to each other. e.g. if predators run faster there is selective pressure for prey to run faster, or if one tree species grows taller, there is selective pressure for other to grow tall. Most environments do change (e.g. due to migration of new species, or natural catastrophes, or climate change, or to sea level change, or continental drift, etc.), so directional selection is common.

2. Stabilising (or Normalising) Selection.

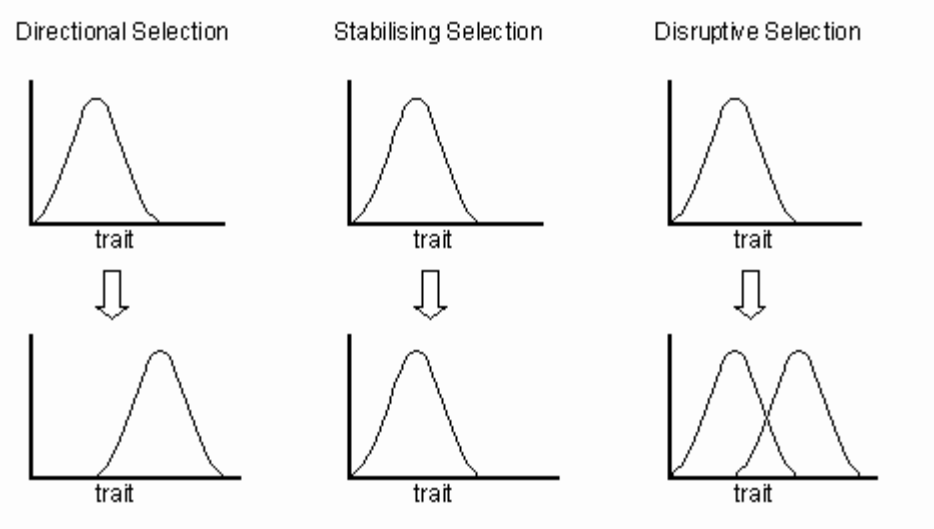
This occurs when the environment doesn't change. Natural selection doesn't have to cause change, and if an environment doesn't change there is no pressure for a well-adapted species to change. Fossils suggest that many species remain unchanged for long periods of geological time. One of the most stable environments on Earth is the deep ocean.

- The Coelocanth. This fish species was known only from ancient fossils and was assumed to have been extinct for 70 million years until a living specimen was found in a trawler net off South Africa in 1938. So this species has not changed in all that time.

3. Disruptive (or Diverging) Selection.

This occurs where an environment changes to become two close but distinct environments.

- Grass plants in Welsh Copper mines. Soil contaminated by copper from the mines in lethal to normal grass plants, but a chance mutation allowed one plant to grow. This plant prospered and reproduced, but only on the contaminated soil. On normal soil it grew more slowly than the normal plants and was easily out-competed. So now there are two varieties growing close together.



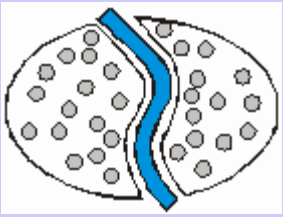
Speciation

A species is defined as a group of interbreeding populations that are reproductively isolated from other groups. Reproductively isolated can mean that sexual reproduction between different species is impossible for physical, ecological, behavioural, temporal or developmental reasons. For example horses and donkeys can apparently interbreed, but the offspring (mule) doesn't develop properly and is infertile. This definition does not apply to asexually reproducing species, and in some cases it is difficult distinguish between a strain and a species.

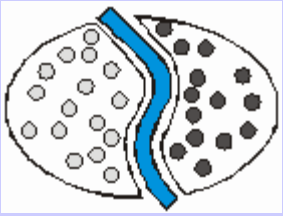
New species usually develop by reproductive isolation (e.g. Albert and Kaibab squirrels of the Grand Canyon).

1. Start with an interbreeding population of one species.

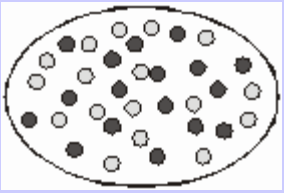
- The population becomes divided by a physical barrier such as water, mountains, desert, or just a large distance. This can happen when some of the population migrates or is dispersed, or when the geography changes catastrophically (e.g. earthquakes, volcanoes, floods) or gradually (erosion, continental drift).



- If the two environments (abiotic or biotic) are different (and they almost certainly will be), then the two populations will experience different selection pressures and will evolve separately. Even if the environments are similar, the populations may change by random genetic drift, especially if the population is small.



- Even if the barrier is removed and the two populations meet again, they are now so different that they can no longer interbreed. They are therefore reproductively isolated and are two distinct species. They may both be different from the original species, if it still exists elsewhere.



It is meaningless to say that one species is absolutely better than another species, only that it is better adapted to that particular environment. A species may be well-adapted to its environment, but if the environment changes, then the species must adapt or die. In either case the original species will become extinct. Since all environments change eventually, it is the fate of all species to become extinct (including our own).

Classification

There are some 10 million species of living organisms (mostly insects), and many more extinct ones, so they need to be classified in a systematic way. In 1753 the Swede Carolus Linnaeus introduced the binomial nomenclature for naming organisms. This consists of two parts: a generic name (with a capital letter) and a specific name (with a small letter), e.g. *Panthera leo* (lion) and *Panthera tigris* (tiger). This system replaced non-standard common names, and is still in use today.

A group of similar organisms is called a taxon, and the science of classification is called taxonomy. In taxonomy groups are based on similar physical or molecular properties, and groups are contained within larger composite groups with no overlap. The smallest group of similar organisms is the species; closely related species are grouped into genera (singular genus), genera into families, families into orders, orders

into classes, classes into phyla (singular phylum), and phyla into kingdoms. So you need to remember KPCOFGS.

This shows how the seven taxons are used to classify humans. As we go through the taxon hierarchy from kingdom to species, the groups get smaller and the animals are more closely related.

	Kingdom Animalia	Phylum Chordata	Class Mammalia	Order Primates	Family Hominidae	Genus *Homo*	Species *sapiens*
Sponge	4						
Earthworm	4						
Insect	4						
Fish	4	4					
Dinosaur E	4	4					
Bird	4	4					
Mouse	4	4	4				
Cat	4	4	4				
Elephant	4	4	4				
Lemur	4	4	4	4			
Monkey	4	4	4	4			
Orang-utan	4	4	4	4			
Gorilla	4	4	4	4	4		
Chimpanzee	4	4	4	4	4		
Australopithecus E	4	4	4	4	4		
Homo Habilis E	4	4	4	4	4	4	
Neanderthal Man E	4	4	4	4	4	4	4
Modern Human	4	4	4	4	4	4	4

E = Extinct

This shows the complete classification of some other species:

	Earthworm	Mushroom	Garlic
Kingdom	Animalia	Fungi	Plantae
Phylum	Annelida	Mycota	Angiospermophyta

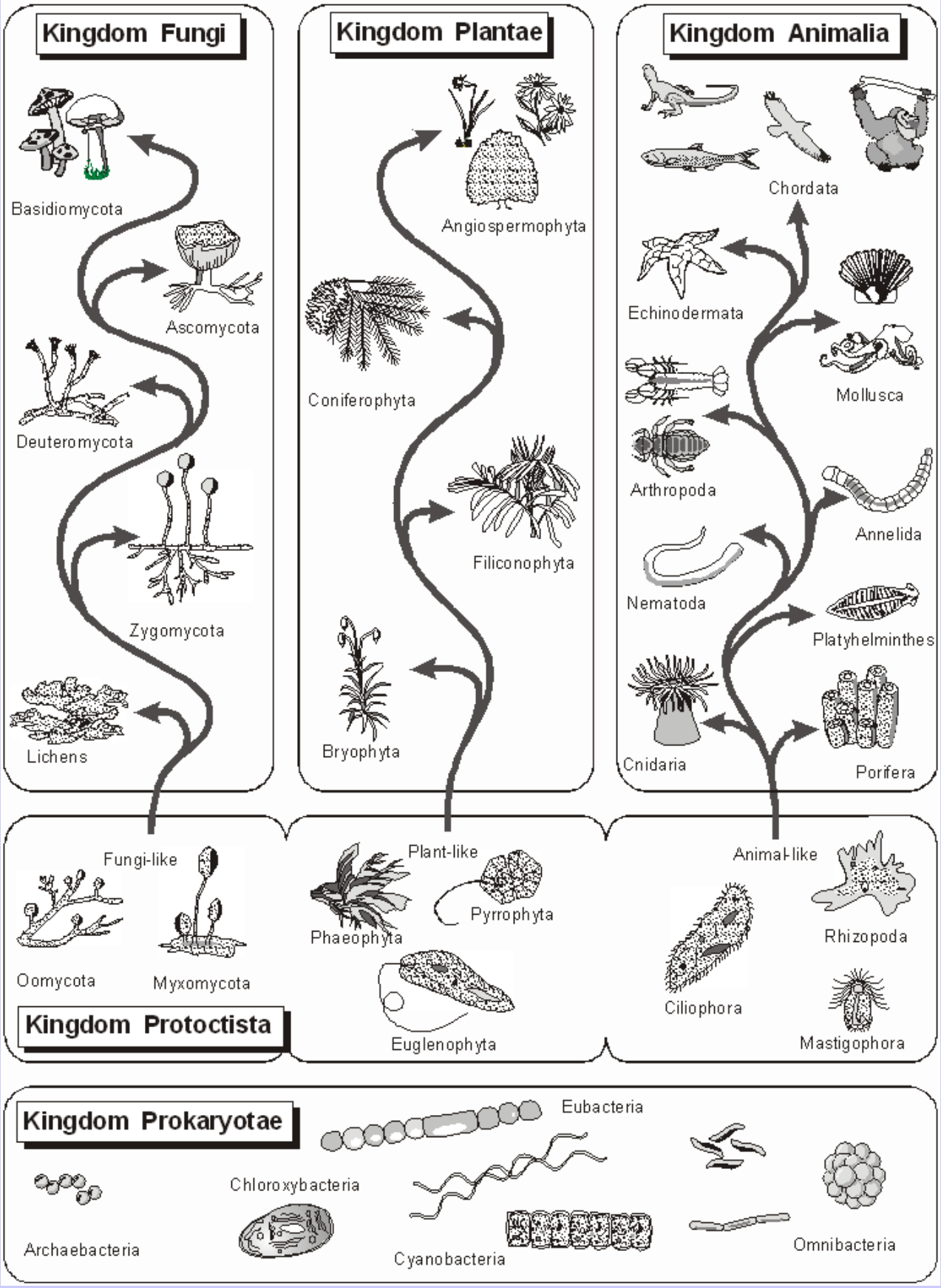
Class	Oligochaeta	Basidiomycota	Monocotyledonea
Order	Terricolae	Agaricales	Liliales
Family	Lumbricidae	Agaricacae	Liliaceae
Genus	*Lumbricus*	*Agaricus*	*Allium*
Species	*terrestris*	*campestris*	*sativum*

The aim of taxonomists today is to develop phylogenies, family trees representing true evolutionary relationships. Historically classification was based on easily observable structures, and gradually this was extended to microscopic and electron-microscopic detail. The recent advances in embryology and molecular biology have given new tools such as patterns of life cycle, larval development, and gene sequences. These have often led to radically different phylogenies (e.g. humans should really be the "third chimpanzee").

The Five Kingdoms

Until the middle of this century, life was divided into two kingdoms, plants and animals. With the greater understanding gained from new techniques this has been revised, and modern classifications recognise far more diversity and are less zoocentric. The classification system used today is that of Whittaker (1959, modified by Margulis), and contains five kingdoms: prokaryotae, protoctista, fungi, plantae and animalia. The greatest division now recognised is not between plants and animals (which are relatively similar), but between the prokaryotes (cells without nuclei) and eukaryotes (cells with nuclei). The three "higher" kingdoms are distinguished by their ecological strategies: absorption (fungi), consumption (animals) and production (plants).

The Five Kingdoms



MODULE 4

Muscles

Contents

Muscles

Muscle

ENGINE FOR SALE
Powerful (100W/kg)
Large Force (200kN/m^2)
Very Efficient (>50%)
Silent Operation
Non-Polluting
Doesn't Overheat (38°C)
Uses a Variety of Fuels
Lasts a Lifetime
Good to Eat
£10-00 per kg at your Supermarket

Muscle is indeed a remarkable tissue. In engineering terms it far superior to anything we have been able to invent, and it is responsible for almost all the movements in animals. There are three types of muscle:

- Skeletal muscle (striated, voluntary)

  This is always attached to the skeleton, and is under voluntary control via the motor neurones of the somatic nervous system. It is the most abundant & best understood type of muscle. It can be subdivided into red (slow) muscle and white (fast) muscle (see module 3).

- Cardiac Muscle

  This is special type of red skeletal muscle. It looks and works much like skeletal muscle, but is not attached to skeleton, and is not under voluntary control (see module 3 for details).

- Smooth Muscle

  This is found in internal body organs such as the wall of the gut, the uterus, blood arteries, the iris, and glandular ducts. It is under involuntary control via the autonomic nervous system or hormones. Smooth muscle usually forms a ring, which

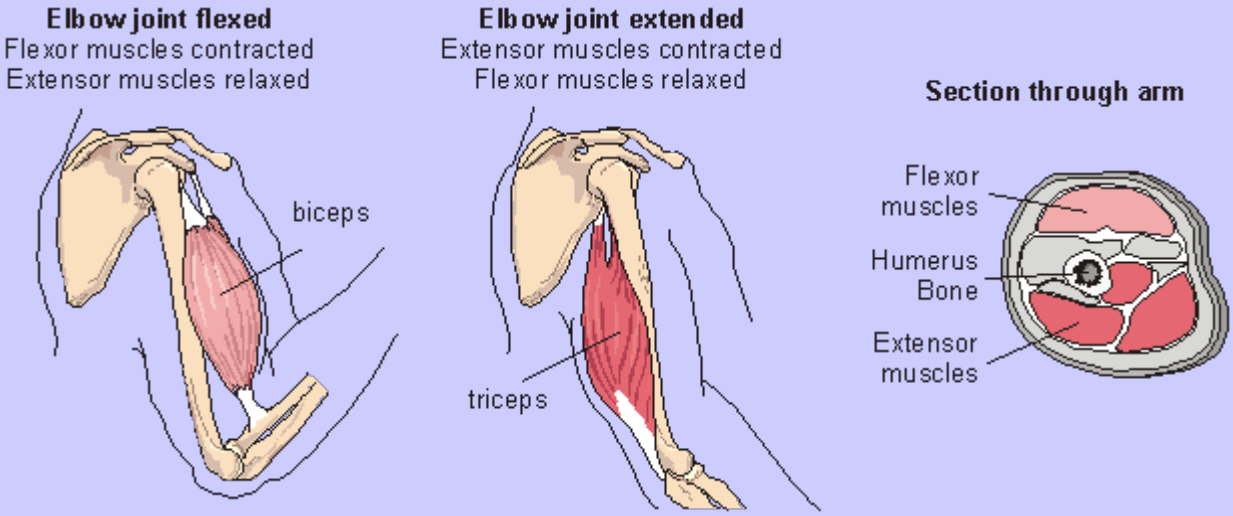
tightens when it contracts, so there is no need of a skeleton to pull against.

Unless mentioned otherwise, the rest of this section is about skeletal muscle.

Muscles and the Skeleton

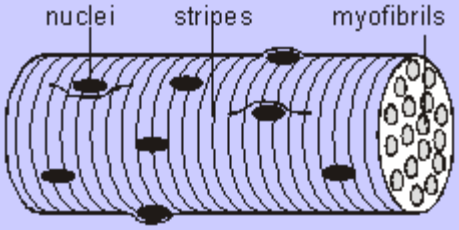
Skeletal muscles cause the skeleton to move (or articulate) at joints. They are attached to the skeleton by tendons, which transmit the muscle force to the bone and can also change the direction of the force. Tendons are made of collagen fibres and are very strong and stiff (i.e. not elastic). The non-moving attachment point (nearest to the trunk) is called the origin, and moving end (furthest from the trunk) is called the insertion. The skeleton provides leverage, magnifying either the movement or the force.

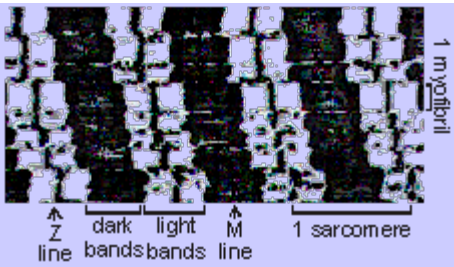
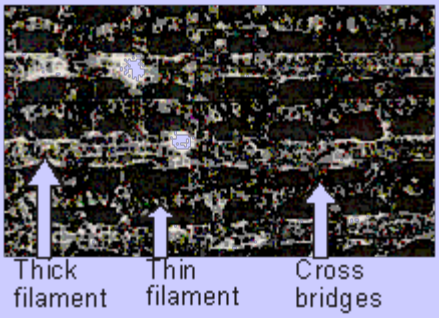
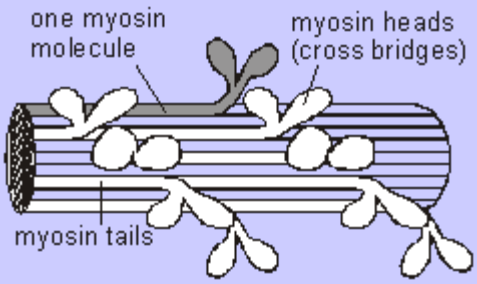
Muscles are either relaxed or contracted. In the relaxed state muscle is compliant (can be stretched), while in the contracted state muscle exerts a pulling force, causing it to shorten or generate force. Since muscles can only pull (not push), they work in pairs called antagonistic muscles. The muscle that bends (flexes) the joint is called the flexor muscle, and the muscle that straightens (extends) the joint is called the extensor muscle. The best-known example of antagonistic muscles are the biceps and triceps muscles, which articulate the elbow joint:



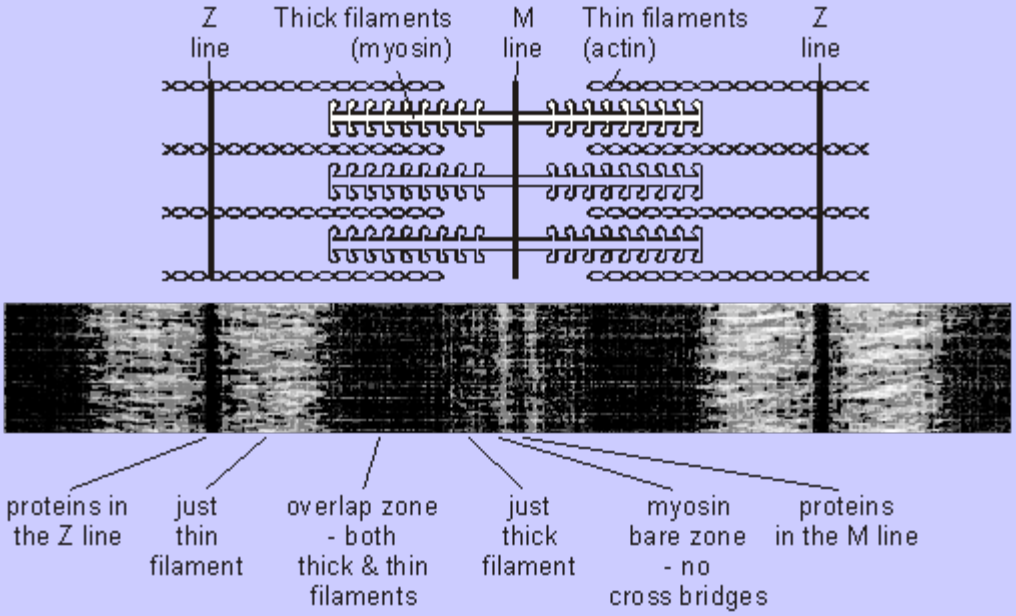
The "relaxed" muscle is actually never completely relaxed. It is always slightly contracted to provide resistance to the antagonistic muscle and so cause a smoother movement. This slightly contracted condition is called tonus, or muscle tone. Most movements also involve many muscles working together, e.g. to bend a finger or to smile. These groups of muscles are called synergistic muscles.

Muscle Structure

	A single muscle (such as the biceps) contains around 1000 muscle fibres running the whole length of the muscle and joined together at the tendons.
	Each muscle fibre is actually a single muscle cell about 100µm in diameter and a few cm long. These giant cells have many nuclei, as they were formed from the fusion of many smaller cells. Their cytoplasm is packed full of myofibrils, bundles of proteins filaments that cause contraction, and mitochondria to provide energy for contraction.
	The electron microscope shows that each myofibril is made up of repeating dark and light bands. In the middle of the dark band is a line called the M line and in the middle of the light band is a line called the Z line. The repeating unit from one Z line to the next is called a

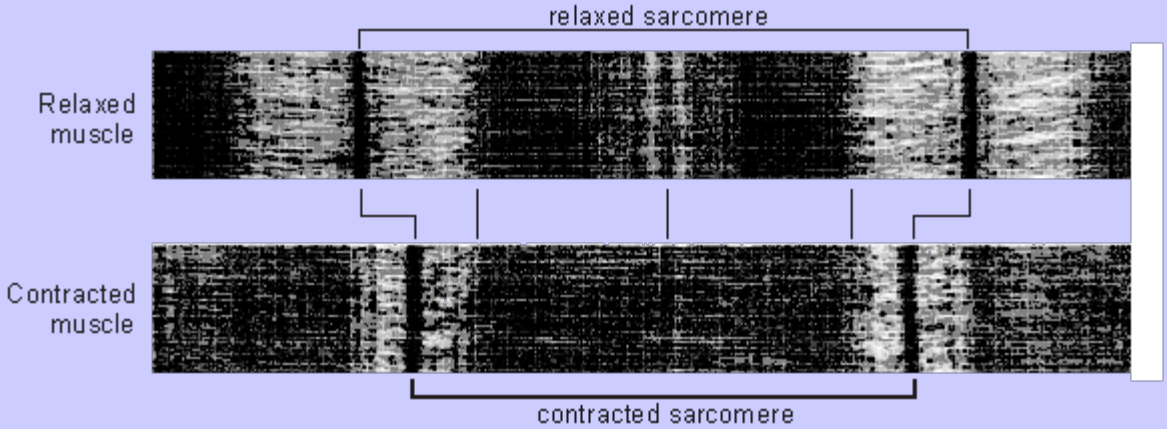
	sarcomere.
	A very high resolution electron micrograph shows that each myofibril is made of parallel filaments. There are two kinds of alternating filaments, called the thick and thin filaments. These two filaments are linked at intervals by blobs called cross bridges, which actually stick out from the thick filaments.
	The thick filament is made of a protein called myosin. A myosin molecule is shaped a bit like a golf club, but with two heads. Many of these molecules stick together to form the thick filament, with the "handles" lying together to form the backbone and the "heads" sticking out in all directions to form the cross bridges.
	The thin filament is made of a protein called actin. Actin is a globular molecule, but it polymerises to form a long double helix chain. The thin filament also contains troponin and tropomyosin, two proteins involved in the control of muscle contraction.

The thick and thin filaments are arranged in a precise lattice to form a sarcomere. The thick filaments are joined together at the M line, and the thin filaments are joined together at the Z line, but the two kinds of filaments are not joined to each other. The position of the filaments in the sarcomere explains the banding pattern seen by the electron microscope:



Mechanism Of Muscle Contraction- the Sliding Filament Theory

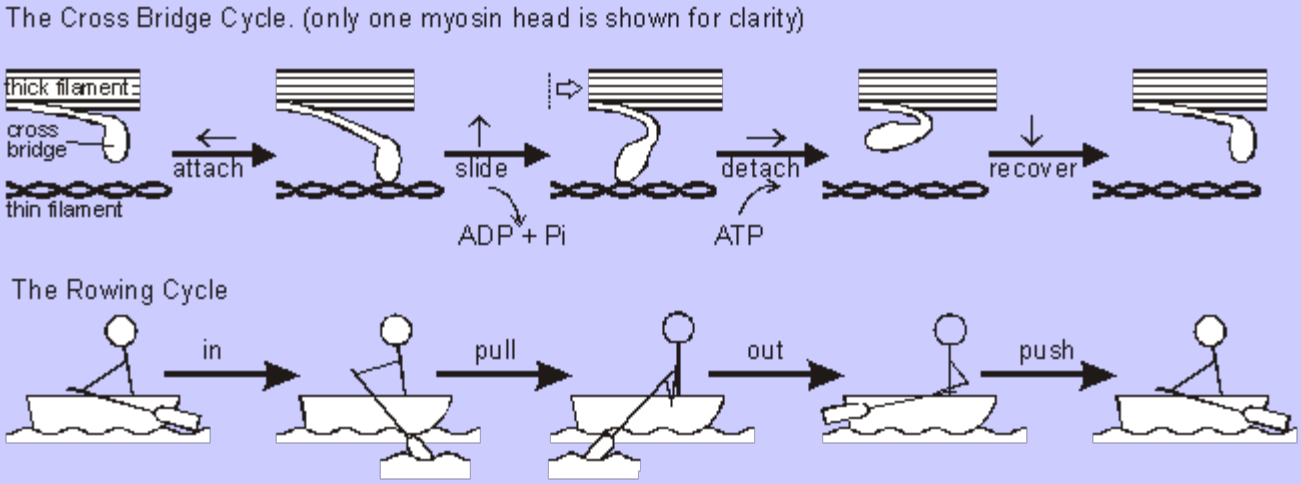
Knowing the structure of the sarcomere enables us to understand what happens when a muscle contracts. The mechanism of muscle contraction can be deduced by comparing electron micrographs of relaxed and contracted muscle:



These show that each sarcomere gets shorter when the muscle contracts, so the whole muscle gets shorter. But the dark band, which represents the thick filament, does not change in length. This shows that the filaments don't contract themselves, but instead they slide past each other. This sliding filament theory was first proposed by Huxley and Hanson in 1954, and has been confirmed by many experiments since.

The Cross Bridge Cycle

What makes the filaments slide past each other? Energy is provided by the splitting of ATP, and the ATPase that does this splitting is located in the myosin cross bridge head. These cross bridges can also attach to actin, so they are able to cause the filament sliding by "walking" along the thin filament. This cross bridge walking is called the cross bridge cycle, and it has 4 steps. One step actually causes the sliding, while the other 3 simply reset the cross bridge back to its starting state. It is analogous to the 4 steps involved in rowing a boat:



1. The cross bridge swings out from the thick filament and attaches to the thin filament. [Put oars in water.]

2. The cross bridge changes shape and rotates through 45°, causing the filaments to slide. The energy from ATP splitting is used for this "power stroke" step, and the products (ADP + Pi) are released. [Pull oars to drive boat through water.]

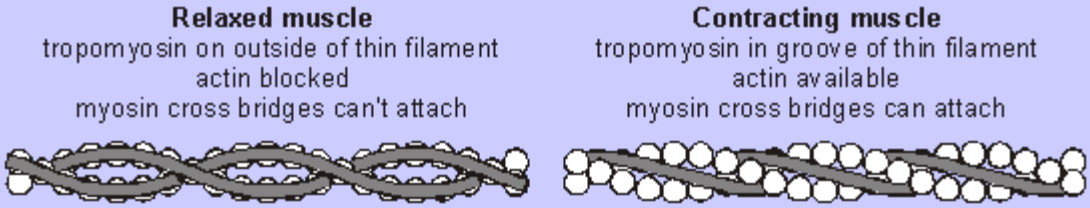
3. A new ATP molecule binds to myosin and the cross bridge detaches from the thin filament. [push oars out of water.]

4. The cross bridge changes back to its original shape, while detached (so as not to push the filaments back again). It is now ready to start a new cycle, but further along the thin filament. [push oars into starting position.]

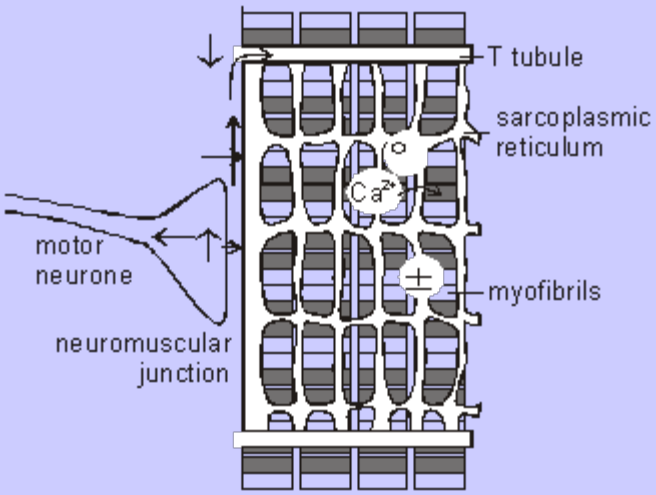
One ATP molecule is split by each cross bridge in each cycle, which takes a few milliseconds. During a contraction, thousands of cross bridges in each sarcomere go through this cycle thousands of times, like a millipede running along the ground. Fortunately the cross bridges are all out of synch, so there are always many cross bridges attached at any time to maintain the force.

Control Of Muscle Contraction

How is the cross bridge cycle switched off in a relaxed muscle? This is where the regulatory proteins on the thin filament, troponin and tropomyosin, are involved. Tropomyosin is a long thin molecule, and it can change its position on the thin filament. In a relaxed muscle is it on the outside of the filament, covering the actin molecules so that myosin cross bridges can't attach. This is why relaxed muscle is compliant: there are no connections between the thick and thin filaments. In a contracting muscle the tropomyosin has moved into the groove of the double helix, revealing the actin molecules and allowing the cross bridges to attach.



Contraction of skeletal muscle is initiated by a nerve impulse, and we can now look at the sequence of events from impulse to contraction (sometimes called excitation contraction coupling).



1. An action potential arrives at the end of a motor neurone, at the neuromuscular junction.

2. This causes the release of the neurotransmitter acetylcholine.

3 This initiates an action potential in the muscle cell membrane.

4. This action potential is carried quickly throughout the large muscle cell by invaginations in the cell membrane called T-tubules.

5. The action potential causes the sarcoplasmic reticulum (large membrane vesicles) to release its store of calcium into the myofibrils.

6. The calcium binds to troponin on the thin filament, which changes shape, moving tropomyosin into the groove in the process.

7. Myosin cross bridges can now attach and the cross bridge cycle can take place.

Relaxation is the reverse of these steps. This process may seem complicated, but it allows for very fast responses so that we can escape from predators and play the piano.

back home

MODULE 4

Nervous Communication

Contents

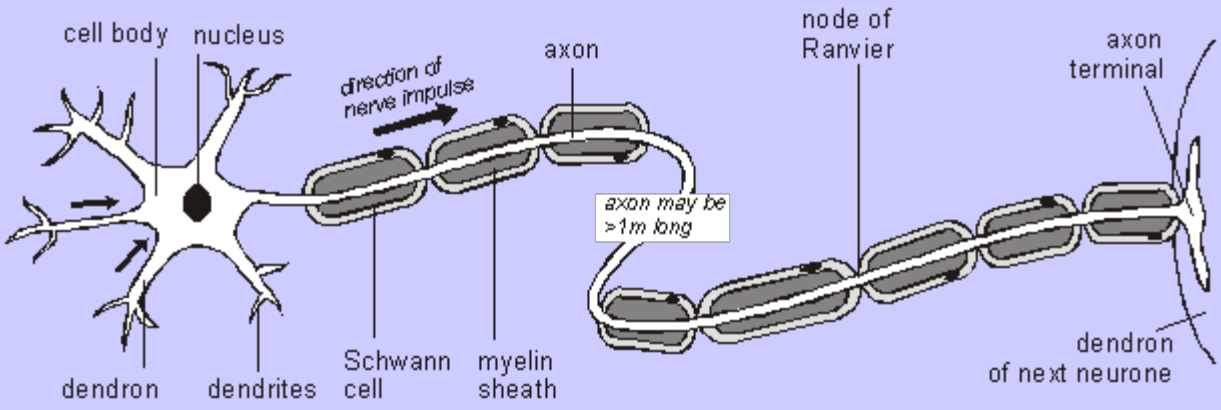
- Nerve Cells
- The Reflex Arc
- The Nerve Impulse
- Synapses
- Drugs
- The Brain

The Human Nervous System

Humans, like all living organisms, can respond to their environment. Humans have two complimentary control systems to do this: the nervous system and the endocrine (hormonal) system. We'll look at the endocrine system later, but first we'll look at the nervous system. The human nervous system controls everything from breathing and producing digestive enzymes, to memory and intelligence.

Nerve Cells

The nervous system composed of nerve cells, or neurones:



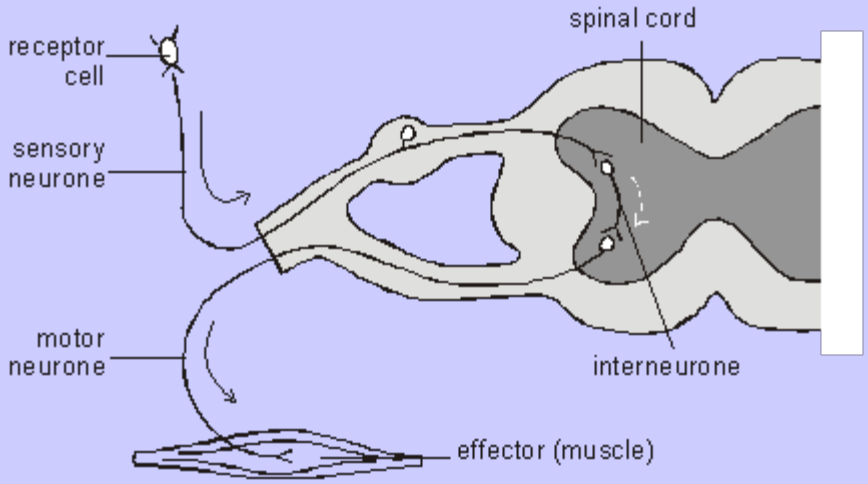
A neurone has a cell body with extensions leading off it. Numerous dendrons and dendrites provide a large surface area for connecting with other neurones, and carry nerve impulses towards the cell body. A single long axon carries the nerve impulse away from the cell body. The axon is only 10µm in diameter but can be up to 4m in length in a large animal (a piece of spaghetti the same shape would be 400m long)! Most neurones have many companion cells called Schwann cells, which wrap their cell membrane around the axon many times in a spiral to form a thick insulating lipid layer called the myelin sheath. Nerve impulse can be passed from the axon of one neurone to the dendron of another at a synapse. A nerve is a discrete bundle of several thousand neurone axons.

Humans have three types of neurone:

- Sensory neurones have long axons and transmit nerve impulses from sensory receptors all over the body to the central nervous system.
- Motor neurones also have long axons and transmit nerve impulses from the central nervous system to effectors (muscles and glands) all over the body.
- Interneurones (also called connector neurones or relay neurones) are usually much smaller cells, with many interconnections.

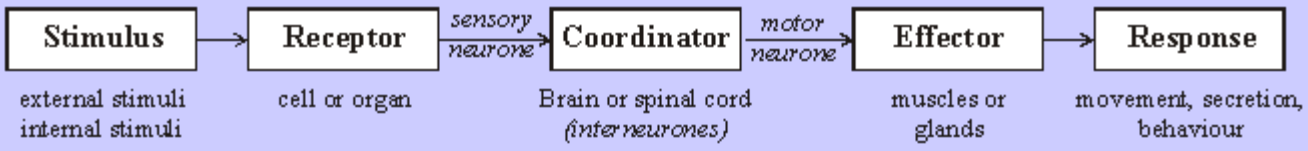
The Reflex Arc

The three types of neurones are arranged in circuits and networks, the simplest of which is the reflex arc.



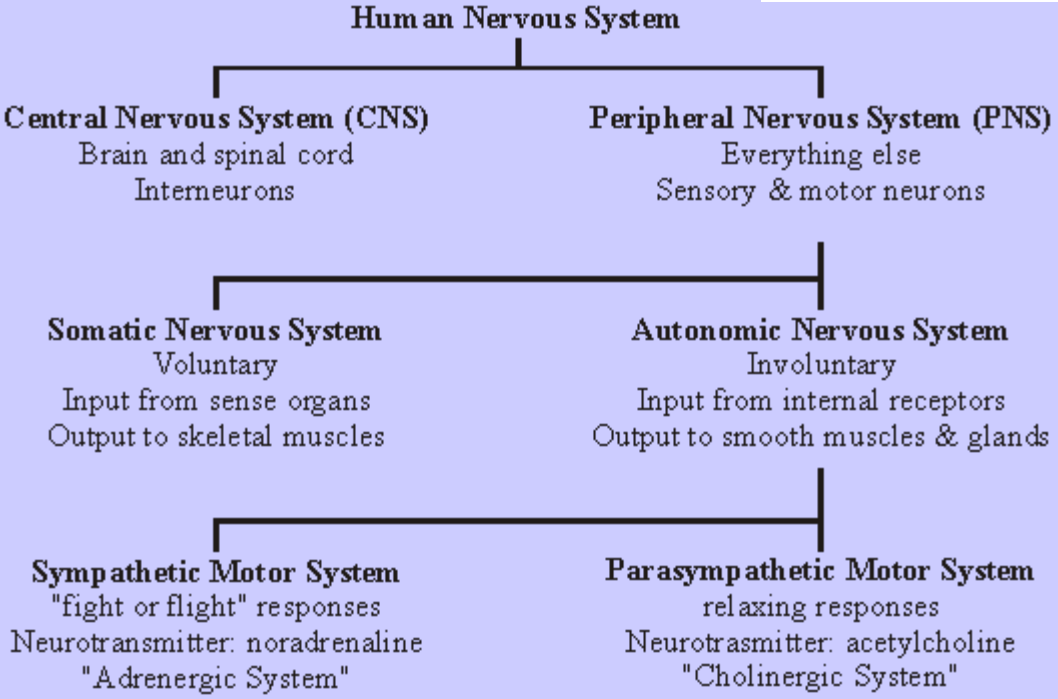
In a simple reflex arc, such as the knee jerk, a stimulus is detected by a receptor cell, which synapses with a sensory neurone. The sensory neurone carries the impulse from site of the stimulus to the central nervous system (the brain or spinal cord), where it synapses with an interneurone. The interneurone synapses with a motor neurone, which carries the nerve impulse out to an effector, such as a muscle, which responds by contracting.

Reflex arc can also be represented by a simple flow diagram:



The Organisation Of The Human Nervous System

The human nervous system is far more complex than a simple reflex arc, although the same stages still apply. The organisation of the human nervous system is shown in this diagram:



It is easy to forget that much of the human nervous system is concerned with routine, involuntary jobs, such as homeostasis, digestion, posture, breathing, etc. This is the job of the autonomic nervous system, and its motor functions are split into two divisions, with anatomically distinct neurones. Most body organs are innervated by two separate sets of motor neurones; one from the sympathetic system and one from the parasympathetic system. These neurones have opposite (or antagonistic) effects. In general the sympathetic system stimulates the "fight or flight" responses to threatening situations, while the parasympathetic system relaxes the body. The details are listed in this table:

Organ	Sympathetic System	Parasympathetic System
Eye	Dilates pupil	Constricts pupil
Tear glands	No effect	Stimulates tear secretion
Salivary glands	Inhibits saliva production	Stimulates saliva production
Lungs	Dilates bronchi	Constricts bronchi
Heart	Speeds up heart rate	Slows down heart rate
Gut	Inhibits peristalsis	Stimulates peristalsis
Liver	Stimulates glucose production	Stimulates bile production
Bladder	Inhibits urination	Stimulates urination

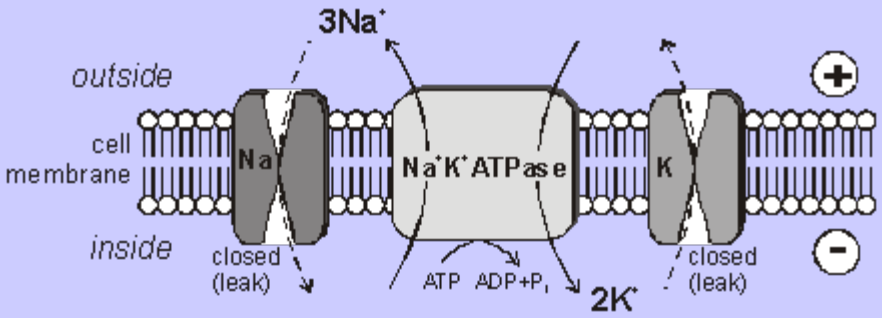
The Nerve Impulse

Neurones and muscle cells are electrically excitable cells, which means that they can transmit electrical nerve impulses. These impulses are due to events in the cell membrane, so to understand the nerve impulse we need to revise some properties of cell membranes.

The Membrane Potential

All animal cell membranes contain a protein pump called the $Na^{+}K^{+}$ATPase. This uses the energy from ATP splitting to simultaneously pump 3 sodium ions out of the cell and 2 potassium ions in. If this was to continue unchecked there would be no

sodium or potassium ions left to pump, but there are also sodium and potassium ion channels in the membrane. These channels are normally closed, but even when closed, they "leak", allowing sodium ions to leak in and potassium ions to leak out, down their respective concentration gradients.

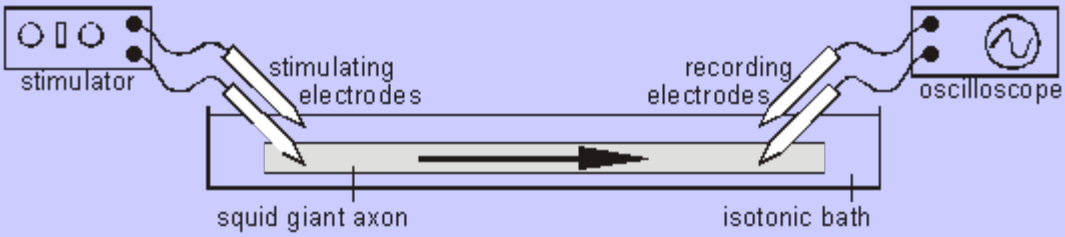


The combination of the Na^+K^+ATPase pump and the leak channels cause a stable imbalance of Na^+ and K^+ ions across the membrane. This imbalance causes a potential difference across all animal cell membranes, called the membrane potential. The membrane potential is always negative inside the cell, and varies in size from –20 to –200 mV in different cells and species. The Na^+K^+ATPase is thought to have evolved as an osmoregulator to keep the internal water potential high and so stop water entering animal cells and bursting them. Plant cells don't need this as they have strong cells walls to prevent bursting.

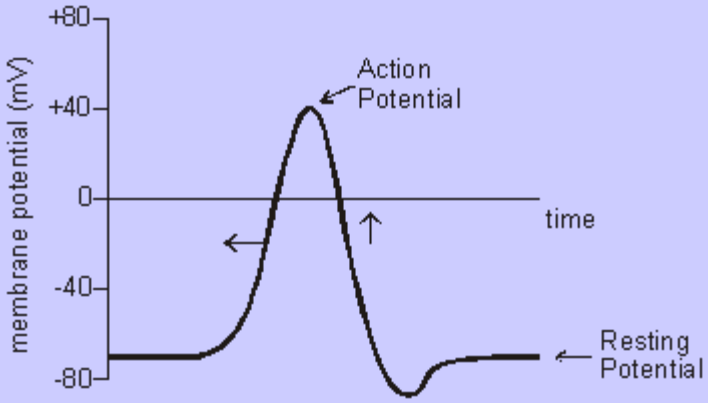
The Action Potential

In nerve and muscle cells the membranes are electrically excitable, which means that they can change their membrane potential, and this is the basis of the nerve impulse. The sodium and potassium channels in these cells are voltage-gated, which means that they can open and close depending on the voltage across the membrane.

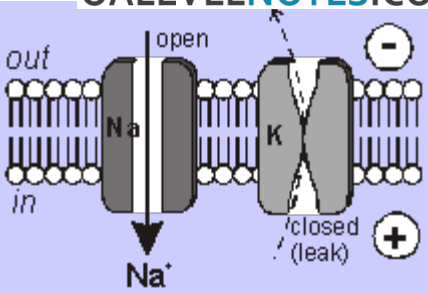
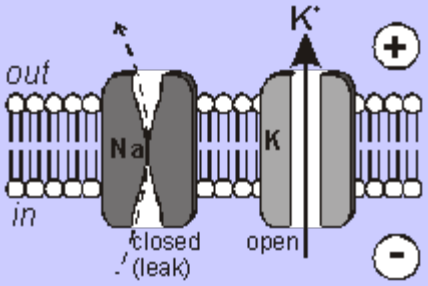
The nature of the nerve impulse was discovered by Hodgkin, Huxley and Katz in Plymouth in the 1940s, for which work they received a Nobel prize in 1963. They used squid giant neurones, whose axons are almost 1mm in diameter, big enough to insert wire electrodes so that they could measure the potential difference across the cell membrane. In a typical experiment they would apply an electrical pulse at one end of an axon and measure the voltage changes at the other end, using an oscilloscope:



The normal membrane potential of these nerve cells is –70mV (inside the axon), and since this potential can change in nerve cells it is called the resting potential. When a stimulating pulse was applied a brief reversal of the membrane potential, lasting about a millisecond, was recorded. This brief reversal is called the action potential:



The action potential has 2 phases called depolarisation and repolarisation.

Depolarisation. The stimulating electrodes cause the membrane potential to change a little. The voltage-gated ion channels can detect this change, and when the potential reaches –30mV the sodium channels open for 0.5ms. The causes sodium ions to rush in, making the inside of the cell more positive. This phase is referred to as a depolarisation since the normal voltage polarity (negative inside) is reversed (becomes positive inside).	
Repolarisation. When the membrane potential reaches 0V, the potassium channels open for 0.5ms, causing potassium ions to rush out, making the inside more negative again. Since this restores the original polarity, it is called repolarisation.	

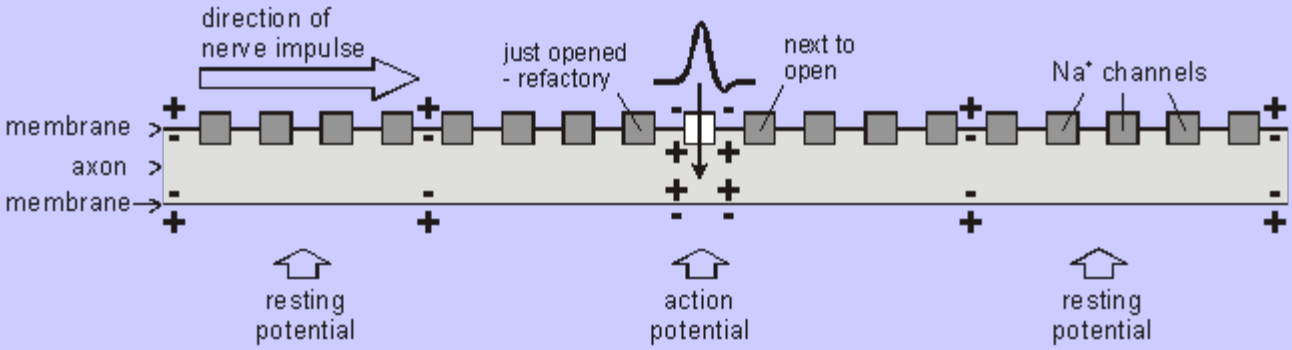
The Na^+K^+ATPase pump runs continuously, restoring the resting concentrations of sodium and potassium ions.

How do Nerve Impulses Start?

In the squid experiments the action potential was initiated by the stimulating electrodes. In living cells they are started by receptor cells. These all contain special sodium channels that are not voltage-gated, but instead are gated by the appropriate stimulus (directly or indirectly). For example chemical-gated sodium channels in tongue taste receptor cells open when a certain chemical in food binds to them; mechanically-gated ion channels in the hair cells of the inner ear open when they are distorted by sound vibrations; and so on. In each case the correct stimulus causes the sodium channel to open; which causes sodium ions to flow into the cell; which causes a depolarisation of the membrane potential, which affects the voltage-gated sodium channels nearby and starts an action potential.

How are Nerve Impulses Propagated?

Once an action potential has started it is moved (propagated) along an axon automatically. The local reversal of the membrane potential is detected by the surrounding voltage-gated ion channels, which open when the potential changes enough.



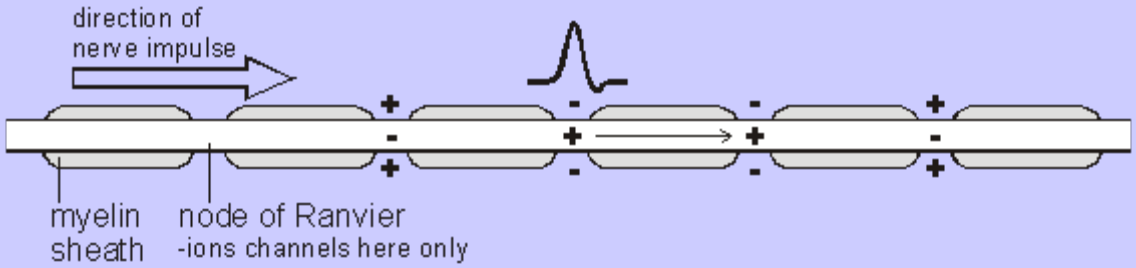
The ion channels have two other features that help the nerve impulse work effectively:

- After an ion channel has opened, it needs a "rest period" before it can open again. This is called the refractory period, and lasts about 2 ms. This means that, although the action potential affects all other ion channels nearby, the upstream ion channels cannot open again since they are in their refractory period, so only the downstream channels open, causing the action potential to move one-way along the axon.
- The ion channels are either open or closed; there is no half-way position. This means that the action potential always reaches +40mV as it moves along an axon, and it is never attenuated (reduced) by long axons. In other word the action potential is all-or-nothing.

How Fast are Nerve Impulses?

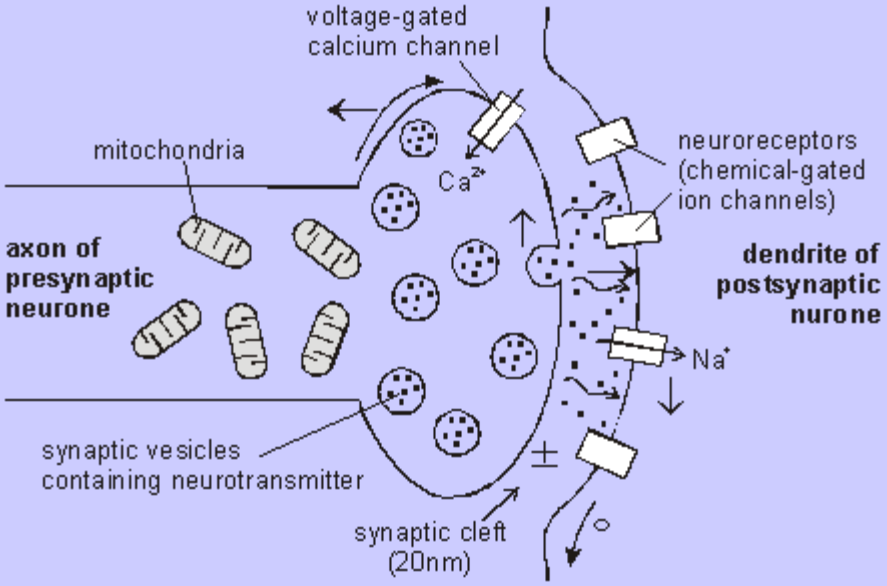
Action potentials can travel along axons at speeds of 0.1-100 m/s. This means that nerve impulses can get from one part of a body to another in a few milliseconds, which allows for fast responses to stimuli. (Impulses are much slower than electrical currents in wires, which travel at close to the speed of light, $3x10^8$ m/s.) The speed is affected by 3 factors:

- Temperature. The higher the temperature, the faster the speed. So homoeothermic (warm-blooded) animals have faster responses than poikilothermic (cold-blooded) ones.
- Axon diameter. The larger the diameter, the faster the speed. So marine invertebrates, who live at temperatures close to 0°C, have developed thick axons to speed up their responses. This explains why squid have their giant axons.
- Myelin sheath. Only vertebrates have a myelin sheath surrounding their neurones. The voltage-gated ion channels are found only at the nodes of Ranvier, and between the nodes the myelin sheath acts as a good electrical insulator. The action potential can therefore jump large distances from node to node (1mm), a process that is called saltatory propagation. This increases the speed of propagation dramatically, so while nerve impulses in unmyelinated neurones have a maximum speed of around 1 m/s, in myelinated neurones they travel at 100 m/s.



Synapses

The junction between two neurones is called a synapse. An action potential cannot cross the synaptic cleft between neurones, and instead the nerve impulse is carried by chemicals called neurotransmitters. These chemicals are made by the cell that is sending the impulse (the pre-synaptic neurone) and stored in synaptic vesicles at the end of the axon. The cell that is receiving the nerve impulse (the post-synaptic neurone) has chemical-gated ion channels in its membrane, called neuroreceptors. These have specific binding sites for the neurotransmitters.



1. At the end of the pre-synaptic neurone there are voltage-gated calcium channels. When an action potential reaches the synapse these channels open, causing calcium ions to flow into the cell.

2. These calcium ions cause the synaptic vesicles to fuse with the cell membrane, releasing their contents (the neurotransmitter chemicals) by exocytosis.

3. The neurotransmitters diffuse across the synaptic cleft.

4. The neurotransmitter binds to the neuroreceptors in the post-synaptic membrane, causing the channels to open. In the example shown these are sodium channels, so sodium ions flow in.

5. This causes a depolarisation of the post-synaptic cell membrane, which may initiate an action potential.

6. The neurotransmitter is broken down by a specific enzyme in the synaptic cleft; for example the enzyme acetylcholinesterase breaks down the neurotransmitter acetylcholine. The breakdown products are absorbed by the pre-synaptic neurone by endocytosis and used to re-synthesise more neurotransmitter, using energy from the mitochondria. This stops the synapse being permanently on.

Different Types of Synapse

The human nervous system uses a number of different neurotransmitter and neuroreceptors, and they don't all work in the same way. We can group synapses into 5 types:

1. Excitatory Ion Channel Synapses.

These synapses have neuroreceptors that are sodium channels. When the channels open, positive ions flow in, causing a local depolarisation and making an action potential more likely. This was the kind of synapse described above. Typical neurotransmitters are acetylcholine, glutamate or aspartate.

2. Inhibitory Ion Channel Synapses.

These synapses have neuroreceptors that are chloride channels. When the channels open, negative ions flow in causing a local hyperpolarisation and making an action potential less likely. So with these synapses an impulse in one neurone can inhibit an impulse in the next. Typical neurotransmitters are glycine or GABA.

3. Non Channel Synapses.

These synapses have neuroreceptors that are not channels at all, but instead are membrane-bound enzymes. When activated by the neurotransmitter, they catalyse the production of a "messenger chemical" inside the cell, which in turn can affect many aspects of the cell's metabolism. In particular they can alter the number and sensitivity of the ion channel receptors in the same cell. These synapses are involved in slow and long-lasting responses like learning and memory. Typical neurotransmitters are adrenaline, noradrenaline (NB adrenaline is called epinephrine in America), dopamine, serotonin, endorphin, angiotensin, and acetylcholine.

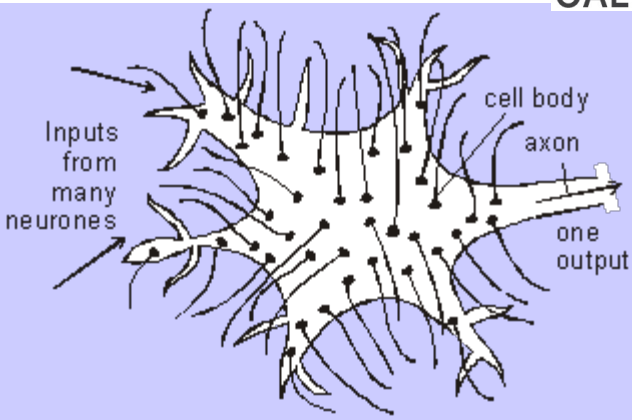
4. Neuromuscular Junctions.

These are the synapses formed between motor neurones and muscle cells. They always use the neurotransmitter acetylcholine, and are always excitatory. We shall look at these when we do muscles. Motor neurones also form specialised synapses with secretory cells.

5. Electrical Synapses.

In these synapses the membranes of the two cells actually touch, and they share proteins. This allows the action potential to pass directly from one membrane to the next. They are very fast, but are quite rare, found only in the heart and the eye.

Summation



One neurone can have thousands of synapses on its body and dendrons. So it has many inputs, but only one output. The output through the axon is called the Grand Postsynaptic Potential (GPP) and is the sum of all the excitatory and inhibitory potentials from all that cell's synapses. If there are more excitatory potentials than inhibitory ones then there will be a GPP, and the neurone will "fire", but if there are more inhibitory potentials than excitatory ones then there will not be a GPP and the neurone will not fire.

This summation is the basis of the processing power in the nervous system. Neurones (especially interneurones) are a bit like logic gates in a computer, where the output depends on the state of one or more inputs. By connecting enough logic gates together you can make a computer, and by connecting enough neurones together to can make a nervous system, including a human brain.

Drugs

Almost all drugs taken by humans (medicinal and recreational) affect the nervous system. From our understanding of the human nervous system we can understand how many common drugs work. Drugs can affect the nervous system in various ways, shown in this table:

Drug action	Effect
Mimic a neurotransmitter	Switch on a synapse
Stimulate the release of a neurotransmitter	Switch on a synapse
Open a neuroreceptor channel	Switch on a synapse
Block a neuroreceptor channel	Switch off a synapse
Inhibit the breakdown enzyme	Switch on a synapse
Inhibit the Na^+K^+ATPase pump	Stop action potentials
Block the Na^+ or K^+ channels	Stop action potentials

Drugs that stimulate a nervous system are called agonists, and those that inhibit a system are called antagonists. By designing drugs to affect specific neurotransmitters or neuroreceptors, drugs can be targeted at different parts of the nervous system. The following paragraph describe the action of some common drugs. You do not need to know any of this, but you should be able to understand how they work.

1. Drugs acting on the central nervous system

In the reticular activating system (RAS) in the brain stem noradrenaline receptors are excitatory and cause wakefulness, while GABA receptors are inhibitory and cause drowsiness. Caffeine (in coffee, cocoa and cola), theophylline (in tea), amphetamines, ecstasy (MDMA) and cocaine all promote the release of noradrenaline in RAS, so are stimulants. Antidepressant drugs, such as the tricyclics, inhibit the breakdown and absorption of noradrenaline, so extending its effect. Alcohol, benzodiazepines (e.g. mogadon, valium, librium), barbiturates, and marijuana all activate GABA receptors, causing more inhibition of RAS and so are tranquillisers, sedatives and depressants. The narcotics or opioid group of drugs, which include morphine, codeine, opium, methadone and diamorphine (heroin), all block opiate receptors, blocking transmission of pain signals in the brain and spinal chord. The brain's natural endorphins appear to have a similar action.

The brain neurotransmitter dopamine has a number of roles, including muscle control, pain inhibition and general stimulation. Some psychosis disorders such as schizophrenia and manic depression are caused by an excess of dopamine, and antipsychotic drugs are used to block the dopamine receptors and so reduce its effects. Parkinson's disease (shaking of head and limbs) is caused by too little dopamine compared to acetylcholine production in the midbrain. The balance can be restored with levodopa, which mimics dopamine, or with anticholinergic drugs (such as procyclidine), which block the muscarinic acetylcholine receptors.

Tetrodotoxin (from the Japanese puffer fish) blocks voltage-gated sodium channels, while tetraethylamonium blocks the voltage-gated potassium channel. Both are powerful nerve poisons. General anaesthetics temporarily inhibit the sodium channels. Strychnine blocks glycine receptors in the brain, causing muscle convulsions and death.

2. Drugs acting on the somatic nervous system

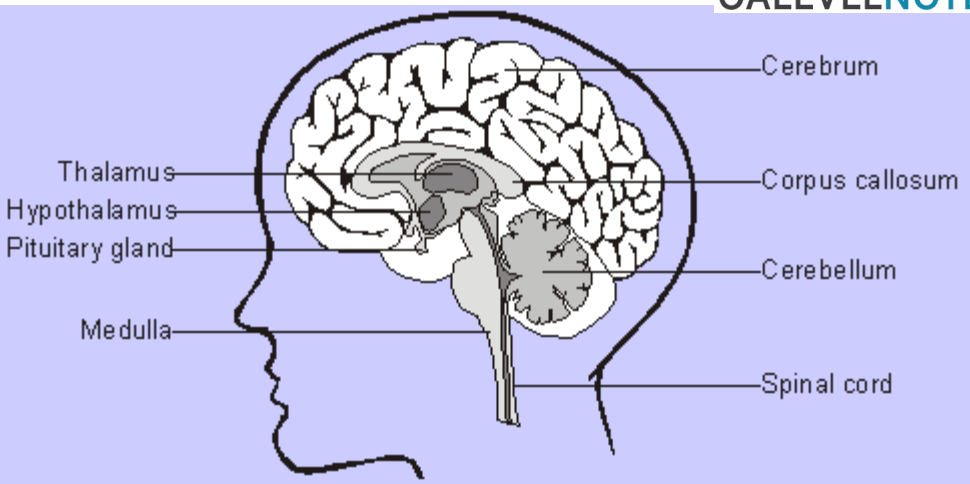
Curare and abungarotoxin (both snake venoms) block the nicotinic acetylcholine receptors in the somatic nervous system, and so relax skeletal muscle. *Myasthenia gravis* (a weakening of the muscles in the face and throat caused by inactive nicotinic acetylcholine receptors) is treated by the drug neostigmine, which inhibits acetylcholinesterase, so increasing the amount of acetylcholine at the neuromuscular junction. Nerve gas and organophosphate insecticides (DDT) inhibit acetylcholinesterase, so nicotinic acetylcholine receptors are always active, causing muscle spasms and death. Damaged tissues release prostaglandins, which stimulate pain neurones (amongst other things). The non-narcotic analgesics such as aspirin, paracetamol and ibuprofen block prostaglandin production at source of pain, while paracetamol has a similar effect in the brain. Local anaesthetics such as procaine block all sensory and motor synapses at the site of application.

3. Drugs acting on the autonomic nervous system

Sympathetic agonists like salbutamol and isoprenaline, activate the adrenergic receptors in the sympathetic system, encouraging smooth muscle relaxation, and are used as bronchodilators in the treatment of asthma. Sympathetic antagonists like the beta blockers block the noradrenaline receptors in the sympathetic nervous system. They cause dilation of blood vessels in the treatment of high blood pressure and migraines, and reduce heartbeat rate in the treatment of angina and abnormal heart rhythms. Parasympathetic antagonists like atropine (from the deadly nightshade *belladonna*) inhibit the muscarinic acetylcholine receptors in parasympathetic system, and are used as eye drops to relax the ciliary muscles in the eye.

The Brain

The human brain is the site of the major coordination in the nervous system. It contains around 10^{10} neurones, each making thousands of connections to others, so the number of pathways through the brain is vast. Different regions of the brain can be identified by their appearance, and it turns out that each region has a different role.



- The medulla controls heart rate, breathing, peristalsis, and reflexes such as swallowing, coughing, sneezing and vomiting.
- The Hypothalamus controls temperature homeostasis, water homeostasis, and controls the release of hormones by the pituitary gland.
- The pituitary gland secretes a range of hormones including LH, FSH, ADH, and growth hormone.
- The Thalamus is a relay station, integrating sensory input and channelling it to the sensory areas of the cerebrum.
- The cerebellum coordinates muscle movement and so controls balance, posture and locomotion (walking, running and jumping).
- The Pineal gland secretes melatonin, the hormone that regulates the biological clock.

These regions of the brain are all involved in involuntary functions, and are connected to the autonomic nervous system. A large part of the brain's processing concerns these routine processes that keep the body working. By contrast, the upper half of the brain, the cerebrum, is responsible for all voluntary activities, and is connected to the somatic nervous system. The cerebrum is divided down the middle by a deep cleft into two cerebral hemispheres. The two halves are quite separate except for the corpus callosum, a bundle of 200 million neurones which run between the two halves. The inside contains fluid and only the outer few mm of the cerebral hemispheres contains neurones, and this is called the cerebral cortex (or just cortex). The cortex is highly folded and so has a large surface area. The cortex is the most complicated, fascinating and least-understood part of the brain.

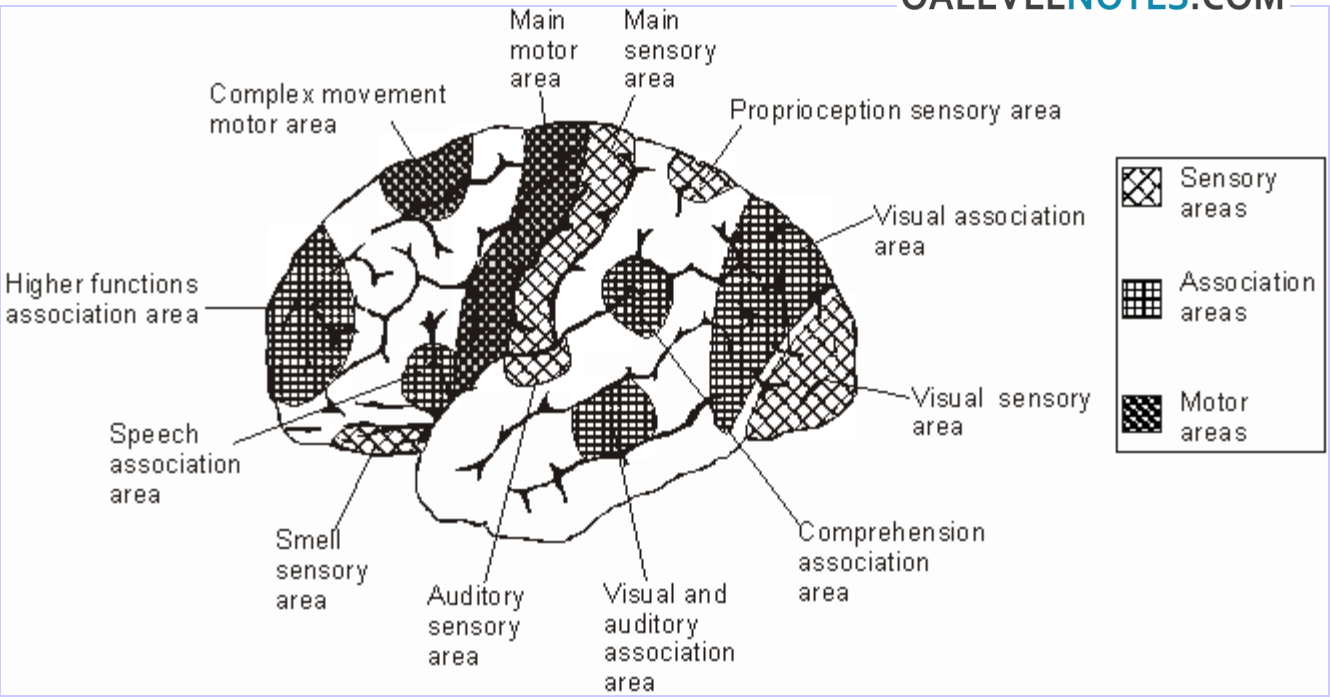
The Cerebral Cortex

Various techniques have been used to investigate the functions of different parts of the brain. Patients with injuries to specific parts of the brain (such as strike victims) can be studies to see which functions are altered. The brain itself has no pain receptors, so during an operation on the brain, it can be studied while the patient is alert. Different parts of the brain can be stimulated electrically to see which muscles in the body respond, or conversely different parts of the body can be stimulated to see which regions of the brain show electrical activity. More recently, the non-invasive technique of magnetic resonance imaging (MRI) has been used to study brain activity of a subject without an operation.

Studies like these have shown that the various functions of the cortex are localised into discrete areas. These areas can be split into three groups:

- Sensory areas, which receive and process sensory input from the sensory organs. There are different sensory areas for each sense organ (visual, auditory, smell, skin, etc.). The sensory neurones are first channelled through the thalamus, and they may also send impulses to other regions of the brain for autonomic processing (such as the iris response).
- Motor areas, which organise and send motor output to skeletal muscles. The motor neurones originate in these areas but are usually processed by the cerebellum before going to the muscles. So the cortex may decide to walk up stairs, but the cerebellum will organise exactly which muscle cells to contract and which to relax.
- Association areas, which are involved in higher processing.

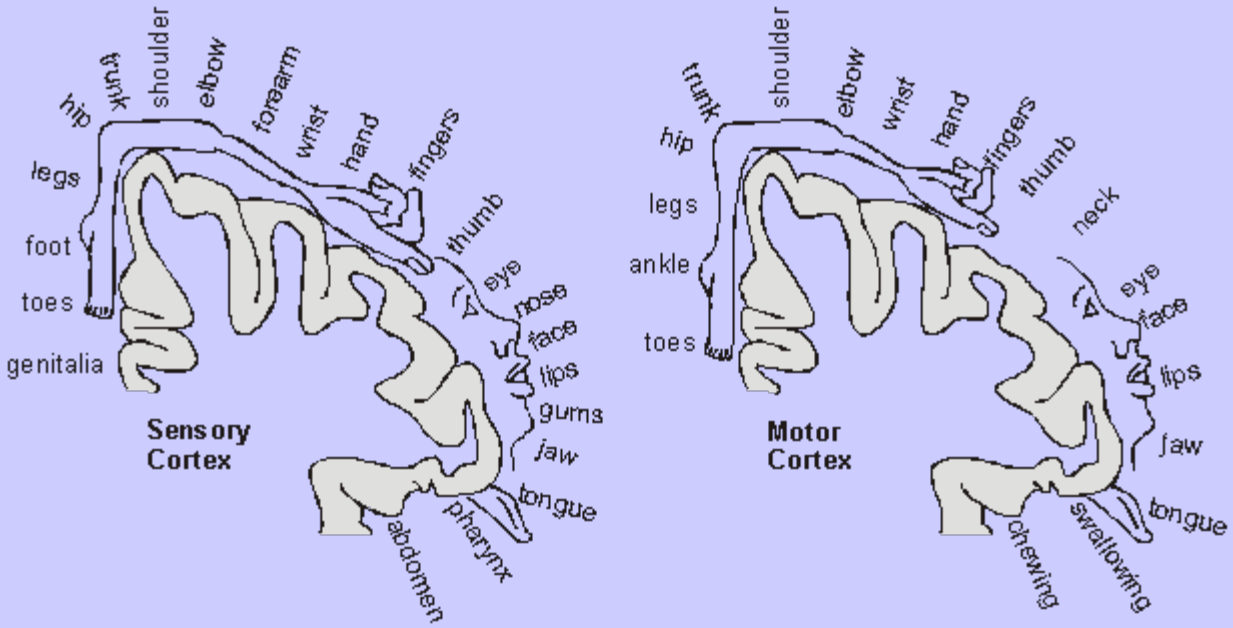
Some of these areas are shown on this map of the surface of the cerebral cortex.



Motor and Sensory Areas

The main motor area controls the main skeletal muscles of the body, and the main sensory area receives input from the various skin receptors all over the body. These two areas are duplicated on the two cerebral hemispheres, but they control the opposite side of the body. So the main sensory and motor areas of the left cerebral hemisphere are linked to the right side of the body, and those of the right cerebral hemisphere are linked to the left side of the body.

These two areas have been studied in great detail, and diagrams can be drawn mapping the part of the cortex to the corresponding part of the body. Such a map (also called a homunculus or "little man") can be drawn for the main sensory and motor areas:



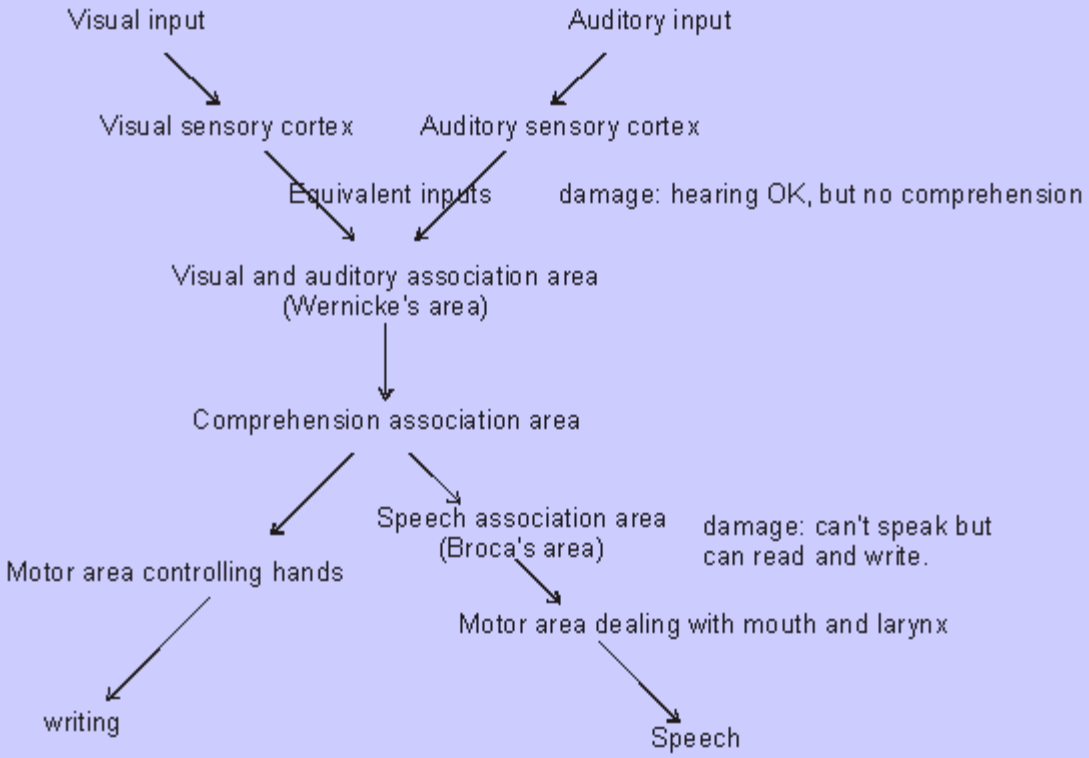
The sensory and motor maps are similar, though not identical, and they show that regions of the body with many sensory (or motor) neurones have correspondingly large areas of the cortex linked to them. So the lips occupy a larger region of the sensory cortex than the shoulder, because they have many more sensory neurones. Similarly, the tongue occupies a larger region of the motor cortex than the trunk because it has more motor neurones controlling its muscles.

Association Areas

While the jobs of the sensory and motor areas are reasonably well defined, the jobs of the association areas are far less clear. The association areas contain multiple copies of the sensory maps and they change as the sensory maps change. These copies are used to compare (or associate) sensory input with previous experiences, and so make decisions. They are therefore involved in advanced skills such as visual recognition, language understanding (aural and read), speech, writing and memory retrieval. The frontal lobes are particularly large in humans, and thought to be responsible for such higher functions as abstract thought, personality and emotion. We'll look briefly at two examples of advanced processing: comprehension and visual processing.

Comprehension

This flow diagram shows how different areas of the cortex work together during a school lesson when a student has to understand the teacher's written and spoken word, write notes, and answer questions.



Unlike the sensory and motor areas, the association areas are not duplicated in the two hemispheres. Association areas in the two hemispheres seem to supervise different skills.

- The right hemisphere has association areas for face recognition, spatial skills and musical sense.
- The left hemisphere has association areas for speech and language, mathematical logical and analytical skills.

These distributions apply to most right-handers, and are often reversed for left-handers. However, even this generalisation is often not true. For example, Broca's area, the speech association areas is quite well-defined and well studied. 95% of right-handers have Broca's area in their left hemisphere while 5% have it in their right. 70% of left-handers have Broca's area in their left hemisphere, 15 in their right, and 15% in both hemispheres! Any reference to "right brain skills" or "left brain skills" should be taken with a large dose of scepticism.

Visual Processing.

The visual sensory area is at the back of the brain and receives sensory input from the optic nerves. Some of the neurones from each optic nerve cross over in the optic chiasma in the middle of the brain, so that neurones from the left half of the retinas of both eyes go to the visual sensory area in the left hemisphere and neurones from the right half of the retinas of both eyes go to the visual sensory area in the right hemisphere. Thus the two hemispheres see slightly different images from opposite side of the visual field, and the differences can be used to help judge distance.

The mechanism of visual processing is complex and not well understood, but it is clear so far that the brain definitely does not work like a digital camera, by forming an image of pixels. Instead it seems to recognise shapes. The neurones in the visual cortex are arranged in 6 layers, each with a different hierarchical function in processing the visual information. The first layer recognises sloping lines, the second recognises complete shapes, the third recognises moving lines, and so on.

MODULE 4

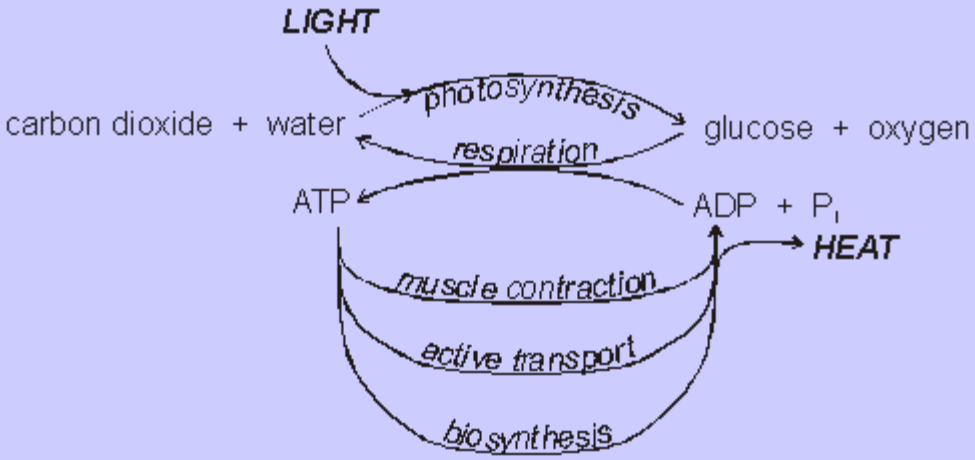
METABOLISM	Respiration
	Photosynthesis

Metabolism

Metabolism refers to all the chemical reactions taking place in a cell. There are thousands of these in a typical cell, and to make them easier to understand, biochemists arrange them into metabolic pathways. The intermediates in these metabolic pathways are called metabolites.

- Reactions that release energy (usually breakdown reactions) are called catabolic reactions (e.g. respiration)
- Reactions that use up energy (usually synthetic reactions) are called anabolic reactions (e.g. photosynthesis).

Photosynthesis and respiration are the reverse of each other, and you couldn't have one without the other. The net result of all the photosynthesis and respiration by living organisms is the conversion of light energy to heat energy.

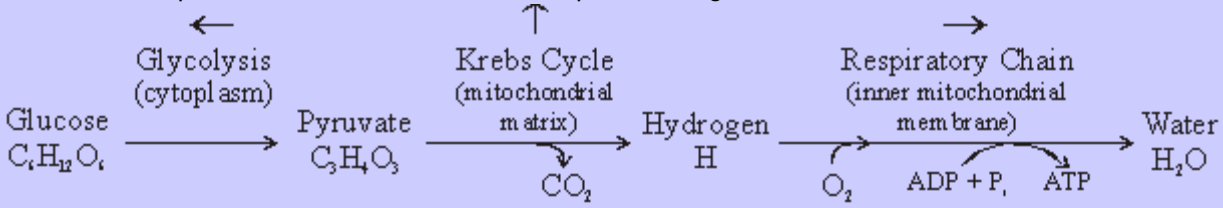


Cellular Respiration

The equation for cellular respiration is usually simplified to:

glucose + oxygen *react to form* carbon dioxide + water (+ energy)

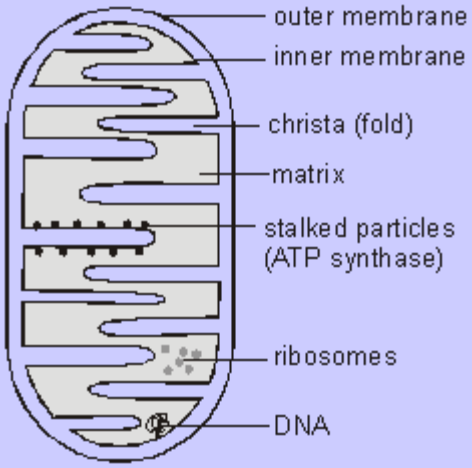
But in fact respiration is a complex metabolic pathway, comprising at least 30 separate steps. To understand respiration in detail we can break it up into 3 stages:



Before we look at these stages in detail, there are a few points from the above summary:

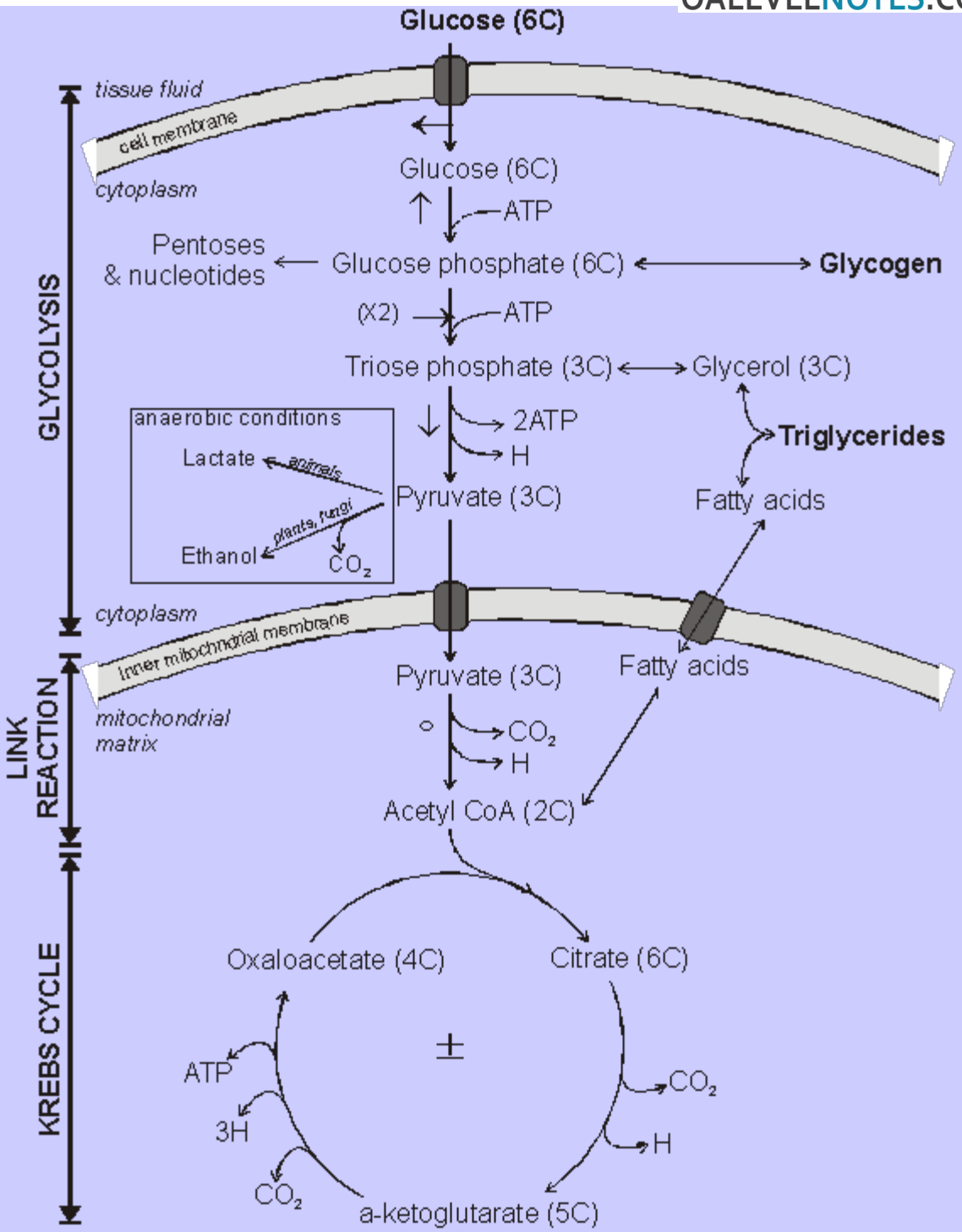
- The different stages of respiration take place in different parts of the cell. This allows the cell to keep the various metabolites separate, and to control the stages more easily.
- The energy released by respiration is in the form of ATP.
- Since this summarises so many separate steps (often involving H^+ and OH^- ions from the solvent water), it is meaningless to try to balance the summary equation.
- The release of carbon dioxide takes place before oxygen is involved. It is therefore not true to say that respiration turns oxygen into carbon dioxide; it is more correct to say that respiration turns glucose into carbon dioxide, and oxygen into water.
- Stage 1 (glycolysis) is anaerobic respiration, while stages 2 and 3 are the aerobic stages.

Mitochondria



Much of respiration takes place in the mitochondria. Mitochondria have a double membrane: the outer membrane contains many protein channels, which let almost any small molecule through; while the inner membrane is more normal and is impermeable to most materials. The inner membrane is highly folded into folds called cristae, giving a larger surface area. The electron microscope reveals blobs on the inner membrane, which were originally called stalked particles. These have now been identified as the enzyme complex that synthesises ATP, are is more correctly called ATP synthase. the space inside the inner membrane is called the matrix, and is where the Krebs cycle takes place (the matrix also contains DNA and some genes are replicated and expressed here).

Details of Respiration



1. Glucose enters cells from the tissue fluid by facilitated diffusion using a specific glucose carrier protein. This carrier can be controlled (gated) by hormones such as insulin, so that uptake of glucose can be regulated.

2. The first step is the phosphorylation of glucose to form glucose phosphate, using phosphate from ATP. Glucose phosphate no longer fits the membrane carrier, so it can't leave the cell. This ensures that pure glucose is kept at a very low concentration inside the cell, so it will always diffuse down its concentration gradient from the tissue fluid into the cell. Glucose phosphate is also the starting material for the synthesis of glycogen.

3. Glucose is phosphorylated again (using another ATP) and split into two triose phosphate (3 carbon) sugars. From now on everything happens twice per original glucose molecule.

4. The triose sugar is changed over several steps to form pyruvate, a 3-carbon compound. In these steps some energy is released to form ATP (the only ATP formed in glycolysis), and a hydrogen atom is also released. This hydrogen atom is very important as it stores energy, which is later used by the respiratory chain to make more ATP. The hydrogen atom is taken up and carried to the respiratory chain by the coenzyme NAD, which becomes reduced in the process.

(oxidised form Õ) NAD + H Õ NADH (? reduced form)

Note: rather than write NADH examiners often simply refer to it as reduced NAD or reduced coenzyme

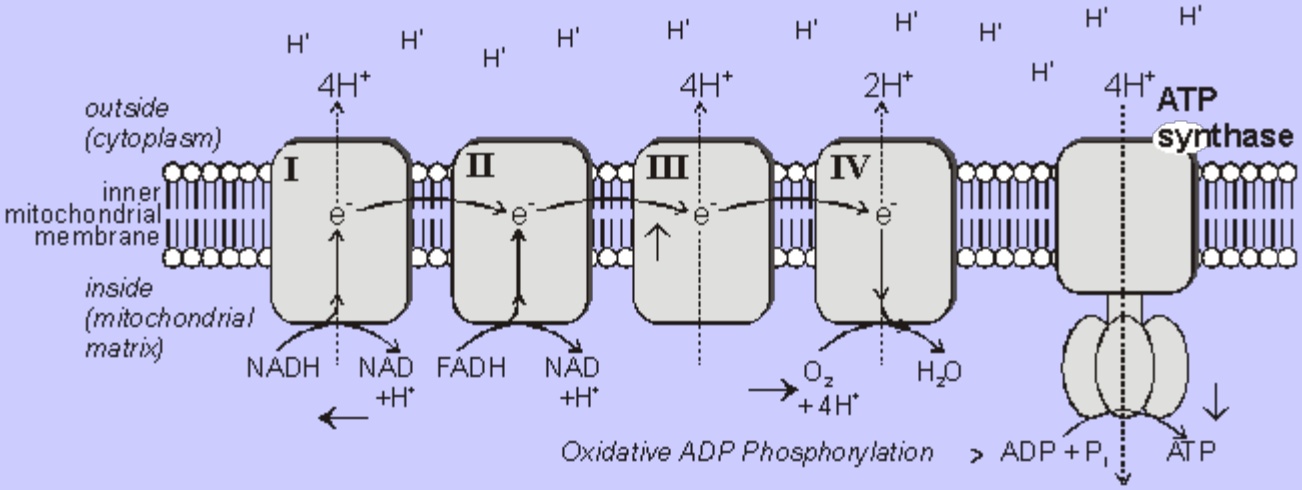
Pyruvate marks the end of glycolysis, the first stage of respiration. In the presence of oxygen pyruvate enters the mitochondrial matrix to proceed with aerobic respiration, but in the absence of oxygen it is converted into lactate (in animals and bacteria) or ethanol (in plants and fungi). These are both examples of anaerobic respiration.

5. Once pyruvate has entered the inside of the mitochondria (the matrix), it is converted to a compound called acetyl CoA. Since this step is between glycolysis and the Krebs Cycle, it is referred to as the link reaction. In this reaction pyruvate loses a CO_2 and a hydrogen to form a 2-carbon acetyl compound, which is temporarily attached to another coenzyme called coenzyme A (or just coA), so the product is called acetyl coA. The CO_2 diffuses through the mitochondrial and cell membranes by lipid diffusion, out into the tissue fluid and into the blood, where it is carried to the lungs for removal. The hydrogen is taken up by NAD again.

6. The acetyl CoA then enters the Krebs Cycle. It is one of several cyclic metabolic pathways, and is also known as the citric acid cycle or the tricarboxylic acid cycle. The 2-carbon acetyl is transferred from acetyl coA to a 4-carbon intermediate (oxaloacetate) to form a 6-carbon intermediate (citrate). Citrate is then gradually broken down in several steps to re-form the 4-carbon intermediate (oxaloacetate), producing carbon dioxide and hydrogen in the process. As before, the CO_2 diffuses out the cell and the hydrogen is taken up by NAD, or by an alternative hydrogen carrier called FAD. These hydrogens are carried to the inner mitochondrial membrane for the final part of respiration.

The Respiratory Chain

The respiratory chain (or electron transport chain) is an unusual metabolic pathway in that it takes place within the inner mitochondrial membrane, using integral membrane proteins. These proteins form four huge trans-membrane complexes. In the respiratory chain the hydrogen atoms from NADH gradually release all their energy to form ATP, and are finally combined with oxygen to form water.



1. NADH molecules bind to Complex I and release their hydrogen atoms as protons (H^+) and electrons (e^-). The NAD molecules then returns to the Krebs Cycle to collect more hydrogen. FADH binds to complex II rather than complex I to release its hydrogen.

2. The electrons are passed down the chain of proteins complexes, each complex binding electrons more tightly than the previous one. In complexes I, II and IV the electrons give up some of their energy, which is then used to pump protons across the inner mitochondrial membrane by active transport through the

complexes.

3. In complex IV the electrons are combined with protons and molecular oxygen to form water, the final end-product of respiration. The oxygen diffused in from the tissue fluid, crossing the cell and mitochondrial membranes by lipid diffusion. Oxygen is only involved at the very last stage of respiration as the final electron acceptor, but without the whole respiratory chain stops.

4. The energy of the electrons is now stored in the form of a proton gradient across the inner mitochondrial membrane. It's a bit like using energy to pump water uphill into a high reservoir, where it is stored as potential energy. And just as the potential energy in the water can be used to generate electricity in a hydroelectric power station, so the energy in the proton gradient can be used to generate ATP in the ATP synthase enzyme. The ATP synthase enzyme has a proton channel through it, and as the protons "fall down" this channel their energy is used to make ATP, spinning the globular head as they go.

This method of storing energy by creating a protons gradient across a membrane is called chemiosmosis. Some poisons act by making proton channels in mitochondrial membranes, so giving an alternative route for protons and stopping the synthesis of ATP. This also happens naturally in the brown fat tissue of new-born babies and hibernating mammals: respiration takes place, but no ATP is made, with the energy being turned into heat instead.

How Much ATP is Made in Respiration?

We can now summarise respiration and see how much ATP is made from each glucose molecule. ATP is made in two different ways:

- Some ATP molecules are made directly by the enzymes in glycolysis or the Krebs cycle. This is called substrate level phosphorylation (since ADP is being phosphorylated to form ATP).
- Most of the ATP molecules are made by the ATP synthase enzyme in the respiratory chain. Since this requires oxygen it is called oxidative phosphorylation. Scientists don't yet know exactly how many protons are pumped in the respiratory chain, but the current estimates are: 10 protons are pumped by NADH; 6 by FADH; and 4 protons are needed by ATP synthase to make one ATP molecule. This means that each NADH can make 2.5 ATPs (10/4) and each FADH can make 1.5 ATPs (6/4). Previous estimates were 3 ATPs for NADH and 2 ATPs for FADH, and these numbers still appear in most textbooks, although they are now know to be wrong. (you don't need to know any numbers anyway so don't worry)
- Two ATP molecules are used at the start of glycolysis to phosphorylate the glucose, and these must be subtracted from the total.

The table below is an "ATP account" for aerobic respiration, and shows that 32 molecules of ATP are made for each molecule of glucose used in aerobic respiration. This is the maximum possible yield; often less ATP is made, depending on the circumstances. Note that anaerobic respiration (glycolysis) only produces 2 molecules of ATP.

Stage	molecules produced per glucose	Final ATP yield old method (interest only)	Final ATP yield new method (interest only)
Glycolysis	2 ATP used	-2	-2
	4 ATP produced (2 per triose phosphate)	4	4
	2 NADH produced (1 per triose phosphate)	6	5
Link Reaction	2 NADH produced (1 per pyruvate)	6	5

Krebs Cycle	2 ATP produced (1 per acetyl coA)	2	2
	6 NADH produced (3 per acetyl coA)	18	15
	2 FADH produced (1 per acetyl coA)	4	3
Total		38	32

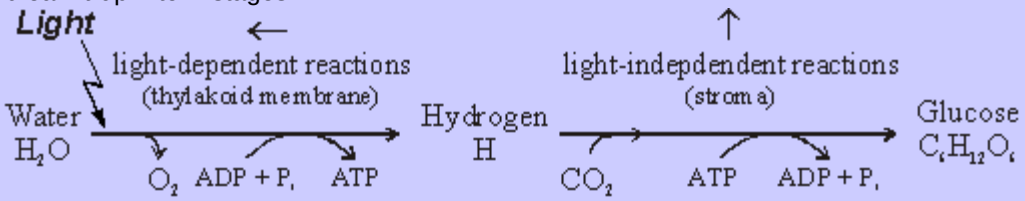
Other substances can also be used to make ATP. Triglycerides are broken down to fatty acids and glycerol, both of which enter the Krebs Cycle. A typical triglyceride might make 50 acetyl CoA molecules, yielding 500 ATP molecules. Fats are a very good energy store, yielding 2.5 times as much ATP per g dry mass as carbohydrates. Proteins are not normally used to make ATP, but in times of starvation they can be broken down and used in respiration. They are first broken down to amino acids, which are converted into pyruvate and Krebs Cycle metabolites and then used to make ATP.

Photosynthesis

Photosynthesis is essentially the reverse of respiration. It is usually simplified to:

carbon dioxide + water (+ light energy) *reacts to form* glucose + oxygen

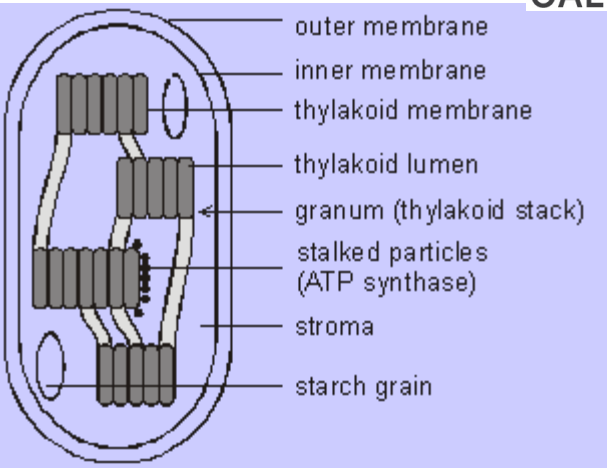
But again this simplification hides numerous separate steps. To understand photosynthesis in detail we can break it up into 2 stages:



- The light-dependent reactions use light energy to split water and make some ATP and energetic hydrogen atoms. This stage takes place within the thylakoid membranes of chloroplasts, and is very much like the respiratory chain, only in reverse.
- The light-independent reactions don't need light, but do need the products of the light-dependent stage (ATP and H), so they stop in the absence of light. This stage takes place in the stroma of the chloroplasts and involve the fixation of carbon dioxide and the synthesis of glucose.

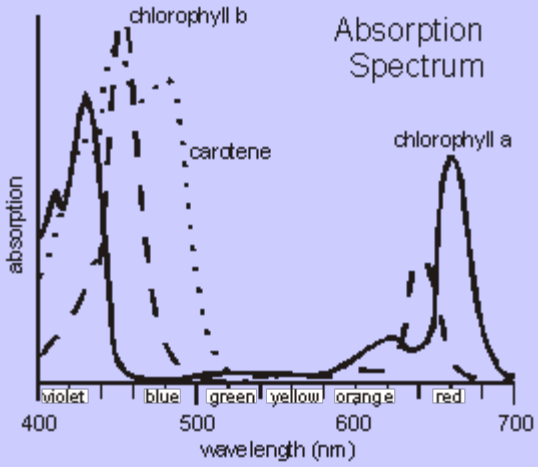
  We shall see that there are many similarities between photosynthesis and respiration, and even the same enzymes are used in some steps.

Chloroplasts

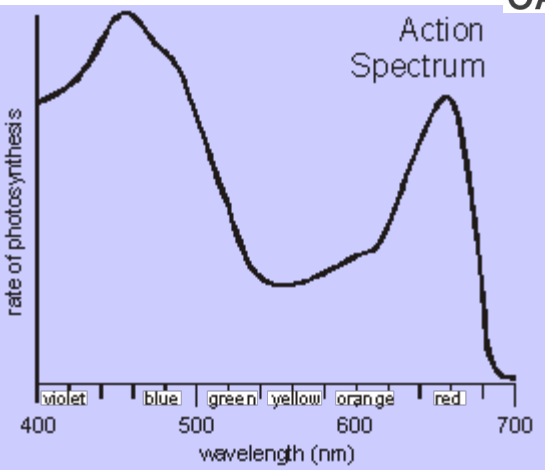


Photosynthesis takes place entirely within chloroplasts. Like mitochondria, chloroplasts have a double membrane, but in addition chloroplasts have a third membrane called the thylakoid membrane. This is folded into thin vesicles (the thylakoids), enclosing small spaces called the thylakoid lumen. The thylakoid vesicles are often layered in stacks called grana. The thylakoid membrane contains the same ATP synthase particles found in mitochondria. Chloroplasts also contain DNA, tRNA and ribososomes, and they often store the products of photosynthesis as starch grains and lipid droplets.

Chlorophyll



Chloroplasts contain two different kinds of chlorophyll, called chlorophyll a and b, together with a number of other light-absorbing accessory pigments, such as the carotenoids and luteins (or xanthophylls). These different pigments absorb light at different wavelengths, so having several different pigments allows more of the visible spectrum to be used. The absorption spectra of pure samples of some of these pigments are shown in the graph on the left. A low absorption means that those wavelengths are not absorbed and used, but instead are reflected or transmitted. Different species of plant have different combinations of photosynthetic pigments, giving rise to different coloured leaves. In addition, plants adapted to shady conditions tend to have a higher concentration of chlorophyll and so have dark green leaves, while those adapted to bright conditions need less chlorophyll and have pale green leaves.

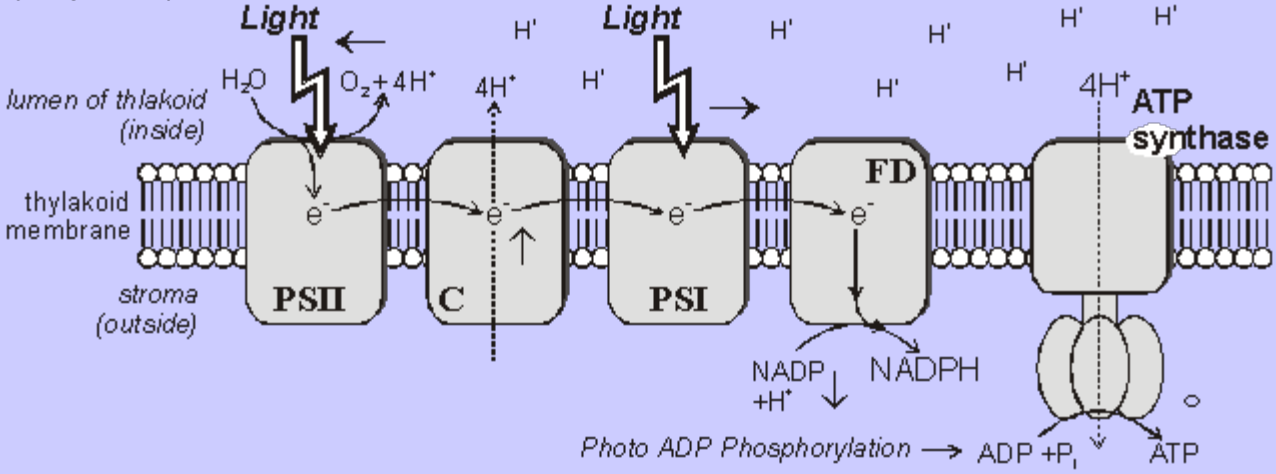


By measuring the rate of photosynthesis using different wavelengths of light, an action spectrum is obtained. The action spectrum can be well explained by the absorption spectra above, showing that these pigments are responsible for photosynthesis.

Chlorophyll is a fairly small molecule (not a protein) with a structure similar to haem, but with a magnesium atom instead of iron. Chlorophyll and the other pigments are arranged in complexes with proteins, called photosystems. Each photosystem contains some 200 chlorophyll molecules and 50 molecules of accessory pigments, together with several protein molecules (including enzymes) and lipids. These photosystems are located in the thylakoid membranes and they hold the light-absorbing pigments in the best position to maximise the absorbance of photons of light. The chloroplasts of green plants have two kinds of photosystem called photosystem I (PSI) and photosystem II (PSII). These absorb light at different wavelengths and have slightly different jobs in the light dependent reactions of photosynthesis.

The Light-Dependent Reactions

The light-dependent reactions take place on the thylakoid membranes using four membrane-bound protein complexes called photosystem I (PSI), photosystem II (PSII), cytochrome complex (C) and ferredoxin complex (FD). In these reactions light energy is used to split water, oxygen is given off, and ATP and hydrogen are produced



1. Chlorophyll molecules in PSII absorb photons of light, exciting chlorophyll electrons to a higher energy level and causing a charge separation within PSII. This charge separation drives the splitting (or photolysis) of water molecules to make oxygen (O_2), protons (H^+) and electrons (e^-):

$$2H_2O \xrightarrow{photolysis} O_2 + 4H^+ + 4e^-$$

Water is a very stable molecule and it requires the energy from 4 photons of light to split 1 water molecule. The oxygen produced diffuses out of the chloroplast and eventually into the air; the protons build up in the thylakoid lumen causing a proton gradient; and the electrons from water replace the excited electrons that have been ejected from chlorophyll.

2. The excited, high-energy electrons are passed along a chain of protein complexes in the membrane, similar to the respiratory chain. They are passed from PSII to C, where the energy is used to pump 4 protons from stroma to lumen; then to PSI, where more light energy is absorbed by the chlorophyll

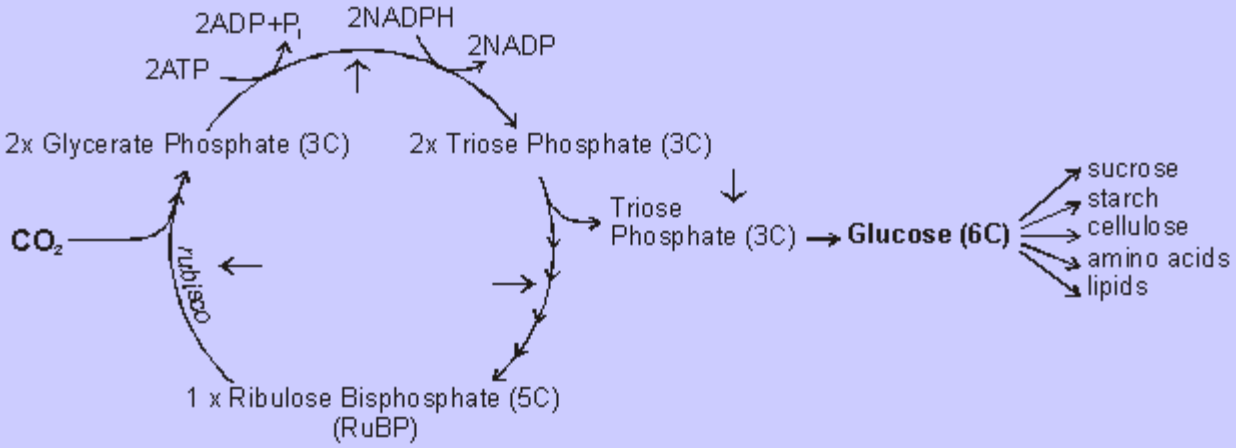
molecules and the electrons are given more energy; and finally to FD.

3. In the ferredoxin complex each electron is recombined with a proton to form a hydrogen atom, which is taken up by the hydrogen carrier NADP. Note that while respiration uses NAD to carry hydrogen, photosynthesis always uses its close relative, NADP.

4. The combination of the water splitting and the proton pumping by the cytochrome complex cause protons to build up inside the thylakoid lumen. This generates a proton gradient across the thylakoid membrane. This gradient is used to make ATP using the ATP synthase enzyme in exactly the same way as respiration. This synthesis of ATP is called photophosphorylation because it uses light energy to phosphorylate ADP.

The Light-Independent Reactions

The light-independent, or carbon-fixing reactions, of photosynthesis take place in the stroma of the chloroplasts and comprise another cyclic pathway, called the Calvin Cycle, after the American scientist who discovered it.



1. Carbon dioxide binds to the 5-carbon sugar ribulose bisphosphate (RuBP) to form 2 molecules of the 3-carbon compound glycerate phosphate. This carbon-fixing reaction is catalysed by the enzyme ribulose bisphosphate carboxylase, always known as rubisco. It is a very slow and inefficient enzyme, so large amounts of it are needed (recall that increasing enzyme concentration increases reaction rate), and it comprises about 50% of the mass of chloroplasts, making the most abundant protein in nature. Rubisco is synthesised in chloroplasts, using chloroplast (not nuclear) DNA.

2. Glycerate phosphate is an acid, not a carbohydrate, so it is reduced and activated to form triose phosphate, the same 3-carbon sugar as that found in glycolysis. The ATP and NADPH from the light-dependent reactions provide the energy for this step. The ADP and NADP return to the thylakoid membrane for recycling.

3. Triose phosphate is a branching point. Most of the triose phosphate continues through a complex series of reactions to regenerate the RuBP and complete the cycle. 5 triose phosphate molecules (15 carbons) combine to form 3 RuBP molecules (15 carbons).

4. Every 3 turns of the Calvin Cycle 3 CO_2 molecules are fixed to make 1 new triose phosphate molecule. This leaves the cycle, and two of these triose phosphate molecules combine to form one glucose molecule using the glycolysis enzymes in reverse. The glucose can then be used to make other material that the plant needs.

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MODULE 4

The Eye

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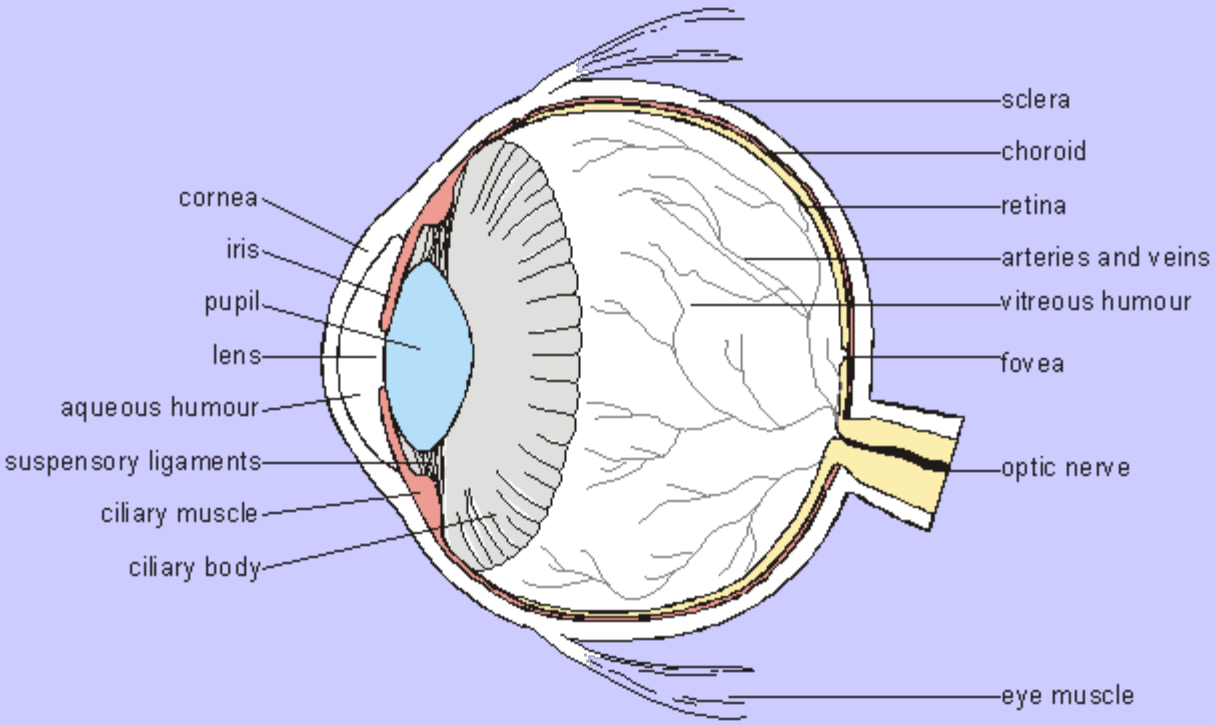
The eye

The Eye

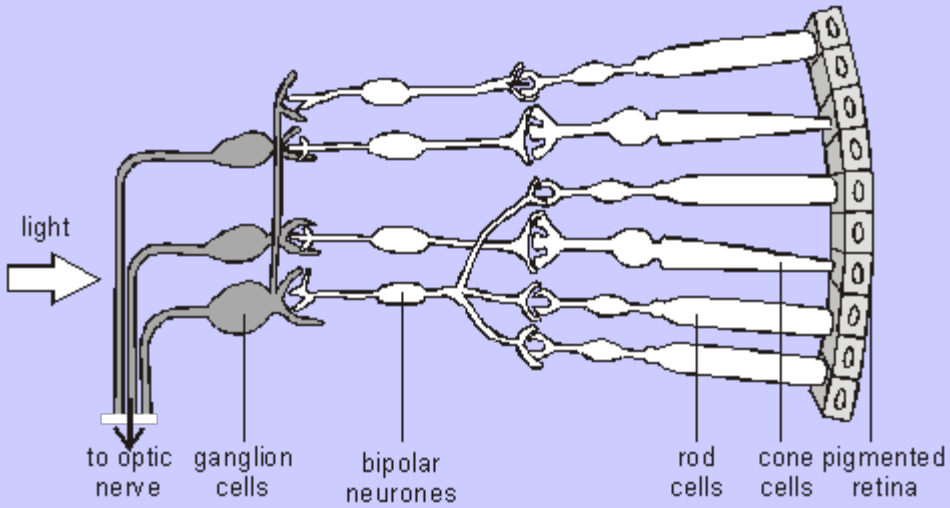


The Sclera	The strong outer layer that hold the eye together. It is soft connective tissue, and the spherical shape of the eye is maintained by the pressure of the liquid inside.
The Choroid	This layer contains the blood vessels that feed every cell of the eye. It also contains the pigmented cells that make the retina appear black.
The Retina	This contains the light-sensitive photoreceptor cells and their associated neurones.
The Cornea	This is a specialised part of the cornea at the front of the eye. It is made of aligned collagen fibres and is transparent and tough.
The Iris	This is made of pigmented cells, which give eye colour, and muscle cells, which control the amount of light entering the eye.
The Lens	This is a transparent, rubbery tissue made of proteins, which crystallise to form a glass-like lens.

The Ciliary body	This supports the lens. It comprises circular muscles and radial elastic fibres called suspensory ligaments. Together theses control the shape of the lens, as described below.
The Humours	These are the names for the fluids inside the eye. The vitreous humour behind the lens is more viscous than the aqueous humour in front of the lens.

The Retina

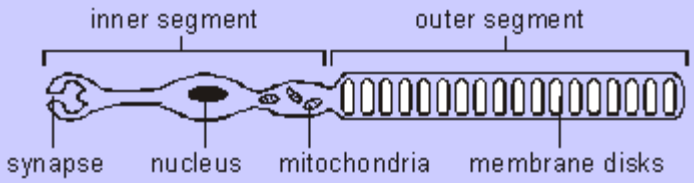
The retina contains the photoreceptor cells and their associated interneurones and sensory neurones. They are arranged as shown in this diagram:



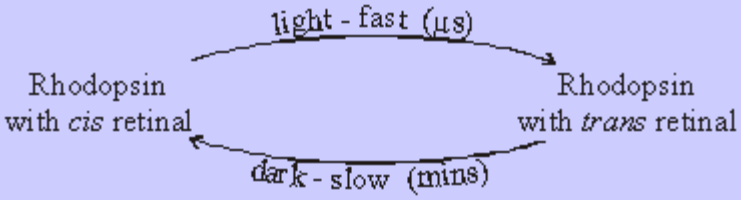
A surprising feature of the retina is that it is back-to-front (inverted). The photoreceptor cells are at the back of the retina, and the light has to pass through several layers of neurones to reach them. This is due to the evolutionary history of the eye, and in fact doesn't matter very much as the neurones are small and transparent. There are two kinds of photoreceptor cells in human eyes: rods and cones, and we shall look at the difference between these shortly. These rods and cones form synapses with special interneurones called bipolar neurones, which in turn synapse with sensory neurones called ganglion cells. The axons of these ganglion cells cover the inner surface of the retina and eventually form the optic nerve (containing about a million axons) that leads to the brain.

Visual Transduction

Visual transduction is the process by which light initiates a nerve impulse. The structure of a rod cell is:



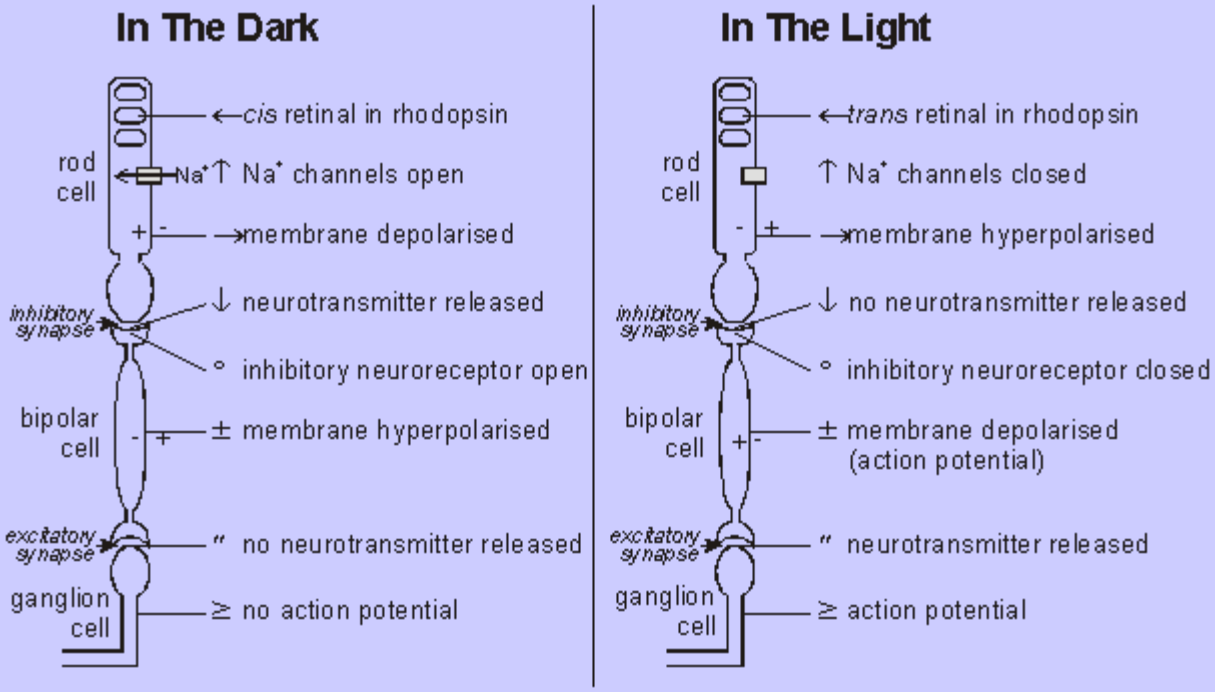
The detection of light is carried out on the membrane disks in the outer segment. These disks contain thousands of molecules of rhodopsin, the photoreceptor molecule. Rhodopsin consists of a membrane-bound protein called opsin and a covalently-bound prosthetic group called retinal. Retinal is made from vitamin A, and a dietary deficiency in this vitamin causes night-blindness (poor vision in dim light). Retinal is the light-sensitive part, and it can exists in 2 forms: a *cis* form and a *trans* form:



In the dark retinal is in the *cis* form, but when it absorbs a photon of light it quickly switches to the *trans* form. This changes its shape and therefore the shape of the opsin protein as well. This process is called bleaching. The reverse reaction (*trans* to *cis* retinal) requires an enzyme reaction and is very slow, taking a few minutes. This explains why you are initially blind when you walk

from sunlight to a dark room: in the light almost all your retinal was in the *trans* form, and it takes some time to form enough *cis* retinal to respond to the light indoors.

The final result of the bleaching of the rhodopsin in a rod cell is a nerve impulse through a sensory neurone in the optic nerve to the brain. However the details of the process are complicated and unexpected. Rod cell membranes contain a special sodium channel that is controlled by rhodopsin. Rhodopsin with *cis* retinal opens it and rhodopsin with *trans* retinal closes it. This means in the dark the channel is open, allowing sodium ions to flow in and causing the rod cell to be depolarised. This in turn means that rod cells release neurotransmitter in the dark. However the synapse with the bipolar cell is an inhibitory synapse, so the neurotransmitter stops the bipolar cell making a nerve impulse. In the light everything is reversed, and the bipolar cell is depolarised and forms a nerve impulse, which is passed to the ganglion cell and to the brain. Fortunately you don't have to remember this, but you should be able to understand it.



Rods and Cones

Why are there two types of photoreceptor cell? The rods and cones serve two different functions as shown in this table:

Rods	Cones
Outer segment is rod shaped	Outer segment is cone shaped
10^9 cells per eye, distributed throughout the retina, so used for peripheral vision.	10^6 cells per eye, found mainly in the fovea, so can only detect images in centre of retina.
Good sensitivity – can detect a single photon of light, so are used for night vision.	Poor sensitivity – need bright light, so only work in the day
Only 1 type, so only monochromatic vision.	3 types (red green and blue), so are responsible for colour vision.
Many rods usually connected to one bipolar cell, so poor visual acuity (i.e. rods are not good at resolving fine detail).	Each cone usually connected to one bipolar cell, so good visual acuity (i.e. cones are used for resolving fine detail such as reading).

Although there are far more rods than cones, we use cones most of the time because they have fine discrimination and can resolve colours. To do this we constantly move our eyes so that images are focused on the small area of the retina called the fovea. You

can only read one word of a book at a time, but your eyes move so quickly that it appears that you can see much more. the more densely-packed the cone cells, the better the visual acuity. In the fovea of human eyes there are 160 000 cones per mm^2, while hawks have 1 million cones per mm^2, so they really do have far better acuity.

Colour Vision

There are three different kinds of cone cell, each with a different form of opsin (they have the same retinal). These three forms of rhodopsin are sensitive to different parts of the spectrum, so there are red cones (10%), green cones (45%) and blue cones (45%). Coloured light will stimulate these three cells differently, so by comparing the nerve impulses from the three kinds of cone, the brain can detect any colour. For example:

- Red light: stimulates red cones mainly
- Yellow light: stimulates red + green cones roughly equally
- Cyan light: stimulates blue and green cones roughly equally
- White light: stimulates all 3 cones equally

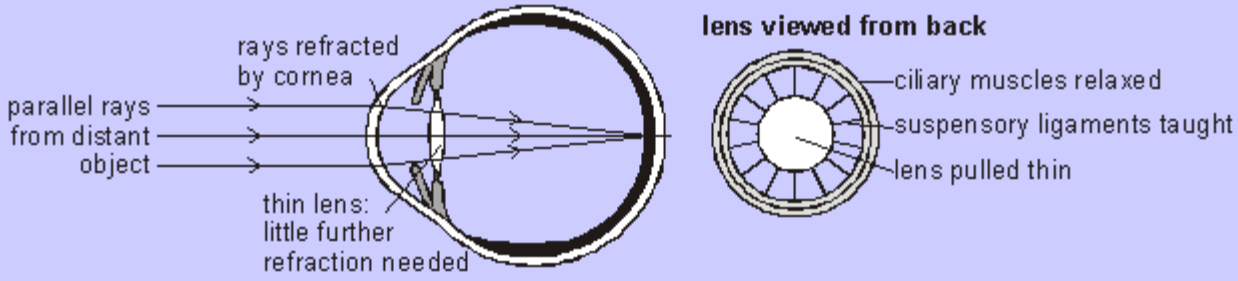
This is called the trichromatic theory of colour vision. The role of the brain in processing visual information is complex and not well understood, but our ability to detect colours depends on lighting conditions and other features of the image.

The red, green and blue opsin proteins are made by three different genes. The green and red genes are on the X chromosome, which means that males have only one copy of these genes (i.e. they're haploid for these genes). About 8% of males have a defect in one or other of these genes, leading to red-green colour blindness. Other forms of colour blindness are also possible, but are much rarer.

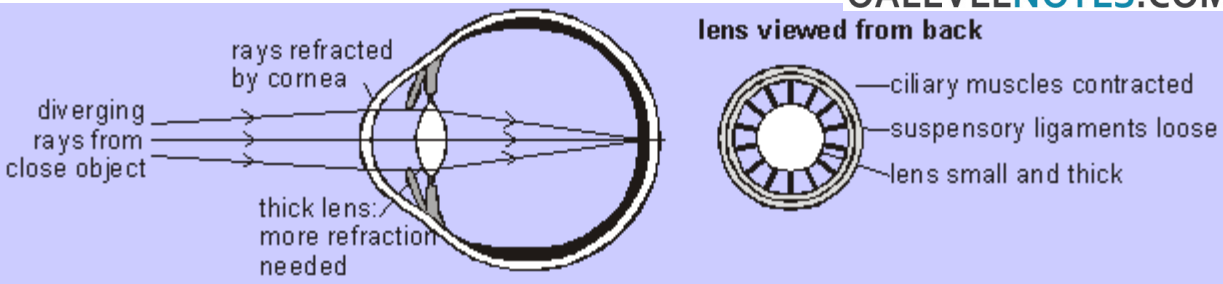
Accommodation

Accommodation refers to the ability of the eye to alter its focus so that clear images of both close and distant objects can be formed on the retina. Cameras do this by altering the distance between the lens and film, but eyes do it by altering the shape and therefore the focal length of the lens. Remember that most of the focusing is actually done by the cornea and the job of the lens to mainly to adjust the focus. The shape of the lens is controlled by the suspensory ligaments and the ciliary muscles.

- Light rays from a distant object are almost parallel so do not need much refraction to focus onto the retina. The lens therefore needs to be thin and "weak" (i.e. have a long focal length). To do this the ciliary muscles relax, making a wider ring and allowing the suspensory ligaments (which are under tension from the pressure of the vitreous humour) to pull the lens out, making it thinner.



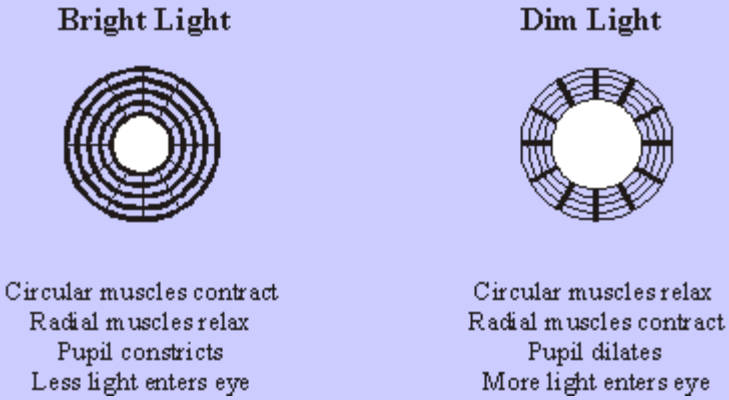
- Light rays from close objects are likely to be diverging, so need more refraction to focus them onto the retina. The lens therefore needs to be thick and "strong" (i.e. have a short focal length). To do this the ciliary muscles contract, making a smaller ring and taking the tension off the suspensory ligaments, which allows the lens to revert to its smaller, fatter shape.



The suspensory ligaments are purely passive, but the ciliary muscles are innervated with motor neurones from the autonomic nervous system, and accommodation is controlled automatically by the brain.

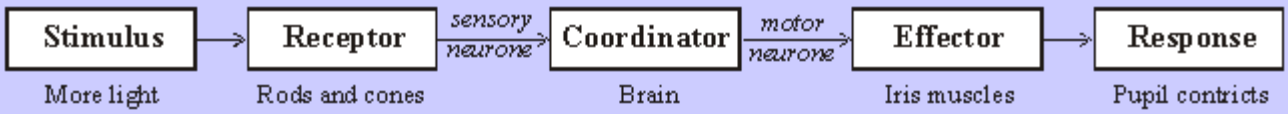
The Iris

The retina is extremely sensitive to light, and can be damaged by too much light. The iris constantly regulates the amount of light entering the eye so that there is enough light to stimulate the cones, but not enough to damage them. The iris is composed of two sets of muscles: circular and radial, which have opposite effects (i.e. they're antagonistic). By contracting and relaxing these muscles the pupil can be constricted and dilated:



The iris is under the control of the autonomic nervous system and is innervated by two nerves: one from the sympathetic system and one from the parasympathetic system. Impulses from the sympathetic nerve cause pupil dilation and impulses from the parasympathetic nerve causes pupil constriction. The drug atropine inhibits the parasympathetic nerve, causing the pupil to dilate. This is useful in eye operations.

The iris is a good example of a reflex arc.



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MODULE 5

AQA(B) A2 Module 5:

Environment

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	Succession
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	Hedgerows
	Fertilisers
	Pesticides
	Eutrophication

These notes may be used freely by A level biology students and teachers, and they may be copied and edited. I would be interested to hear of any comments and corrections.

Neil C Millar (nmillar@cwcom.net) 10/6/01

Module 5 Specification

Ecosystems

A population is all the organisms of one species in a habitat. Populations of different species form communities. These communities are found in a particular habitat and are based on dynamic feeding relationships. The relationship of pyramids of number, biomass and energy to their corresponding food chains and webs

Energy Flow through Ecosystems

Photosynthesis is the major route by which energy enters an ecosystem. Energy is transferred through the trophic levels in

food chains and food webs and is dissipated. Quantitative consideration of the efficiency of energy transfer between trophic levels.

Material Cycles in Ecosystems

Complex organic molecules are broken down in an ecosystem by microorganisms. Carbon dioxide and inorganic ions are thus made available for re-use.

- The role of microorganisms in the carbon cycle
- The role of microorganisms in the nitrogen cycle in sufficient detail to illustrate the processes of saprophytic nutrition, deamination, nitrification, nitrogen fixation and denitrification. (Names of individual species not required.)

Population Ecology

An ecosystem supports a certain size of population of any one species. This population size may vary as a result of

- the effect of abiotic factors
- interactions between organisms
- inter-and intra-specific competition
- predation.

Ecological Niche

Within a habitat a species occupies a niche governed by adaptation to food and/or prevailing abiotic forces.

Succession

In natural and suitable conditions land will gradually become colonised by a range of herbaceous plants, then by shrubs and finally by trees as a climax community. There is change in the communities with time, because of the interaction between species and their environment. At each stage certain species can be recognised which change the environment so that it becomes more suitable for other species. Candidates should be able to describe one example of succession.

Ecological Impact of Farming

There is a balance of food production and conservation.

- The impact of monoculture and the removal of hedgerows on the environment.
- The effects of organic effluent, nitrates and phosphates on aquatic ecosystems, including eutrophication and effects on biochemical oxygen demand.
- Biodegradable and non-biodegradable pesticides. The bioaccumulation of pesticides in food webs.
- Farms may be managed in ways that help to ensure sustainability and reduce the impact on wildlife, such as the use of organic fertilisers, prevention of erosion, control of pesticide use and maintenance of habitat variety.

Evaluate evidence and make balanced judgements between the need to meet the demands for increased food production by agriculture and the need to conserve the environment.

Practical Ecology

Studied an ecosystem in the field and be familiar with the uses, roles and limitations of

- frame quadrats
- line transects
- measurement of abiotic factors such as pH, light and temperature.

Candidates should understand the principles involved in the use of standard deviation and the chi-squared test in reporting the results of ecological studies.

Ecosystems

Ecology is the study of living organisms and their environment. Its aim it to explain why organisms live where they do.

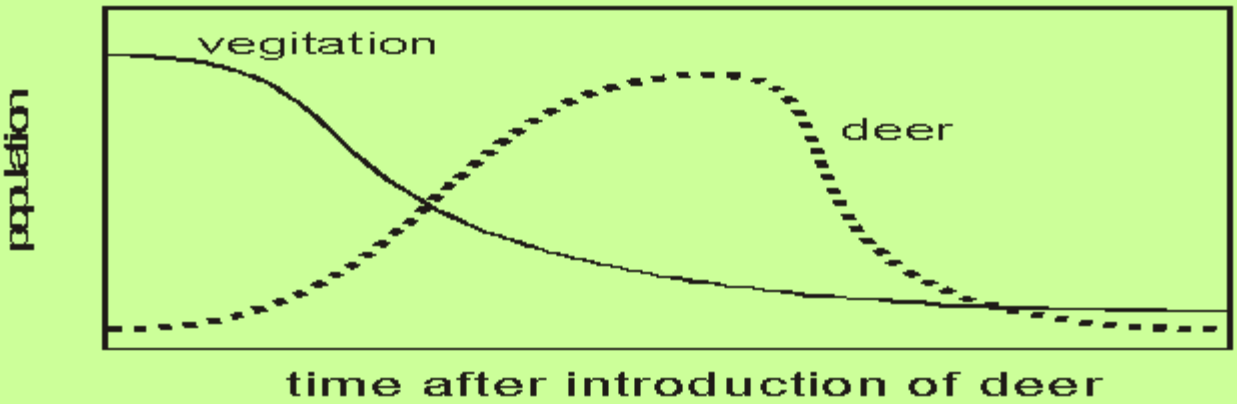
To do this ecologists study ecosystems, areas that can vary in size from a pond to the whole planet.

Ecosystem	A reasonably self-contained area together with all its living organisms.
Habitat	The physical or abiotic part of an ecosystem, i.e. a defined area with specific characteristics where the organisms live, e.g. oak forest, deep sea, sand dune, rocky shore, moorland, hedgerow, garden pond, etc.
Community	The living or biotic part of an ecosystem, i.e. all the populations of all the different species living in one habitat.
Biotic	Any living or biological factor.
Abiotic	Any non-living or physical factor.
Population	The members of the same species living in one habitat.
Species	A group of organisms that can interbreed and produce fertile offspring.

Energy and Matter

Before studying ecosystems, it is important to appreciate the difference between energy and matter. Energy and matter are quite different things and cannot be inter-converted.

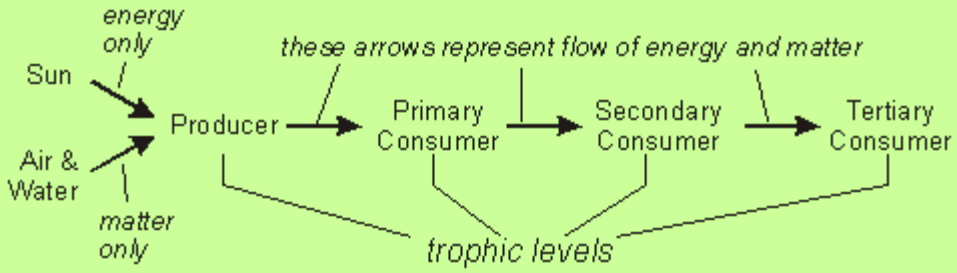
- Energy comes in many different forms (such as heat, light, chemical, potential, kinetic, etc.) which can be inter-converted, but energy can never be created, destroyed or used up. If we talk about energy being "lost", we usually mean as heat, which is radiated out into space. Energy is constantly arriving on earth from the sun, and is constantly leaving the earth as heat, but the total amount of energy on the earth is constant.
- Matter comes in three states (solid, liquid and gas) and again, cannot be created or destroyed. The total amount of matter on the Earth is constant. Matter (and especially the biochemicals found in living organisms) can contain stored chemical energy, so a cow contains biomass (matter) as well as chemical energy stored in its biomass.



All living organisms need energy and matter from their environment. Matter is needed to make new cells (growth) and to create now organisms (reproduction), while energy is needed to drive all the chemical and physical processes of life, such as biosynthesis, active transport and movement.

Food Chains and Webs

The many relationships between the members of a community in an ecosystem can be described by food chains and webs. Each stage in a food chain is called a trophic level, and the arrows represent the flow of energy and matter through the food chain. Food chains always start with photosynthetic producers (plants, algae, plankton and photosynthetic bacteria) because, uniquely, producers are able to extract both energy and matter from the abiotic environment (energy from the sun, and 98% of their matter from carbon dioxide in the air, with the remaining 2% from water and minerals in soil). All other living organisms get both their energy and matter by eating other organisms.

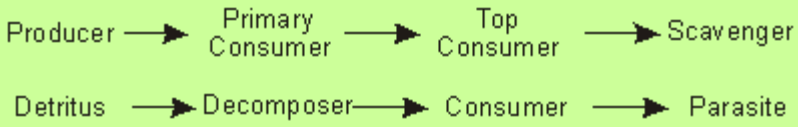


Although this represents a "typical" food chain, with producers being eaten by animal consumers, different organisms use a large range of feeding strategies (other than consuming), leading to a range of different types of food chain. Some of these strategies are defined below, together with other terms associated with food chains.

Producer	An organism that produces food from carbon dioxide and water using photosynthesis. Can be plant, algae, plankton or bacteria.
Consumer	An animal that eats other organisms
Herbivore	A consumer that eats plants (= primary consumer).
Carnivore	A consumer that eats other animals (= secondary consumer).
Top carnivore	A consumer at the top of a food chain with no predators.
Omnivore	A consumer that eats plants or animals.
Vegetarian	A human that chooses not to eat animals (humans are omnivores)
Autotroph	An organism that manufactures its own food (= producer)
Photoautotroph	An organism that manufactures its own food using light energy
Chemoautotroph	An organism that manufactures its own food using energy derived from chemical reactions (e.g. sulphur reducing bacteria)
Heterotroph	An organism that obtains its energy and mass from other organisms (=consumers + decomposers)
Plankton	Microscopic aquatic organisms.
Phytoplankton	"Plant plankton" i.e. microscopic aquatic producers.
Zooplankton	"Animal plankton" i.e. microscopic aqautic consumers.
Predator	An animal that hunts and kills animals for food.

Prey	An animal that is hunted and killed for food.
Scavenger	An animal that eats dead animals, but doesn't kill them
Detritus	Dead and waste matter that is not eaten by consumers
Decomposer	An organism that consumes detritus (= detrivores + saprophytes)
Detrivore	An animal that eats detritus.
Saprophyte	A microbe (bacterium or fungus) that lives on detritus.
Symbiosis	Organisms living together in a close relationship (= parasitism, mutualism, pathogen).
Mutualism	Two organisms living together for mutual benefit.
Commensalism	Relationship in which only one organism benefits
Parasite	An organism that feeds on a larger living host organism, harming it
Pathogen	A microbe that causes a disease.

So food chains need not end with a consumer, and need not even start with a producer, e.g.:

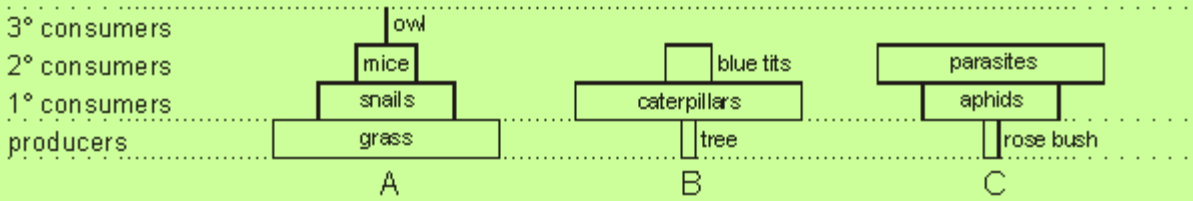


Ecological Pyramids

In general as you go up a food chain the size of the individuals increases and the number of individuals decreases. These sorts of observations can be displayed in ecological pyramids, which are used to quantify food chains. There are three kinds:

1. Pyramids of Numbers.

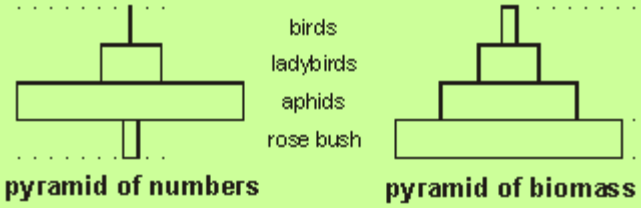
These show the numbers of organisms at each trophic level in a food chain. The width of the bars represent the numbers, or the bars may be purely qualitative. The numbers should be normalised for a given area for a terrestrial habitat (usually m^2), or volume for an aquatic habitat (m^3). Pyramids of numbers are most often triangular shaped, but can be almost any shape. In the pyramids below, A shows a typical pyramid of numbers for carnivores; B shows the effect of a single large producer such as a tree; and C shows a typical parasite food chain.



2. Pyramids of Biomass

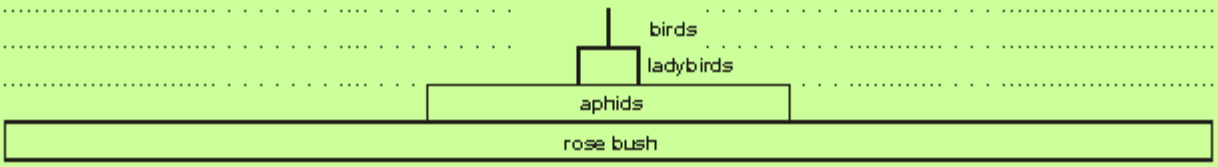
These convey more information, since they consider the total mass of living organisms (i.e. the biomass) at each

trophic level. The biomass should be dry mass (since water stores no energy) and is measured in kg m^{-2}. The biomass may be found by drying and weighing the organisms at each trophic level, or by counting them and multiplying by an average individual mass. Pyramids of biomass are always pyramid shaped, since if a trophic level gains all its mass from the level below, then it cannot have more mass than that level (you cannot weigh more than you eat). The "missing" mass, which is not eaten by consumers, becomes detritus and is decomposed.



3. Pyramids of Energy

Food chains represent flows of matter and energy, so two different pyramids are needed to quantify each flow. Pyramids of energy show how much energy flows into each trophic level in a given time, so the units are usually something like kJ m^{-2} y^{-1}. Pyramids of energy are always pyramidal (energy cannot be created), and always very shallow, since the transfer of energy from one trophic level to the next is very inefficient The "missing" energy, which is not passed on to the next level, is lost eventually as heat.

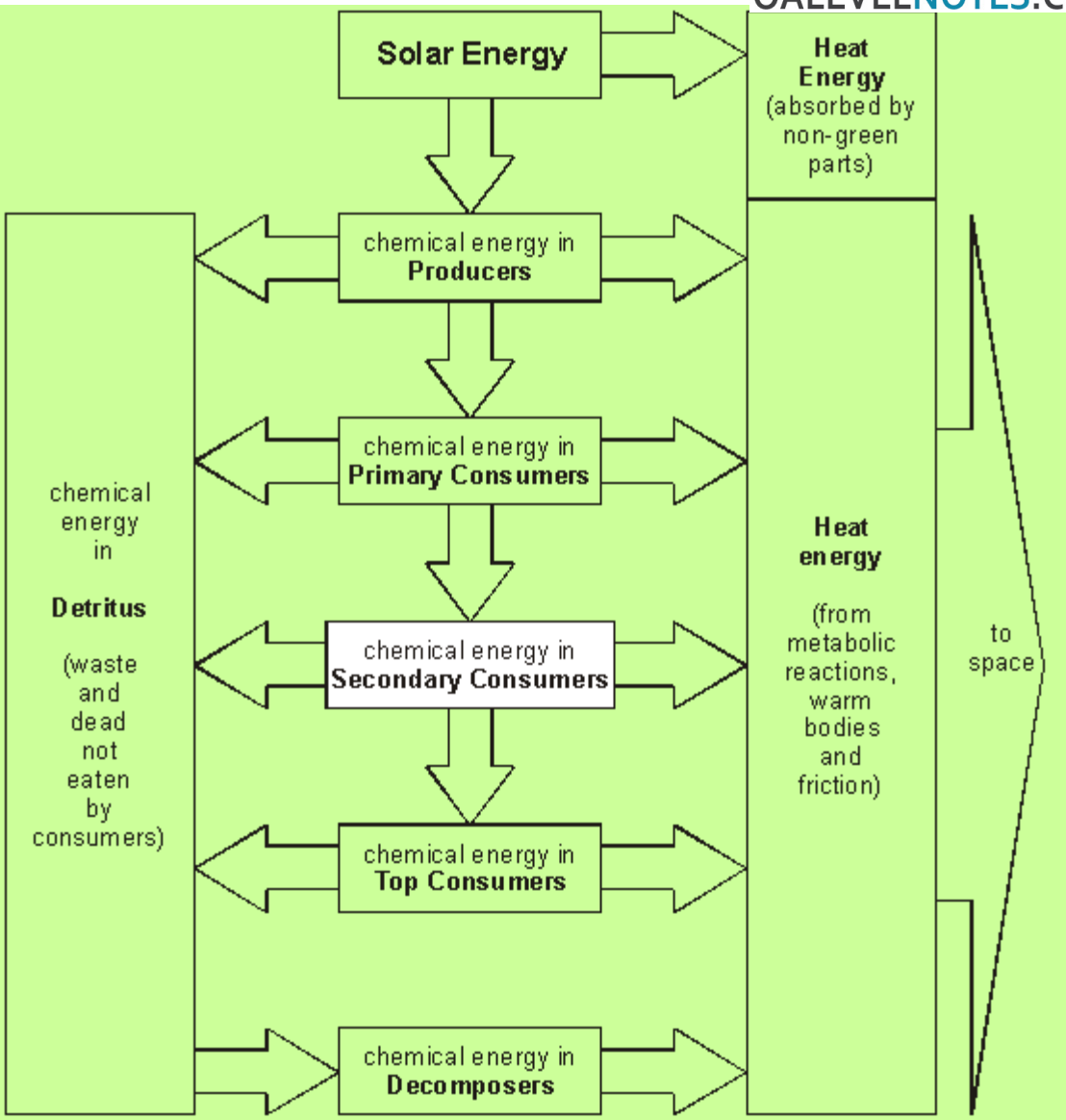


Energy Flow in Ecosystems

Three things can happen to the energy taken in by the organisms in a trophic level:

- It can be passed on to the next trophic level in the food chain when the organism is eaten.
- It can become stored in detritus. This energy is passed on to decomposers when the detritus decays.
- It can be converted to heat energy by inefficient chemical reactions, radiated by warm bodies, or in friction due to movement. The heat energy is lost to the surroundings, and cannot be regained by living organisms.

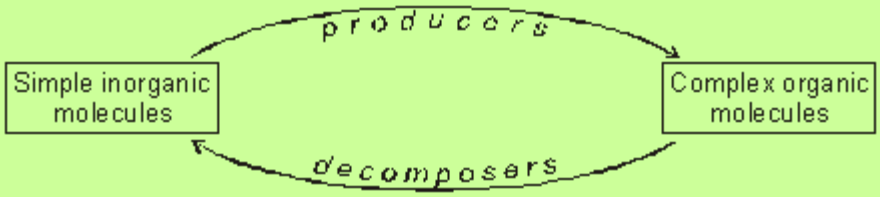
These three fates are shown in this energy flow diagram:



Eventually all the energy that enters the ecosystem will be converted to heat, which is lost to space.

Material Cycles in Ecosystems

Matter cycles between the biotic environment and in the abiotic environment. Simple inorganic molecules (such as CO_2, N_2 and H_2O) are assimilated (or fixed) from the abiotic environment by producers and microbes, and built into complex organic molecules (such as carbohydrates, proteins and lipids). These organic molecules are passed through food chains and eventually returned to the abiotic environment again as simple inorganic molecules by decomposers. Without either producers or decomposers there would be no nutrient cycling and no life.



The simple inorganic molecules are often referred to as nutrients. Nutrients can be grouped as: major nutrients (molecules containing the elements C, H and O, comprising >99% of biomass); macronutrients (molecules containing elements such as N, S, P, K, Ca and Mg, comprising 0.5% of biomass); and micronutrients or trace elements (0.1% of

biomass). Macronutrients and micronutrients are collectively called minerals. While the major nutrients are obviously needed in the largest amounts, the growth of producers is usually limited by the availability of minerals such as nitrate and phosphate.

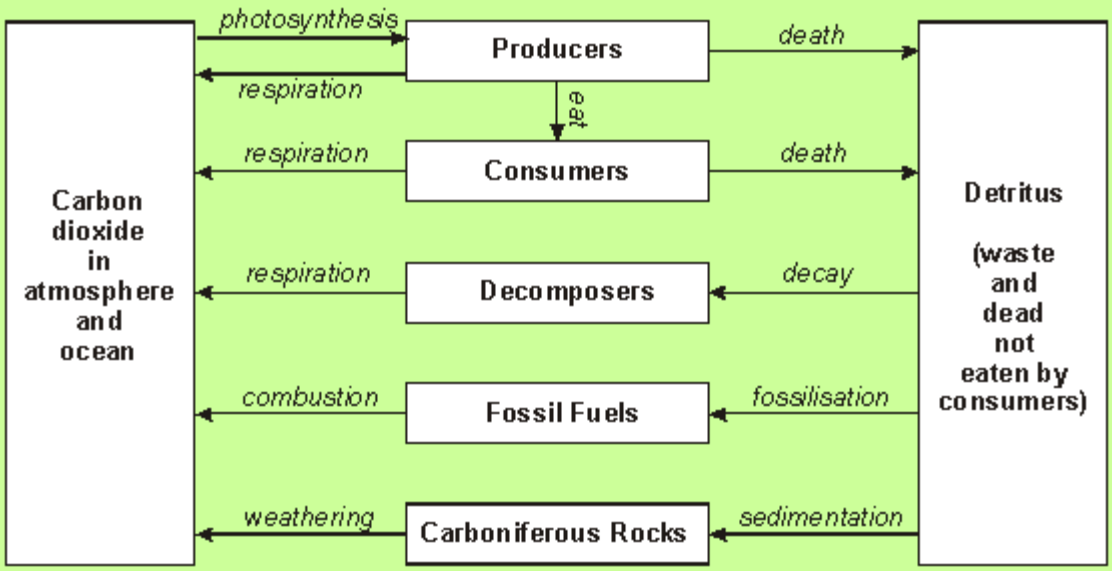
There are two groups of decomposers:

- Detrivores are animals that eat detritus (such as earthworms and woodlice). They digest much of the material, but like all animals are unable to digest the cellulose and lignin in plant cell walls. They break such plant tissue into much smaller pieces with a larger surface area making it more accessible to the saprophytes. They also assist saprophytes by excreting useful minerals such as urea, and by aerating the soil.

- Saprophytes (or decomposers) are microbes (fungi and bacteria) that live on detritus. They digest it by extracellular digestion, and then absorb the soluble nutrients. Given time, they can completely break down any organic matter (including cellulose and lignin) to inorganic matter such as carbon dioxide, water and mineral ions.

Detailed material cycles can be constructed for elements such as carbon, nitrogen, oxygen or sulphur, or for compounds such as water, but they all have the same basic pattern as the diagram above. We shall only study the carbon and nitrogen cycles in detail.

The Carbon Cycle



As this diagram shows, there are really many carbon cycles here with time scales ranging from minutes to millions of years. Microbes play the major role at all stages.

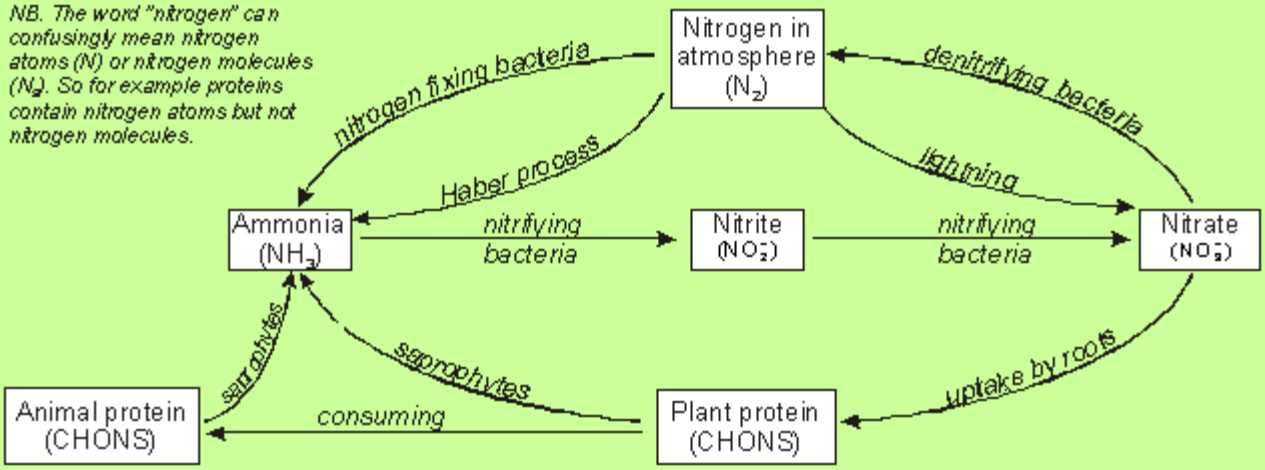
- Far more carbon is fixed by microscopic marine producers (algae and phytoplankton) from CO_2 dissolved in the oceans than by terrestrial plants from CO_2 in the air.

- A large amount of the fixed carbon is used by marine zooplankton to make calcium carbonate shells. These are not eaten by consumers and cannot easily be decomposed, so turn into carboniferous rocks (chalk, limestone, coral, etc). 99% of the Earth's carbon is in this form.

- The decomposers are almost all microbes such as fungi and bacteria. Most of the detritus is in the form of cellulose and other plant fibres, which higher organisms cannot digest. Only a few bacteria posses the cellulase enzymes required to break down plant fibres. Herbivorous animals such as cows and termites depend on these bacteria in their guts.

- Much of the CO_2 that was fixed during the carboniferous era (300 MY ago) was sedimented and turned into fossil fuels. The recent mining and burning of fossil fuels has significantly altered the carbon cycle by releasing the carbon again, causing a 15% increase in CO_2 in just 200 years. Many people believe this is largely responsible for global warming.

The Nitrogen Cycle



Microbes are involved at most stages of the nitrogen cycle:

Nitrogen Fixation. 78% of the atmosphere is nitrogen gas (N_2), but this is inert and can't be used by plants or animals. Nitrogen fixing bacteria reduce nitrogen gas to ammonia ($N_2 + 6H$ Õ $2NH_3$), which dissolves to form ammonium ions (NH_4^+). This process uses the enzyme nitrogenase and ATP as a source of energy. The nitrogen-fixing bacteria may be free-living in soil or water, or they may live in colonies inside the cells of root nodules of leguminous plants such as clover or peas. This is an example of mutualism as the plants gain a source of useful nitrogen from the bacteria, while the bacteria gain carbohydrates and protection from the plants. Nitrogen gas can also be fixed to ammonia by humans using the Haber process, and a small amount of nitrogen is fixed to nitrate by lightning.

Nitrification. Nitrifying bacteria can oxidise ammonia to nitrate in two stages: first forming nitrite ions $(NH_4^+ \rightarrow NO_2^-)$ then forming nitrate ions $(NO_2^- \rightarrow NO_3^-)$. These are chemosynthetic bacteria, which means they use the energy released by nitrification to live, instead of using respiration. Plants can only take up nitrogen in the form of nitrate.

Denitrification. The anaerobic denitrifying bacteria convert nitrate to N_2 and NO_x, which is then lost to the air. This represents a constant loss of "useful" nitrogen from soil, and explains why nitrogen fixation by the nitrifying bacteria and fertilisers are so important.

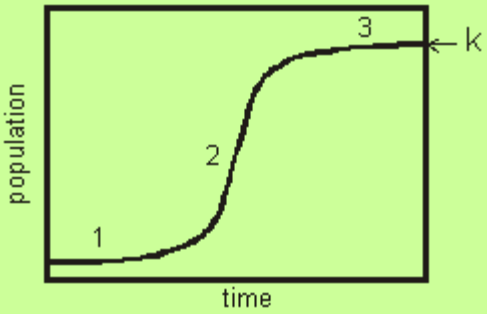
Ammonification. Microbial saprophytes break down proteins in detritus to form ammonia in two stages: first they digest proteins to amino acids using extracellular protease enzymes, then they remove the amino groups from amino acids using deaminase enzymes.

Population Ecology

Population Ecology in concerned with the question: why is a population the size it is? This means understanding the various factors that affect the population.

Population Growth

When a species is introduced into a new environment its population grows in a characteristic way. This growth curve is often seen experimentally, for example bees in a hive, sheep in Tasmania, bacteria in culture. The curve is called a logistic or sigmoid growth curve.



The growth curve has three phases, with different factors being responsible for the shape of each phase. The actual factors depend on the ecosystem, and this can be illustrated by considering two contrasting examples: yeast in a flask (reproducing asexually), and rabbits in a field (reproducing sexually).

	Yeast in a flask	Rabbits in a field
1. Lag phase	Little growth while yeast starts transcribing genes and synthesising appropriate enzymes for new conditions.	Little growth due to small population. Individuals may rarely meet, so few matings. Long gestation so few births.
2. Rapid Growth Phase	Rapid exponential growth. No limiting factors since relatively low density.	Rapid growth, though not exponential. Few limiting factors since relatively low density.
3. Stable Phase	Slow growth due to accumulation of toxic waste products (e.g. ethanol) or lack of sugar.	Slow growth due to intraspecific competition for food/territory, predation, etc.

At the end of phase 3 the population is stable. This population is called the carrying capacity of the environment (K), and is the maximum population supported by a particular ecosystem.

Factors Affecting Population Size

Many different factors interact to determine population size, and it can be very difficult to determine which factors are the most important. Factors can be split into two broad group: abiotic factors and biotic factors. We'll look at 7 different factors.

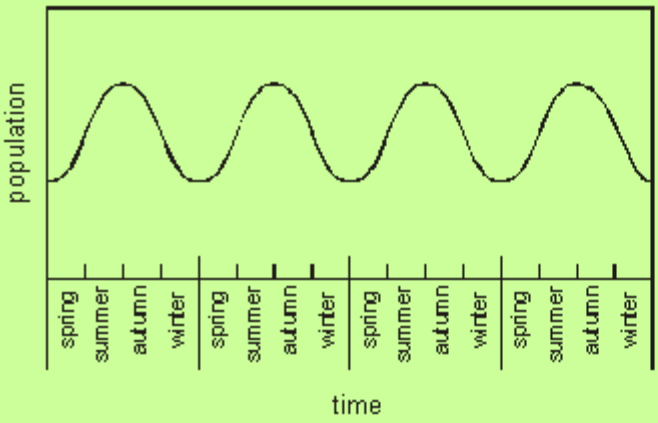
1. Abiotic Factors

The population is obviously affected by the abiotic environment such as: temperature; water/humidity; pH; light/shade; soil (edaphic factors); mineral supply; current (wind/water); topography (altitude, slope, aspect); catastrophes (floods/fire/frost); pollution. Successful species are generally well adapted to their abiotic environment.

In harsh environments (very cold, very hot, very dry, very acid, etc.) only a few species will have successfully adapted to the conditions so they will not have much competition from other species, but in mild environments lots of different species could live there, so there will be competition. In other words in harsh environments abiotic factors govern who survives, while in mild environments biotic factors (such as competition) govern who survives.

2. Seasons

Many abiotic factors vary with the seasons, and this can cause a periodic oscillation in the population size.



Explanation:

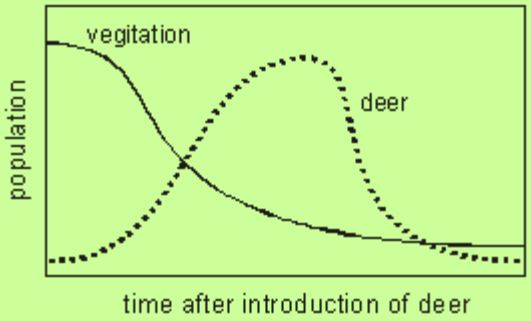
warm weather → more reproduction → population increases

cold weather → less reproduction → population decreases

This is only seen in species with a short life cycle compared to the seasons, such as insects. Species with long life cycles (longer than a year) do not change with the seasons like this.

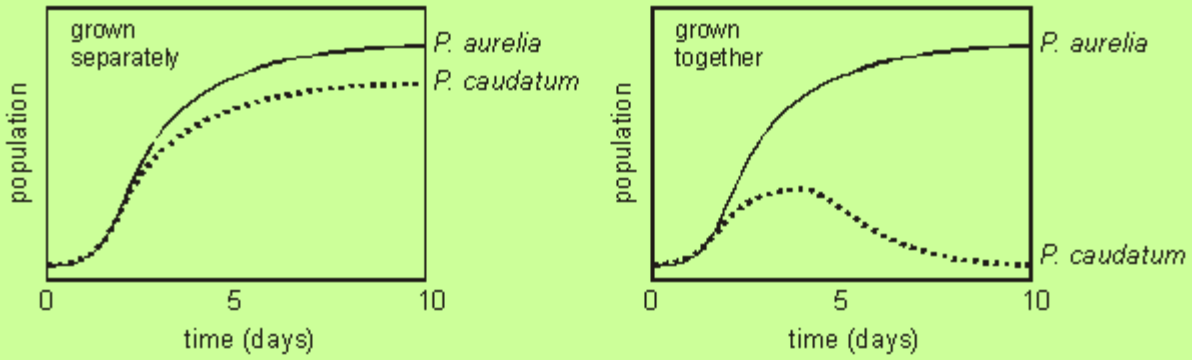
3. Food Supply

A population obviously depends on the population of its food supply: if there is plenty of food the population increases and vice versa. For example red deer introduced to an Alaskan island at first showed a population increase, but this large population grazed the vegetation too quickly for the slow growth to recover, so the food supply dwindled and the deer population crashed.



4. Interspecific Competition

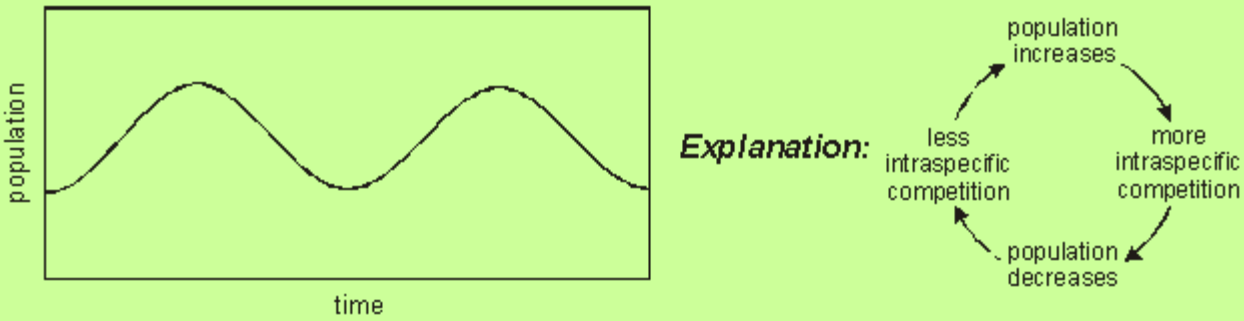
Interspecific competition is competition for resources (such as food, space, water, light, etc.) between members of different species, and in general one species will out-compete another one. This can be demonstrated by growing two different species of the protozoan *Paramecium* in flasks in a lab. They both grow well in lab flasks when grown separately, but when grown together *P.aurelia* out-competes *P.caudatum* for food, so the population of *P.caudatum* falls due to interspecific competition:



5. Intraspecific Competition

Intraspecific competition is competition for resources between members of the same species. This is more significant than interspecific competition, since member of the same species have the same niche and so compete for exactly the same resources.

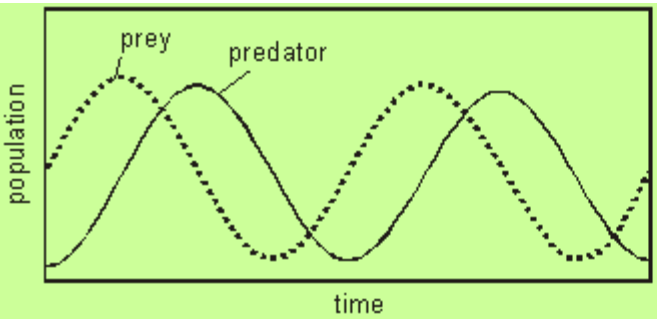
Intraspecific competition tends to have a stabilising influence on population size. If the population gets too big, intraspecific population increases, so the population falls again. If the population gets too small, intraspecific population decreases, so the population increases again:

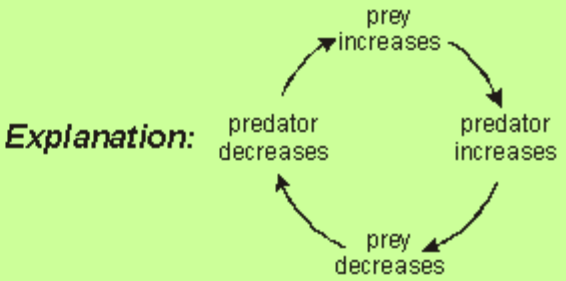


Intraspecific competition is also the driving force behind natural selection, since the individuals with the "best" genes are more likely to win the competition and pass on their genes. Some species use aggressive behaviour to minimise real competition. Ritual fights, displays, threat postures are used to allow some individuals (the "best") to reproduce and exclude others (the "weakest"). This avoids real fights or shortages, and results in an optimum size for a population.

6. Predation

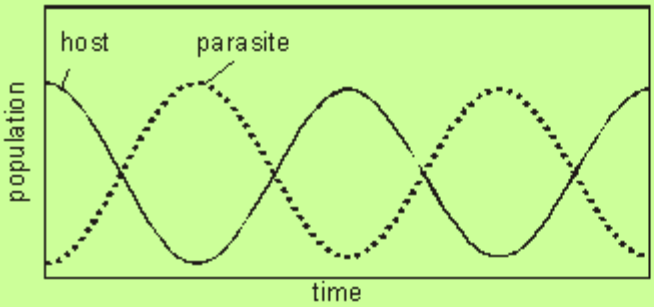
The populations of predators and their prey depend on each other, so they tend to show cyclical changes. This has been famously measured for populations of lynx (predator) and hare (prey) in Canada, and can also be demonstrated in a lab experiment using two species of mite: *Eotetranchus* (a herbivore) and *Typhlodromus* (a predator). If the population of the prey increases, the predator will have more food, so its population will start to increase. This means that more prey will be eaten, so its population will decrease, so causing a cycle in both populations:

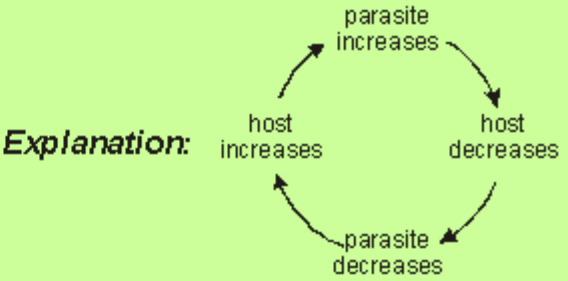




7. Parasitism and Disease

Parasites and their hosts have a close symbiotic relationship, so their populations also oscillate. This is demonstrated by winter moth caterpillars (the host species) and wasp larvae (parasites on the caterpillars). If the population of parasite increases, they kill their hosts, so their population decreases. This means there are fewer hosts for the parasite, so their population decreases. This allows the host population to recover, so the parasite population also recovers:

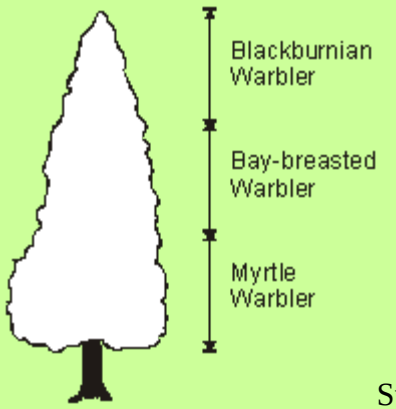




A similar pattern is seen for pathogens and their hosts.

The Ecological Niche

A population's niche refers to its role in its ecosystem. This usually means its feeding role in the food chain, so a particular population's niche could be a producer, a predator, a parasite, a leaf-eater, etc. A more detailed description of a niche should really include many different aspects such as its food, its habitat, its reproduction method etc, so gerbils are desert seed-eating mammals; seaweed is an inter-tidal autotroph; fungi are asexual soil-living saprophytes. Identifying the different niches in an ecosystem helps us to understand the interactions between populations. Members of the same population always have the same niche, and will be well-adapted to that niche, e.g. nectar feeding birds have long thin beaks.



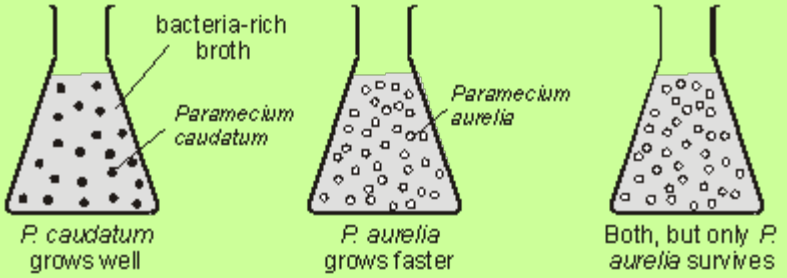
Species with narrow niches are called specialists (e.g. anteater). Many different specialists

can coexist in the same habitat because they are not competing, so this can lead to high diversity, for example warblers in a coniferous forest feed on insects found at different heights. Specialists rely on a constant supply of their food, so are generally found in abundant, stable habitats such as the tropics.

Species with broad niches are called generalists (e.g. common crow). Generalists in the same habitat will compete, so there can only be a few, so this can lead to low diversity. Generalists can cope with a changing food supply (such as seasonal changes) since they can switch from one food to another or even one habitat to another (for example by migrating).

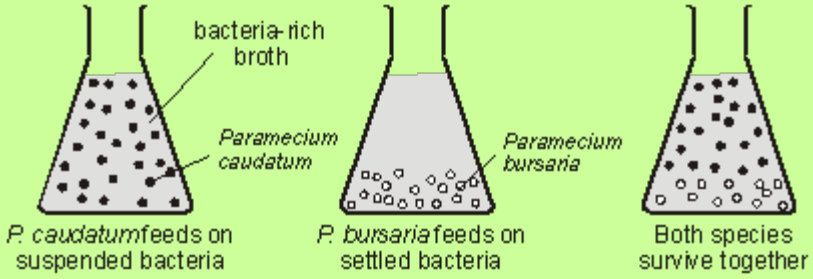
The niche concept was investigated in some classic experiments in the 1930s by Gause. He used flasks of different species of the protozoan *Paramecium*, which eats bacteria.

Experiment. 1:



Conclusion: These two species of *Paramecium* share the same niche, so they compete. *P. aurelia* is faster-growing, so it out-competes *P. caudatum*.

Experiment. 2:



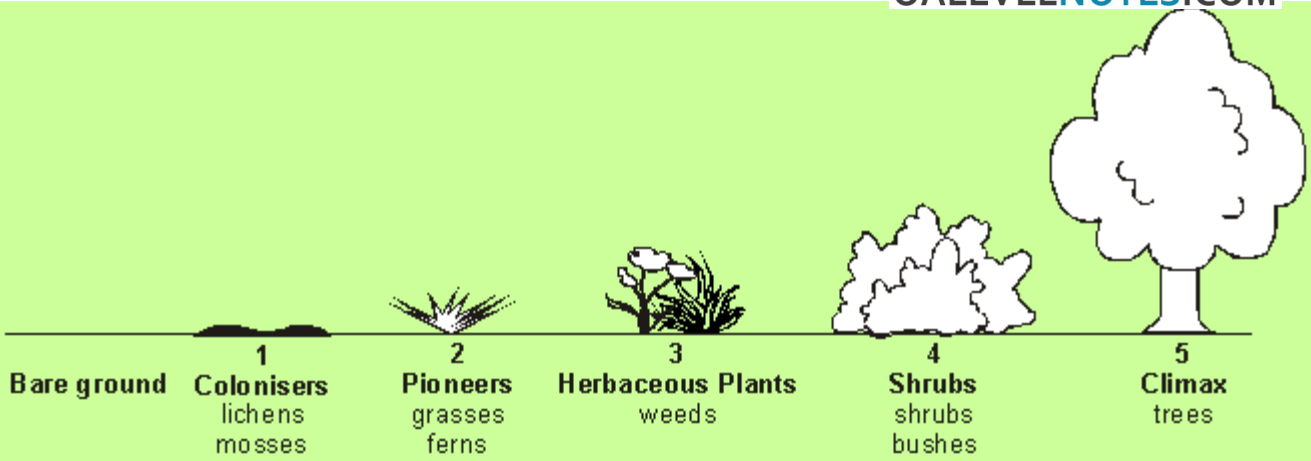
Conclusion: These two species of *Paramecium* have slightly different niches, so they don't compete and can coexist.

It is important to understand the distribution in experiment 2. *P. caudatum* lives in the upper part of the flask because only it is adapted to that niche and it has no competition. In the lower part of the flask both species could survive, but only *P. bursaria* is found because it out-competes *P. caudatum*. If *P. caudatum* was faster-growing it would be found throughout the flask.

The niche concept is summarised in the competitive exclusion principle: Two species cannot coexist in the same habitat if they have the same niche.

Succession

Ecosystems are not fixed, but constantly change with time. This change is called succession. Imagine a lifeless area of bare rock. What will happen to it as time passes?



1. Very few species can live on bare rock since it stores little water and has few available nutrients. The first colonisers are usually lichens, which are a mutualistic relationship between an alga and a fungus. The alga photosynthesises and makes organic compounds, while the fungus absorbs water and minerals and clings to the rock. Lichens are such good colonisers that almost all "bare rock" is actually covered in a thin layer of lichen. Mosses can grow on top of the lichens. Between then these colonisers start to erode the rock and so form a thin soil. Colonisers are slow growing and tolerant of extreme conditions.

2. Pioneer species such as grasses and ferns grow in the thin soil and their roots accelerate soil formation. They have a larger photosynthetic area, so they grow faster, so they make more detritus, so they form better soil, which holds more water.

3. Herbaceous Plants such as dandelion, goosegrass ("weeds") have small wind-dispersed seeds and rapid growth, so they become established before larger plants.

4. Larger plants (shrubs) such as bramble, gorse, hawthorn, broom and rhododendron can now grow in the good soil. These grow faster and so out-compete the slower-growing pioneers.

5. Trees grow slowly, but eventually shade and out-compete the shrubs, which are replaced by shade-tolerant forest-floor species. A complex food web is now established with many trophic levels and interactions. This is called the climax community.

These stages are called seral stages, or seral communities, and the whole succession is called a sere. Each organism modifies the environment, so creating opportunities for other species. As the succession proceeds the community becomes more diverse, with more complex food webs being supported. The final seral stage is stable (assuming the environment doesn't change), so succession stops at the climax stage. In England the natural climax community is oak or beech woodland (depending on the underlying rock), and in the highlands of Scotland it is pine forests. In Roman times the country was covered in oak and beech woodlands with herbivores such as deer, omnivores such as bear and carnivores such as wolves and lynxes. It was said that a squirrel could travel from coast to coast without touching ground.

Humans interfere with succession, and have done so since Neolithic times, so in the UK there are few examples of a natural climax left (except perhaps small areas of the Caledonian pine forest in the Scottish Highlands). Common landscapes today like farmland, grassland, moorland and gardens are all maintained at pre-climax stages by constant human interventions, including ploughing, weeding, herbicides, burning, crop planting and grazing animals. These are examples of an artificial climax, or plagioclimax.

- Primary succession starts with bare rock or sand, such as behind a retreating glacier, after a volcanic eruption, following the silting of a shallow lake or seashore, on a new sand dune, or on rock scree from erosion and weathering of a mountain.

- Secondary succession starts with soil, but no (or only a few) species, such as in a forest clearing, following a

forest fire, or when soil is deposited by a meandering river.

Ecological Impact of Farming

One of the main reasons for studying ecology is to understand the impact humans are having on the planet. The huge increases in human population over the last few hundred years has been possible due to the development of intensive farming, including monoculture, selective breeding, huge farms, mechanisation and the use of chemical fertilisers and pesticides. However, it is apparent that this intensive farming is damaging the environment and is becoming increasingly difficult to sustain. Some farmers are now turning to environmentally-friendly organic farming. We'll examine 5 of the main issues and their possible solutions.

1. Monoculture

Until the middle of the 20th century, farms were usually small and mixed (i.e. they grew a variety of crops and kept animals). About a third of the population worked on farms. The British countryside was described by one observer in 1943 as "*an attractive patchwork with an infinite variety of small odd-shaped fields bounded by twisting hedges, narrow winding lanes and small woodlands*". Today the picture is quite different, with large uninterrupted areas of one colour due to specialisation in one crop - monoculture. Monoculture increases the productivity of farmland by growing only the best variety of crop; allowing more than one crop per year; simplifying sowing and harvesting of the crop; and reducing labour costs.

However, monoculture has a major impact on the environment:

- Using a single variety of crop reduces genetic diversity and renders all crops in a region susceptible to disease.
- Fertilisers are required to maintain soil fertility. This is expensive and can pollute surrounding groundwater due to leaching.
- Pesticides are required to keep crops healthy. Again this is expensive and potentially polluting.
- Monoculture reduces species diversity. This has many knock-on effects such as allowing a pest species to get out of control, fewer plants due to lack of pollinating insects and loss of species that may be useful to humans.
- Less attractive countryside.

Some farmers are now returning to traditional crop rotations, where different crops are grown in a field each year. This breaks the life cycles of pests (since their host is changing); improves soil texture (since different crops have different root structures and methods of cultivation); and can increase soil nitrogen (by planting nitrogen-fixing legumes).

2. Hedgerows

Hedges have been planted since Anglo-Saxon times to mark field boundaries and to contain livestock. As they have matured they have diversified to contain a large number of different plant and animal species, some found nowhere else in the UK. Since the second world war much of the hedgerow has been removed because:

- As mixed farms converted to arable farms, hedgerows are no longer needed to contain livestock.
- Many small farms have been amalgamated into large farms, allowing larger fields, which in turn allows greater mechanisation and lower labour costs. One farmer found that by removing 1.5 miles of hedges, he increased his arable land by 3 acres and reduced harvesting time by one third.
- Hedgerows reduce the space available for planting crops, and their roots compete with those of crops for water and minerals in the soil.
- Hedgerows provide shelter for pests such as rabbits and insects, and they are a reservoir of weeds and disease.
- Hedgerows need to be maintained, which is a skilled job, costing time and money.

However it has now become clear that hedgerows served an important place in the ecology of Britain.

- They provide habitats for at least 30 species of trees and shrubs, 65 species of nesting birds, 1500 species of insects and 600 species of wildflowers. These in turn provide food for small mammals.
- They act as corridors, allowing animals to move safely between woodlands.
- Some of the animals they shelter are predators of plant pests, so they may reduce pests, not increase them.
- They are efficient windbreaks, providing shelter for animals and plants, and reducing soil erosion. During storms in recent years large amounts of topsoil was blown away from large unsheltered fields.
- They provide habitats for pollinating insects, so removing hedgerows can indirectly reduce the populations of other local plant species.
- In the UK we have surpluses of many crops, and farmers can receive grants to reduce their food production.

The importance of hedgerows is now being recognised, and farmers can now receive grants to plant hedgerows. However it takes hundreds of years for new hedgerows to mature and develop the same diversity as the old ones.

3. Fertilisers

Since the rate of plant growth in usually limited by the availability of mineral ions in the soil, then adding more of these ions as fertiliser is a simple way to improve yields, and this is a keystone of intensive farming. The most commonly used fertilisers are the soluble inorganic fertilisers containing nitrate, phosphate and potassium ions (NPK). Inorganic fertilisers are very effective but also have undesirable effects on the environment. Since nitrate and ammonium ions are very soluble, they do not remain in the soil for long and are quickly leached out, ending up in local rivers and lakes and causing eutrophication. They are also expensive.

An alternative solution, which does less harm to the environment, is the use of organic fertilisers, such as animal manure (farmyard manure or FYM), composted vegetable matter, crop residues, and sewage sludge. These contain the main elements found in inorganic fertilisers (NPK), but in organic compounds such as urea, cellulose, lipids and organic acids. Of course plants cannot make use of these organic materials in the soil: their roots can only take up inorganic mineral ions such as nitrate, phosphate and potassium. But the organic compounds can be digested by soil organisms such as animals, fungi and bacteria, who then release inorganic ions that the plants can use (refer to the nitrogen cycle). Some advantages of organic fertilisers are:

- Since the compounds in organic fertilisers are less soluble than those in inorganic fertilisers, the inorganic minerals are released more slowly as they are decomposed. This prevents leaching and means they last longer.
- The organic wastes need to be disposed of anyway, so they are cheap. Furthermore, spreading on to fields means

they will not be dumped in landfill sites, where they may have caused uncontrolled leaching.

- The organic material improves soil structure by binding soil particles together and provides food for soil organisms such as earthworms. This improves drainage and aeration.

Some disadvantages are that they are bulky and less concentrated in minerals than inorganic fertilisers, so more needs to be spread on a filed to have a similar effect. They may contain unwanted substances such as weed seeds, fungal spores, heavy metals. They are also very smelly!

4. Pesticides

To farmers, a pest is any organism (animal, plant or microbe) that damages their crops. Some form of pest control has always been needed, whether it is chemical (e.g. pesticides), biological (e.g. predators) or cultural (e.g. weeding or a scarecrow). Chemicals pesticide include:

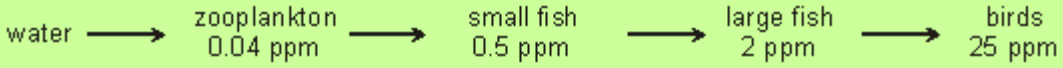
- herbicides anti-plant chemicals
- insecticides anti-insect chemicals
- fungicides anti-fungal chemicals
- bactericides anti-bacterial chemicals

Pesticides have to be effective against the pest, but have no effect on the crop. They may kill the pests, or just reduce their population by slowing growth or preventing reproduction. Intensive farming depends completely on the use of pesticides, and some wheat crops are treated with 18 different chemicals to combat a variety of weeds, fungi and insects. In addition, by controlling pests that carry human disease, they have saved millions of human lives. However, with their widespread use and success there are problems, the mains ones being persistence and bioaccumulation.

Both of these are illustrated by DDT (DichloroDiphenylTrichloroethane), an insecticide used against the malaria mosquito in the 1950s and 60s very successfully, eradicating malaria from southern Europe. However the population of certain birds fell dramatically while it was being used, and high concentrations of DDT were found in their bodies, affecting calcium metabolism and causing their egg shells to be too thin and fragile. DDT was banned in developed countries in 1970, and the bird populations have fully recovered. Alternative pesticides are now used instead, but they are not as effective, and continued use of DDT may have eradicated malaria in many more places.

Persistence. This refers to how long a pesticide remains active in the environment. Some chemicals are broken down by decomposers in the soil (they're biodegradable) and so are not persistent, while others cannot be broken down by microbes (they're non biodegradable) and so continue to act for many years, and are classed as persistent pesticides. The early pesticides (such DTT) were persistent and did a great deal of damage to the environment, and these have now largely been replaced with biodegradable insecticides such as carbamates and pyrethroids.

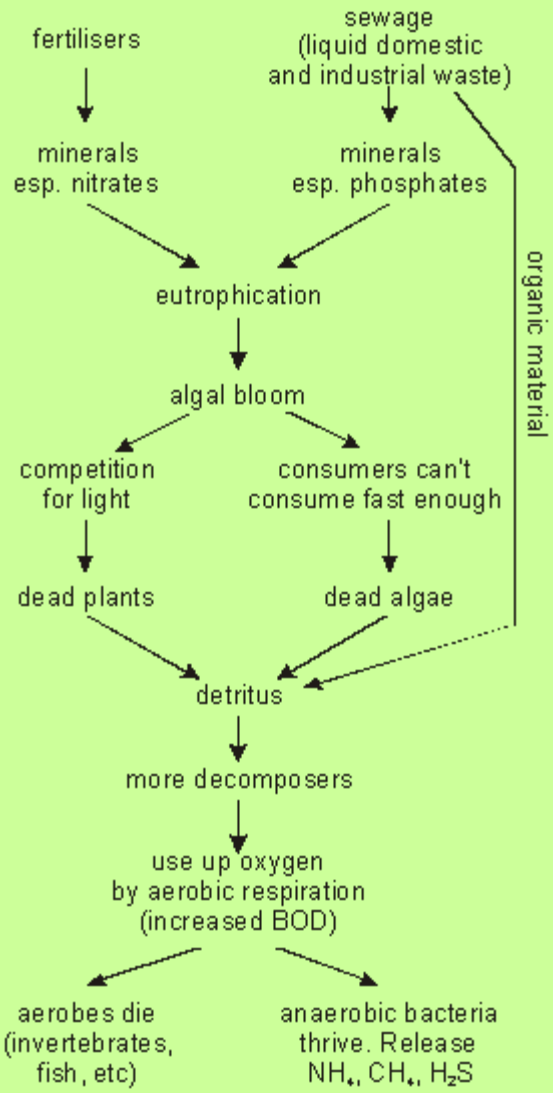
Bioaccumulation (or Biomagnification). This refers to the built-up of a chemical through a food chain. DDT is not soluble in water and is not excreted easily, so it remains in the fat tissue of animals. As each consumer eats a large mass of the trophic level below it, DTT accumulates in the fat tissue of animals at the top of the food chain. This food chain shows typical concentrations of DDT found in a food chain (in parts per million, ppm):



The high concentration of DDT in birds explains why the toxic effects of DDT were first noticed in birds.

5. Eutrophication

Eutrophication refers to the effects of nutrients on aquatic ecosystems. These naturally progress from being oligotrophic (clean water with few nutrients and algae) to eutrophic (murky water with many nutrients and plants) and sometimes to hypertrophic (a swamp with a mass of plants and detritus). This is in fact a common example of succession. In the context of pollution "eutrophication" has come to mean a sudden and dramatic increase in nutrients due to human activity, which disturbs and eventually destroys the food chain. The main causes are fertilisers leaching off farm fields into the surrounding water course, and sewage (liquid waste from houses and factories). These both contain dissolved minerals, such as nitrates and phosphates, which enrich the water.



Since producer growth is generally limited by availability of minerals, a sudden increase in these causes a sudden increase in producer growth. Algae grow faster than larger plants, so they show a more obvious "bloom", giving rise to spectacular phenomena such as red tides. Algae produce oxygen, so at this point the ecosystem is well oxygenated and fish will thrive.

However, the fast-growing algae will out-compete larger plants for light, causing the plants to die. The algae also grow faster than their consumers, so many will die without being consumed, which is not normal. These both lead to a sudden increase in detritus. Sewage may also contain organic matter, which adds to the detritus.

Decomposing microbes can multiply quickly in response to this, and being aerobic they use up oxygen faster than it can be replaced by photosynthesis or diffusion from the air. The decreased oxygen concentration kills larger aerobic animals and encourages the growth of anaerobic bacteria, who release toxic waste products.

Biochemical Oxygen Demand (BOD). This measures the rate of oxygen consumption by a sample of water, and therefore gives a good indication of eutrophication. A high BOD means lots of organic material and aerobic microbes, i.e. eutrophication. The method is simple: a sample of water is taken and its O_2 concentration is measured using an oxygen meter. The sample is then left in the dark for 5 days at 20°C, and the O_2 is measured again. The BOD is then calculated from: original O_2 concentration – final O_2 concentration. The more oxygen used up over the 5 days (in $mg.dm^{-3}$) the higher the BOD, and the higher the BOD the more polluted the water is. This table shows some typical BOD values.

	BOD (mg.dm^{-3})
clean water	3
polluted water	10
cleaned sewage	20 (legal max)
raw sewage	300

Aquatic ecosystems can slowly recover from a high BOD as oxygen dissolves from the air, but long-term solutions depend on reducing the amount of minerals leaching into the water. This can be achieved by applying inorganic fertilisers more carefully, by using organic fertilisers, by using low-phosphate detergents, and by removing soluble minerals by precipitation in modern sewage plants. As a last resort eutrophic lakes can be dredged to remove mineral-rich sediment, but this is expensive and it takes a long time for the ecosystem to recover. This has been done in the Norfolk Broads.

MODULE 8

A2 Option Module 8 BEHAVIOUR AND POPULATIONS

Introduction: This option module extends the study of nervous and hormonal physiology in Module 4 to the behaviour of whole organisms. There is also consideration of reproductive behaviour and human growth and development, with an emphasis on the underlying principles of hormonal control. The study of human populations is developed to include a range of public health issues. Candidates are expected to understand the biological background to these issues and to be able to evaluate possible strategies for improvement. In the assessment of this module a knowledge and understanding of relevant content from Modules 1 to 5 will be assumed.

CLICK TO JUMP TO THE SECTION YOU DESIRE

- Specification table
- Patterns of behaviour
- Reproductive behaviour
- Pregnancy
- Human growth and development
- Human populations and health

Specification table:

Patterns of behaviour

Innate behaviour	The principal differences between innate and learned behaviour.
Taxes and kineses	Taxes and kineses as examples of innate behaviour.
Reflex actions	The nature of simple reflex behaviour, such as in reflex escape responses. The linking of a number of simple reflexes to produce a more complex pattern of behaviour as shown by the reflexes involved in the feeding of a new-born human infant.
Modified reflexes	The modification of reflex behaviour by learning as shown by the development of conscious control of bladder emptying.
Learned behaviour	Habituation and imprinting. Classical conditioning, illustrated by the work of Pavlov on the control of salivation in dogs. Operant conditioning, illustrated by the work of Skinner on rats. The importance of reinforcement stimuli and rewards in learning . Candidates should be able to explain examples of behaviour in terms of classical conditioning and of operant conditioning and to evaluate parallels between animal and human behaviour.

Reproductive behaviour

Courtship	Courtship behaviour as a necessary precursor to successful mating. The roles of species recognition, pair bond formation, sexual selection and synchronisation of breeding behaviour. Sign stimuli and innate releaser mechanisms as components in simple courtship patterns. The role of hormones and pheromones in courtship behaviour. Candidates should be able to analyse individual components in simple courtship patterns, and evaluate comparisons between the behaviour of humans and other animals.
Territorial behaviour	The advantages of defending a territory, in relation to breeding success.
The menstrual cycle	The roles of FSH, LH, oestrogen and progesterone in controlling the human menstrual cycle. The effect of oestrogen and progesterone on the uterine endometrium. The role of negative feedback in regulating hormone concentrations.
Contraception	The use of oral contraceptives based on oestrogen and progesterone in controlling fertility. Candidates should be able to evaluate the different methods of birth control. The treatment of female infertility with extracted and synthetic hormones and with drugs such as clomiphene which stimulate hormone activity. The key stages in in vitro fertilisation:

Infertility	• the use of fertility drugs to stimulate ovulation; • the collection of mature egg cells and their incubation with sperms; • the insertion of embryos into the uterus.

Pregnancy

Conception	Fertilisation, including capacitation, the role of the acrosome and formation of the fertilisation membrane.
Hormones and pregnancy	The roles of human chorionic gonadotrophin (HCG) and progesterone in controlling the events of pregnancy. Confirmation of pregnancy by determining HCG and progesterone levels.
The placenta	The structure of the placenta in relation to its role in the supply of substances to, and the removal of waste products from, the developing foetus.
Physiological changes in the mother	The changes in the following which take place during the course of a normal pregnancy and their physiological significance: • body mass; • plasma volume, red-blood-cell mass and cardiac output; • kidney function.

Human growth and development

Patterns of human growth	The pattern of growth of the whole body, reproductive organs and the brain from infancy to adulthood. Candidates should be able to represent graphically and interpret data relating to growth and growth rate.
Hormonal control	The roles of thyroxine, growth hormone and sex hormones in the control of human growth from infancy to maturity. Puberty and the principal physical changes associated with it. The evolutionary importance of a long pre-puberty stage in the human lifespan.
Ageing	The contributions to ageing of changes in physiological function, degeneration of tissue, accumulation of genetic error, and malfunction of the immune system.

Human populations and health

Population size and structure	Population growth rates, pyramids, survival rates and life expectancy. Candidates should be able to: • interpret population growth curves, survival curves and age pyramids; • calculate population growth rate from data on birth rate, death rate, emigration and immigration; • relate changes in the size and structure of human populations to different stages in demographic transition.
Social conditions	The influence of food supply, safe drinking water and effective sewage disposal on mortality.
Infectious disease	Pathogens, including certain bacteria, viruses and fungi, as the cause of infectious disease. Transmission of pathogens by droplet infection and contact, or in food and water. Natural immunity as production of antibodies in response to antigens. Immunological memory. (Details of the mechanisms of the immune response not required.) Artificial immunity by vaccination. The limitations of vaccination related to variability of antigens in pathogens. The herd immunity effect. Candidates should be able to: • interpret information relating to the incidence and mortality of diseases; • evaluate the effectiveness of immunisation programmes and changes in social conditions in preventing epidemics.
Effects of lifestyle on health	The constitution and importance of a balanced diet. The effects of excess fat and salt intakes, and of deficiency of mineral ions (calcium, iron and iodine) and vitamins (vitamins A, C and D). The relationships between diet, exercise and cardiovascular disease. Atheroma formation, formation of blood clots, aneurysm, myocardial infarction and cerebrovascular accident. The relationships between air pollution and smoking and chronic bronchitis, emphysema and lung cancer. The development and effects on lung function of bronchitis, emphysema and lung cancer. The relationship between ultra-violet light and malignant skin tumors. Tumor growth and metastasis. Candidates should be able to explain the biological effects of the disorders listed, and to evaluate measures that might be taken to reduce the risk factors. The principles involved in the use of x-rays, endoscopy, ultrasound and genetic techniques in diagnosis and screening programmes. Candidates should be able to:

Screening programmes	suggest the most appropriate technique to use in the diagnosis or screening of a particular condition; evaluate the issues arising from the use of screening programmes for inherited conditions.

Patterns of behaviour

Behaviour

Behaviour is what an animal does and how it does it. To some extent all behaviour has a genetic basis but in general, behaviour is a response to some environmental stimulus. Ethology is the correct term for the study of behaviour in its natural habitat. It is mostly a descriptive science.

There are two types of behaviour innate and learned.

- Innate behaviour – little influence from the environment – does not need to be learnt, varies little within species. (inflexible)
- Learned behaviour – develops from an animals experience of its environment – not passed on genetically

Some behaviours are a blend of both so classification is not always so easy

Innate Behaviours

- inherited, instinctive:
- programmed by genes
- highly stereotyped (similar each time in many individuals)

Types of innate behaviour:

1. Kinesis: "change the speed of random movement in response to environmental stimulus"
2. Taxis: "a directed movement toward or away from a stimulus; positive and negative taxes
3. Reflex: "movement of a body part in response to stimulus".
4. Fixed Action Pattern (FAP): "stereotyped and often complex series of movements., responses to a specific stimulus - Releaser"

Kinesis: An orienting behaviour which is non-directional. Here an animal reduces it's rate of movement or increases its rate of turning as the intensity of the stimulus increases (e.g. woodlice slow down and turn more in the dark). This action has the effect of keeping the organism in an area it finds favourable and making it move away from areas it finds unfavourable.
Taxis: An orienting behaviour which is directional. Here an animal turns towards or away from a stimulus such as light. Can be positive or negative. Blowfly larvae (maggots) show negative phototaxis.
Reflex: A simple reflex is movement of a body part in response to stimulus. It is a rapid, innate automatic response to a stimulus. We looked at the nerve pathway involved in a reflex in module 4 and that helps explain why they are quick and the response does not vary. Watch out for synoptic questions on reflexes.

Reflexes can be linked together to produce more complicated behaviours. The example of this that you have to learn is breast-feeding in humans.

There are several reflexes involved in the sequence.

Reflex	Behaviour
Rooting reflex (baby)	Also called nipple-seeking behaviour. When the breast touches the baby it will turn its head with its mouth open until it finds the nipple.
Sucking reflex (baby)	When the baby attaches to the nipple it begins to suck.
Let-down reflex (mother)	The stimulation of the nipples by the baby sucking causes the reflex release of the hormone oxytocin. This hormone triggers smooth muscle contraction in the mammary glands causing the release of milk

Although reflexes are defined as unconscious actions that are performed in their entirety and are automatic –

they can in some instances be modified. The most obvious example of this is the control of the sphincters which govern urination and defaecation.

The reflex that empties the bladder is as follows. The full bladder is the stimulus which causes the sphincter muscles around the base of the urethra to relax, these muscles are connected to the autonomic nervous system – to modify it this muscular relaxation has to be prevented.

Learned behaviours develop during an animals lifetime and are not passed on genetically to its offspring. They vary from very simple to the complex social interactions in primates and whales. Since learned behaviours are not “hardwired” they can often be adapted – this adaptation of behaviour forms the basis of animal training.

When a reflex is modified it is because the stimulus that causes the reflex also causes sensory information to be sent to the brain. When the learning has occurred this information causes inhibitory signals to be sent from the CNS preventing the normal reflex response

Learned Behaviour

Learned Behaviour can be divided into different categories:

- Habituation
- Imprinting
- Conditioning:
- classical conditioning
- operant conditioning
- Insight, reasoning

Habituation is perhaps the simplest form of learned behaviour. This is where an animal that normally responds to a certain stimulus learns to stop responding to the same stimulus when it is repeatedly stimulated without reason. For example some spiders lie in wait for prey to one side of their web and when something gets trapped. On the web the spider detects the vibrations of the web a rushed out to kill its prey. This response can be made to occur by simply tapping the web with a pen. However after a few stimuli the spider ceases to respond. We say it has become habituated.

Young geese (goslings) do not immediately recognise their mother but they imprint on her. There is a sensitive period during the first few days of a goslings life in which it will follow and become attached to any large object, of course in nature this is the mother but in some experiments it has been humans or even a red watering can. When goslings are distressed they will run to whatever object they have imprinted on which usually will be advantageous as it would be their mother but not so helpful if the object was the red watering can.

It breeding programs to replenish rare or endangered animals care is taken to avoid imprint onto humans and habituation to the presence of humans. In fact habituation to human presence is one of the factors that makes zoo and captive bred animals very different to their wild counterparts and is an obstacle to reintroduction.

Conditioning involves the formation of new connections between stimuli and responses, the table below shows a summary of this

Type of conditioning	Summary
Classical	A stimulus leads to a response. Here a new stimulus is given at the same time as the first after time the response occurs even if only the second stimulus is given.
Operant	Trial and reward learning.

Classical conditioning was first shown by the work of Pavlov with dogs. He collected saliva from dogs and noted that when presented with the sight and smell of their food they began to salivate in preparation of eating. Pavlov began to ring a bell each time the dog was shown their food. After a while Pavlov found that the dogs salivated when the bell was rung regardless of whether food was present. The dog had become

conditioned it associated a bell with the arrival of food.

Habituation

Habituation is a reduction in a previously displayed response when a stimulus is repeatedly applied with no reward or punishment following.

If you make an unusual sound in the presence of the family dog, it will respond - usually by turning its head toward the sound. If the stimulus is given repeatedly and nothing either pleasant or unpleasant happens to the dog, it will soon cease to respond. This lack of response is not a result of fatigue or sensory adaptation and is long-lasting; when fully habituated, the dog will not respond to the stimulus even though weeks or months have elapsed since it was last presented.

Imprinting

If newly-hatched geese are exposed to a moving object of reasonable size and emitting reasonable sounds, they will begin to follow it just as they would normally follow their mother.

This is called imprinting.

The time of exposure is quite critical. A few days after hatching, imprinting no longer occurs. Prior to this time, though, the results can be quite remarkable. A gosling imprinted to a moving box or clucking person will try to follow this object for the rest of its life. In fact, when the gosling reaches sexual maturity, it will make the imprinted object - rather than a member of its own species - the goal of its sexual drive.

Much of our knowledge of imprinting was learned from the research of Konrad Lorenz

The Conditioned Response

The conditioned response is probably the simplest form of learned behaviour. It is a response that - as a result of experience - comes to be caused by a stimulus different from the one that originally triggered it. The Russian physiologist Ivan Pavlov found that placing meat powder in a dog's mouth would cause it to salivate.

This unconditioned stimulus (US) is probably a simple inborn reflex involving taste receptors, sensory neurons, networks of interneurons in the brain, and motor neurons running to the salivary glands.

Pavlov found that if he rang a bell every time he put the meat powder in the dog's mouth, the dog eventually salivated upon hearing the bell alone. This is the conditioned response.

The dog has learned to respond to a substitute stimulus, the conditioned stimulus (CS).

We assume that the physiological basis of the conditioned response is the transfer, by appropriate neurons, of nervous activity in the auditory areas of the brain to the motor neurons controlling salivation. This involves the development of new circuits, which - we may also assume - is characteristic of all forms of learning.

We use the term "operant conditioning" to describe one type of associative learning. Operant conditioning is also termed trial and reward learning. The classic experiments into operant conditioning were carried out by Skinner, where he trained rats and pigeons to press a lever in order to obtain a food reward ("skinners box). In such experiments, the subject is able to generate certain motor-output *responses* (e.g. running around, cleaning, resting, pressing the lever). The experimentor chooses a certain action (e.g. pressing the lever) to act as the response and to pair with an *unconditioned stimulus* (e.g. a food reward). After a training period, the subject will show the *conditioned response* (e.g. pressing the lever) if the response-unconditioned stimulus association has been memorized.

Pheremones

Pheromones are chemicals released by an organism into its environment enabling it to communicate with other members of its own species.

Humans may have pheromones

It has long been noticed that women living close together (e.g., college roommates) develop synchronous menstrual cycles.

This is thought to be because they release two (as yet uncharacterised) primer pheromones

- one prior to ovulation that tends to speed up the onset of ovulation in others

one after ovulation that tends to delay the onset of ovulation in other women.

Both pheromones are released from the armpits.

The pheromones are not detected consciously as odours, but presumably trigger the hormonal changes that mediate the menstrual cycle.

Reproductive behaviour	

Courtship

Courtship Behaviour:

- Attraction of mate, (possibly from a considerable distance)
- Allows species recognition
- Allows sex recognition
- Stimulates sexual behaviour / egg production
- Allows recognition of sexually mature / receptive individuals
- Enables choice of fittest individuals

In birds courtship behaviours can include action such as:

- Head Wagging
- Sky Pointing
- Hop Display
- Wing Waving
- Bowing
- Presenting Nest Material

In establishing breeding partners and defending territories members of the same species rarely fight. Instead they take part in behaviour that is stylised and aimed at avoiding the need to fight

Aggressive encounters between individuals of the same species

- Song, Roar etc.
- Display
- Charging
- Pushing & Shoving
- Displacement

Why not just fight?

- Risk of Injury
- Expenditure of energy
- Conclusion predictable

Do they ever fight?....Yes...When

- The stakes are high i.e. it is a life or death situation
- The outcome may not be clear

TYPES OF MATING RELATIONSHIPS

MONOGAMOUS: Male and female form exclusive bond, may be for one breeding season or for life.

POLYGAMOUS: Animals have several mates at the same time ~ 2 classes:

- Males have several female mates = polygynous. e.g. red deer
- Females have several male mates = polyandrous. e.g. starlings

THE HORMONAL CONTROL OF THE FEMALE MENSTRUAL CYCLE

Pituitary Hormones - released from the pituitary gland in the brain

- FSH: Follicle Stimulating Hormone
- LH: Lutenising Hormone

Ovarian Hormones - released from the ovaries (the examiners usually think of oestrogen as been released from the follicle and progesterone as been released from the corpus luteum - however there is actually some overlap)

- Oestrogen; This repair the uterine lining.
- Progesterone; This maintain the uterine lining

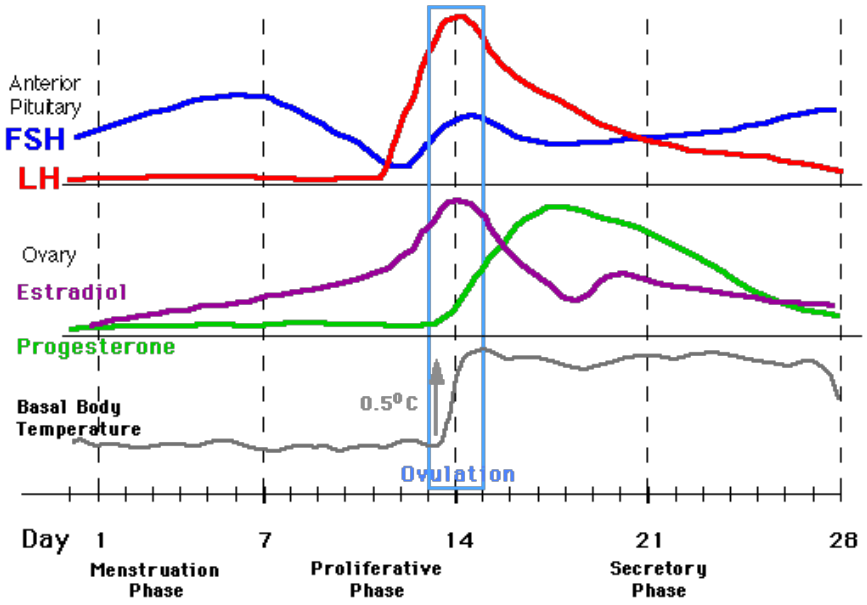
THE SEQUENCE

- FSH stimulates growth of the follicle.
- The developing follicle in the ovary produces oestrogens
- Rising oestrogen levels inhibit FSH and promote LH production
- LH stimulates follicle development and its conversion into the corpus luteum
- Rising oestrogen levels stimulates an increase in FSH
- A surge of FSH and LH brings about ovulation
- LH stimulates progesterone production
- Progesterone inhibits FSH and LH

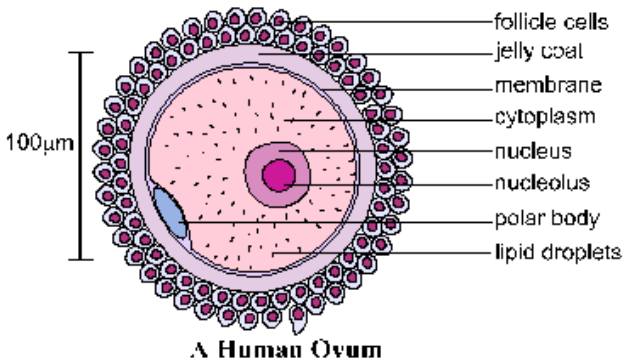
SUMMARY OF EFFECTS

HORMONE	EFFECTS
FSH	• stimulates the growth & development of the follicle • stimulates secretion of oestrogen • enhances effect of LH in stimulating ovulation
LH	• stimulates the final development of the follicle • stimulates ovulation • stimulates the development of the corpus luteum • stimulates production of progesterone • stimulates repair of uterine lining

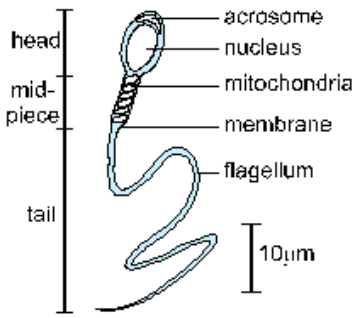
Oestrogen	at high conc. inhibits FSH, however during 'pituitary hormone surge' it stimulates further FSH production • as conc. peaks stimulates release of LH • maintains uterine lining
Progesterone	• inhibits release of FSH • inhibits release of LH • fall in conc. results in menstruation • fall in conc. removes inhibition of FSH and a new cycle begins.



These diagrams of human gametes illustrate the differences between male and female.



A Human Ovum



A Human Sperm

Fertilisation Summary:

Fertilisation is the fusion of two gametes to form a zygote. In humans this takes place near the top of the

oviduct. Hundreds of sperm reach the egg and use their tails to swim through the follicle cells (shown in this photo). When they reach the jelly coat surrounding the ovum they bind to receptors and this stimulates the rupture of the acrosome membrane in the sperms, releasing digestive enzymes, which make a path through the jelly coat. When a sperm reaches the ovum cell the two membranes fuse and the sperm nucleus enters the cytoplasm of the ovum. This triggers a series of reactions in the ovum that cause the jelly coat to thicken and harden, preventing any other sperm from entering the ovum. The sperm and egg nuclei then fuse, forming a diploid zygote.

Fertilisation Detail:
Copulation and Fertilization

For fertilization to occur, sperm must be deposited in the vagina within a few days before or a day or two after ovulation. Sperm transfer is accomplished by copulation.

Semen is a fluid containing the sperm and liquid added by the seminal vesicles, Cowper's glands, and the prostate gland. These fluids provide a source of energy (fructose) and perhaps in other ways provide an optimum chemical environment for the sperm. The semen passes through the urethra and is expelled into the vagina.

Once deposited within the vagina, the sperm proceed on their journey into and through the uterus and on up into the fallopian tubes. It is here that fertilization may occur if an "egg" is present (strictly speaking, it is still a secondary oocyte until after completion of meiosis II).

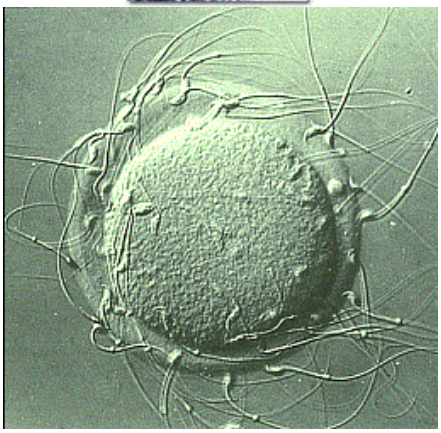
Although sperm can swim several millimetres each second, their trip to and through the fallopian tubes may be assisted by muscular contraction of the walls of the uterus and the tubes. There is some evidence that the egg may release a chemical attractant for sperm. In any case, sperm may reach the egg within 15 minutes of ejaculation. The trip is massive for the sperm and many don't make it. An average human ejaculate contains several hundred million sperm but only a few hundred reach the egg. And of these, only one will succeed in entering the egg and fertilizing it.

Before sperm can fertilise an egg a process called capacitation must take place. This is where a coating surrounding the sperm is removed it occurs over a period of a few hours and is triggered by the conditions within the female reproductive tract. Once capacitation has occured the acrosome is capable of releasing its enzymes.

Fertilization begins with the binding of a sperm cell to the outer coating of the egg (called the zona pellucida). Enzymes released by the acrosome at the tip of the sperm head digest a path through the zona and enable the sperm to enter the cytoplasm of the egg.

Once a single sperm has penetrated, the cell membrane of the egg calcium ions move into the egg cell. This causes exocytosis of cortical granules from the egg. The granules fuse with the zona pellucida, forming a fertilisation membrane. This prevents the entry of other sperm. The other sperm die within 48 hours. Thus the cortical reaction ensures that only one sperm fertilizes the egg.

Soon the head of the successful sperm enlarges. At the same time, the egg (secondary oocyte) completes meiosis II. The male and female nuclei move toward each other. Their nuclear envelopes disintegrate. A spindle is formed, and a full diploid set of chromosomes assembles on it. The fertilized egg or zygote is now ready for its first mitosis.



Pregnancy	

Pregnancy

Embryonic development begins while the fertilized egg is still within the fallopian tube. The developing embryo travels down the tube, reaching the uterus in about a week. As a result of repeated mitotic divisions and some migration of cells, a hollow ball of cells is formed called the blastocyst. Approximately one week after fertilization, the blastocyst embeds itself in the endometrium, a process called implantation. With implantation, pregnancy is established.

The blastocyst has two parts the inner cell mass and the trophoblast. Between them these two parts will develop into the:

- baby
- amnion
- placenta
- umbilical cord

and secrete the pregnancy hormone human chorionic gonadotropin (HCG).

Human Chorionic Gonadotropin

HCG behaves much like LH because it stimulates the corpus luteum to secrete progesterone but has one crucial difference: it is NOT inhibited by a rising level of progesterone. So HCG prevents the deterioration of the corpus luteum at the end of the fourth week and enables pregnancy to continue beyond the end of the normal menstrual cycle.

Because only the implanted embryo makes HCG, its early appearance in the urine of pregnant women provides the basis for the most widely used test for pregnancy (which can provide a positive signal even before menstruation would have otherwise begun).

As pregnancy continues, the placenta becomes a major source of progesterone, and its presence is essential to maintain pregnancy.

The Pregnancy Test

This test is usually the first test conducted when you suspect that you may be pregnant. There are a variety of home testing kits available over-the-counter and all detect a protein hormone called human chorionic gonadotropin (hCG). When an egg is fertilized, the embryo begins to produce hCG. Levels of hCG increase after conception and can be detected in the mother's urine. By 10 days after conception, hCG levels are about 25 milli-International Units (mIU).

Typically, the home test is a urine test for hCG:

1. You collect a sample of urine. You would usually use the first urine in the morning, when hCG levels are the most concentrated, or wave the test wand through the urine stream.
2. If you collected the urine, you can either dip the test wand into the cup or place a drop on the test wand.
3. The test wands or dipsticks have a plastic coating embedded with antibodies to hCG.
4. The test wands also have a second antibody to hCG linked with some colour tag (e.g., coloured latex beads, enzyme that produces a colour reaction).
5. If sufficient levels of hCG are present in the urine (more than 25 mIU), then the hCG will bind with the second antibody and cause a colour reaction to occur (i.e., a positive test result).

If a positive test occurs, you generally call your doctor and a second test is performed at the office to confirm the pregnancy. The doctor may also order a blood test to determine the precise quantity of hCG present, which can be used to assess the baby's health.

The placenta

The placenta grows tightly fused to the wall of the uterus. Its blood vessels, supplied by the foetal heart, are literally bathed in the mother's blood. Although there is normally no mixing of the two blood supplies, the placenta does facilitate the transfer of a variety of materials between the foetus and the mother.

TABLE SHOWING EXCHANGE OF MATERIALS ACROSS THE PLACENTA

MOTHER TO FOETUS	FOETUS TO MOTHER
• Oxygen • Glucose • Amino acids • Lipids, fatty acids and glycerol • Vitamins • Ions; Na, Cl, Ca, Fe • Alcohol, nicotine + other drugs • Viruses • Antibodies	• Carbon dioxide • Urea • Other waste products

The placenta is an organ of exchange and therefore requires a large surface area – to achieve this it has chorionic villi (the cells of which have microvilli and many mitochondria)

The metabolic activity of the placenta is almost as great as that of the foetus itself.

The placenta is also an endocrine organ and it secretes hCG, progesterone and oestrogen

During pregnancy prenatal diagnosis of genetic disorders can be made using the procedures of amniocentesis and chorionic villus sampling (CVS) – see later screening section for details.

PHYSIOLOGICAL CHANGES TO THE MOTHER DURING PREGNANCY

Physiological and anatomical alterations develop in many organ systems during the course of pregnancy and delivery. Early changes are due, in part, to the metabolic demands brought on by the foetus, placenta and uterus and, in part, to the increasing levels of pregnancy hormones, particularly those of progesterone and oestrogen. Later anatomical changes, starting in mid-pregnancy, are caused by mechanical pressure from the expanding uterus.

Cardiovascular System

The pregnancy-induced changes in the cardiovascular system develop primarily to meet the increased metabolic demands of the mother and foetus.

Blood Volume

Increases progressively from 6-8 weeks and reaches a maximum at approx. 32-34 weeks with little change afterwards. Most of the added volume of blood is accounted for by an increased capacity of the uterine, breast, renal, muscle and adipose tissues. The increase in plasma volume (40-50%) is relatively greater than that of red cell mass (20-30%) resulting in a decrease in haemoglobin concentration. Intake of supplemental iron and folic acid is necessary to restore haemoglobin levels to normal (12 g/dl).
The increased blood volume serves two purposes. It helps maternal and foetal exchanges and it reduces the impact of maternal blood loss at delivery. Typical losses of 300-500 ml for vaginal births are thus compensated with the so-called "autotransfusion" of blood from the contracting uterus.

Cardiac Output

Increases to a similar degree as the blood volume. During the first trimester cardiac output is 30-40% higher than the non-pregnant output. Steady rises occur from about 7 litres/minute at 8-11 weeks to 9 litres/minute at 36-39 weeks; they are due, to an increase in stroke volume (35%) and also to a more rapid heart rate (15%).

Cardiac Size

There are size changes. The heart is enlarged by both chamber dilation and hypertrophy.

Blood Pressure

Systemic arterial pressure is never increased during normal gestation. In fact, by midpregnancy, a slight decrease in diastolic pressure can be recognized. Pulmonary arterial pressure also maintains a constant level.

Renal System

Kidney Function

Blood flow through the kidney can increase from 25-50% and blood urea also increases as foetal urea is added via the placenta. The kidney accommodates for these changes by increasing in size (length can increase by 1cm). Volume of urine production is not greatly increased (though frequency of urination usually is) therefore the concentration of urine is normally increased.

Body Mass

The average weight gain during pregnancy is about 12kg (or 25-35 pounds). The table below shows some typical mass changes that may occur if I became pregnant (ok I know it's impossible but it gives an idea of proportion)

SOURCE	TYPICAL INCREASE IN MASS (LB.)
Uterus	2.4
Breasts	1.0
Blood	3.1
Water	4.2
Fat	8.3
Amniotic Fluid	2.0
Placenta	1.6
Foetus	7.5

Birth and Lactation

Exactly what brings about the onset of labour is still not completely understood. Probably hormonal control is responsible. The first result of labour is the opening of the cervix. With continued powerful contractions, the amnion ruptures and the amniotic fluid (the "waters") flows out through the vagina. The baby follows, and its umbilical cord can be cut. Shortly after the baby, the placenta and the remains of the umbilical cord (the "afterbirth") are expelled.

At the time of birth, and for a few days after, the mother's breasts contain a fluid called colostrum. It is rich in calories and protein, including antibodies that provide passive immunity for the newborn infant.

Three or four days after delivery, the breasts begin to secrete milk.

- Its synthesis is stimulated by the pituitary hormone prolactin.
- Its release is stimulated by a rise in the level of oxytocin when the baby begins sucking the nipples.
- Milk contains an inhibitory peptide. If the breasts are not fully emptied, the peptide accumulates and inhibits milk production. This is an example of negative feedback.

Contraception

As you can see from the process of sexual reproduction, there are several ways to prevent the sperm and egg from coming together. These methods of contraception fall into the following categories:

- Not engaging in sexual activity - abstinence
- Preventing a follicle from developing - birth control pills
- Placing a barrier between sperm and egg - condoms (male/female), cervical caps, diaphragms
- Killing the sperm - spermicides
- Surgery - blocking the sperm or egg with surgical procedures like tubal ligations (in women) or vasectomies (in men)
- Timing - avoiding intercourse during the period of maximum fertility

Chronic bronchitis

Chronic bronchitis is a clinical diagnosis where there is cough producing sputum on most days for 3 months of the year for 2+ years which is not due to another respiratory illness. The disorder is characterised by excess mucus secretion.

Emphysema

Emphysema is defined by its pathology and is characterised by destruction of respiratory tissue and permanent enlargement of the unit of the lung distal to the terminal bronchiole. This can be detected by endoscopic examination.

In the past much importance has been placed on the distinction between chronic bronchitis and emphysema. In the majority of patients both conditions co-exist, usually in heavy cigarette smokers.

Aetiology and prevalence

Chronic bronchitis and emphysema are responsible for personal disability and misery of 10,000's of patients. Respiratory disorders are an important cause of death and of these chronic bronchitis and emphysema constitute a large proportion of these.

Atmospheric pollution and occupational dust exposure are minor aetiological factors in chronic bronchitis and the dominant causal agent is cigarette smoke. Smoking also causes emphysema.

Mechanism of airflow obstruction

In chronic bronchitis and emphysema the fundamental cause of reduced ventilatory capacity and breathlessness is the limitation of expiratory airflow. In emphysema a more important mechanism is the narrowing and collapse of airways during expiration as a consequence of loss of the lung elastic recoil which normally keeps airways open. In emphysema there is also collapse of alveolar walls causing reduced surface area for gas exchange.

Physical signs

In predominantly emphysematous patients, inspiratory airways resistance is not increased and inspiration is therefore quiet, whereas patients with predominantly chronic bronchitis have noisy breathing. To control airways collapse on expiration, patients with emphysema apply a positive pressure to the bronchial tree by the technique of purse-lipped breathing.

Human growth and development

Cessation of cigarette smoking

Human Growth & Development

Tobacco smoke damages the bronchial tree and produces airflow limitation by a number of different actions. Smokers are predisposed to bronchial infection and consequent inflammation. It is therefore not surprising that chronic bronchitis and emphysema are found in 15% of middle-aged males who smoke moderately or heavily but are rare in non-smokers, and that deaths from bronchitis increase with the amount smoked.

- Growth occurs during gestation, childhood and adolescence.
- Growth rate = change in size per unit time.
- Growth varies with different tissues and organs.
- Allometric Growth - different parts of body grow at different rates, causing body proportions to change between infancy and adulthood.

If patients with chronic bronchitis and emphysema stop smoking the rate of decline in pulmonary function is reduced to that of non-smokers. Indeed, if patients stop smoking early in their disease there is improvement in pulmonary function. However, before the disease, stopping smoking will reduce cough.

CRITERIA: Having a productive cough for at least 3 months during 2 successive years

- SYMPTOMS: Productive cough, breathlessness
- Smoking and air pollution paralyse the cilia in the bronchial tubes so mucus builds up in clumps that are coughed up (that's the productive cough). The lining of the bronchial tubes becomes irritated and inflamed.

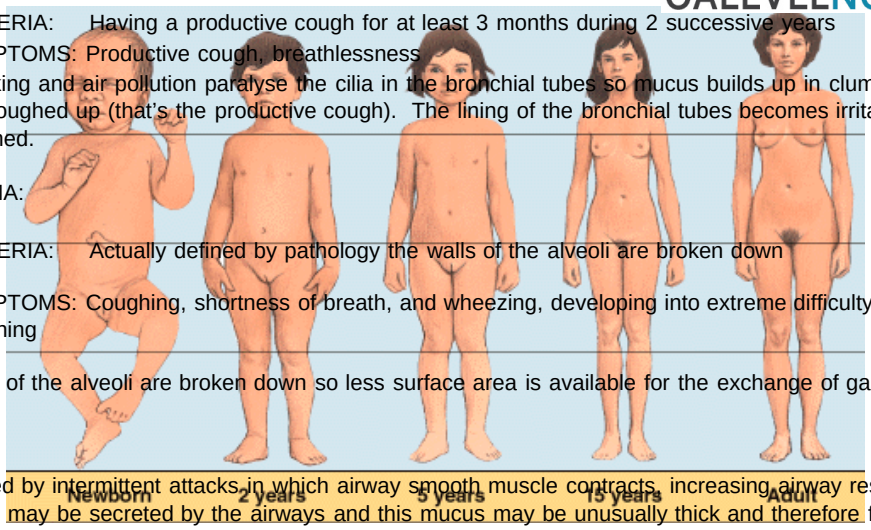
EMPHYSEMA:

- CRITERIA: Actually defined by pathology the walls of the alveoli are broken down
- SYMPTOMS: Coughing, shortness of breath, and wheezing, developing into extreme difficulty in breathing
- Walls of the alveoli are broken down so less surface area is available for the exchange of gases.

ASTHMA:

Characterised by intermittent attacks in which airway smooth muscle contracts, increasing airway resistance. More mucus may be secreted by the airways and this mucus may be unusually thick and therefore further increase airway resistance.

Stages of Human Growth



Gestation — Relatively short for humans because of our large brains and cranium, mean longer gestation would cause birth problems.

Childhood — Pre-pubertal period gives us time to grow, develop and learn (acquire skills and knowledge). This phase is relatively longer in humans than in other animals (we have extended period of dependency). During childhood boys and girls grow at the same rate this growth is controlled by hormones. The pituitary gland secretes a hormone called pituitary growth hormone (PGH). Also it secretes a hormone called thyroid stimulating hormone (TSH) which stimulates the thyroid gland to secrete thyroxin. Thyroxin and PGH both stimulate growth.

Adolescence — During puberty the pituitary gland produces LH and FSH which cause the ovaries to produce oestrogen and the testes to produce testosterone. These hormones are the sex hormones and cause the development of secondary sexual characteristics. The growth spurt that occurs during puberty is due to increased levels of PGH.

Ageing

- Senescence = The deterioration of bodily functions and the appearance of features associated with old age.

A CASE STUDY OF THE # OF DEATHS OF CIGARETTE SMOKERS ("OBSERVED [OBS.] DEATHS") COMPARED WITH THE NUMBER TO BE EXPECTED AMONG NONSMOKERS OF THE SAME AGES ("EXPECTED [EXP.] DEATHS")

CAUSE OF DEATH	OBS. DEATHS	EXP. DEATHS	EXCESS DEATHS	% CHANGE
Total deaths (all causes)	7316	4651	2665	57
Heart disease	3864	2398	1466	61
Cerebrovascular lesions	556	428	138	30
Other circulatory diseases	173	97	76	78
Lung cancer	397	37	360	973
Cancer of mouth/ larynx/oesophagus	91	18	73	406
Other cancers	972	686	286	42
G.I. tract Ulcers & liver Cirrhosis	183	68	115	169
Pulmonary disease (except cancer)	231	81	150	185
All other diseases	486	453	33	7
Accident, violence, suicide	363	385	-22	-6

(Data from E. C. Hammond and D. Dorn, 1966.)

CANCER:

A cancer is an uncontrolled proliferation of cells.

- In some the rate is fast, in others, slow, but in all cancers the cells never stop dividing.
- This distinguishes cancers - malign tumours - from benign growths like moles where their cells eventually stop dividing.
- Cancers are clones. No matter how many trillions of cells are present in the cancer, they are all descended from a single ancestral cell.
- Cancers begin as a primary tumour. At some point, however, cells break away from the primary tumour and - travelling in blood and lymph - establish metastases in other locations of the body. Metastasis is what usually kills the patient.
- Cancer cells contain mutated genes known as oncogenes. The mutations are found in genes that are involved in mitosis; that is, in genes that control the cell cycle.

Probable Sequence:

- A single cell in a tissue suffers a mutation in a gene involved in mitosis.

Physiological effects of ageing

- Effects on skin collagenase produced, collagen structure altered loss of elasticity due to x-links in elastin + collagen
- accumulation of genetic errors
- Degeneration of tissues – due to 'wear & tear' organ function metabolic rate lung capacities
- Immune system, efficiency decreases + incidence of autoimmune diseases

Human populations and health

- This results in giving that cell a slight growth advantage over other cells in the tissue.
- A cell that develops into a clone, some if its descendants suffer a second mutation
- This further deregulates the cell cycle of that cell and its descendants.
- As the rate of mitosis in that clone increases, the chances of further DNA damage increases.
- Eventually the growth of that clone becomes completely unregulated.
- The result: full blown cancer.

Colon Cancer: An example.

- Begins with the development of polyps in the epithelium of the colon. Polyps are benign growths
- As time passes, the polyps may get bigger.
- At some point, nests of malignant cells may appear within the polyps
- If the polyp is not removed, some of these malignant cells will escape from the primary tumour and metastasise throughout the body.
- Examination of the cells at the earliest, polyp, stage, reveals that they contain oncogenes.

Cancers become more common as one gets older.

This explains why cancer has become such a common cause of death during the twentieth century. It probably has very little to do with exposure to the chemicals of modern living and everything to do with the increased longevity that has been such a remarkable feature of this century.

A population where most members only survive to reproductive age is a population increasingly condemned to death from such "organic" diseases as cancer.

Causes of Cancer

- anything that damages DNA, that is anything that is mutagenic
- radiation that can penetrate to the nucleus and interact with DNA
- chemicals that can penetrate to the nucleus and damage DNA. Chemicals that cause cancer are called carcinogens.
- anything that stimulates the rate of mitosis. This is because a cell is most susceptible to mutations when it is replicating its DNA during the S phase of interphase.
- certain hormones (e.g. hormones stimulating mitosis in the breast & prostate glands)
- certain viruses

Disease

Radiation and cancer

High doses of radiation cause cancer. Various studies, including excellent ones on the survivors of Hiroshima and Nagasaki, show that a pop^{n}. exposed to a dose of 12,500 mrem will have a measurable increase (about 1%) in the incidence of cancer. Note that the measurements are made on a pop^{n}. not on individuals. We can never say that a particular individual exposed to a particular dose of radiation will develop cancer. The induction of cancer is a chance event unlike radiation sickness which is completely predictable. The element of chance arises because cancer is an event that occurs in a single cell unlucky enough to suffer damage to specific genes mutating them to oncogenes. However, the energy needed to cause mutations is very low. So if you expose a sufficiently large number of cells to even tiny doses of radiation, some cell is going to be unlucky.

Screening and diagnostic tests

- Biochemical tests

- Immunological tests
- Screening
- Biopsies
- Cytological examinations
- Culturing microorganisms
- Genetic analysis
- X-rays
- Ultrasound
- Endoscopy
- Blood pressure measurement
- Sight and hearing tests

Screening

Looking for signs of the disease before acute symptoms are evident. Based on the premise that early detection can lead to a complete cure.

Genetic analysis

Amniocentesis = the method which removes a small sample of amniotic fluid from the uterus. Done with a needle. The fluid contains some foetal cells on which genetic analysis can be carried out

Chorionic villus sampling = the method of obtaining a tissue sample from the area of the placenta of the early embryo. Done in conjunction with an ultrasound probe.

Genetic analysis could be by karyotyping or use of a genetic probe

X-rays

Mainly used on bones. X-rays are a form of ionising radiation so care is required as it can damage DNA. Can be used to detect some abnormalities to soft tissue. Barium is opaque to X-rays and can be ingested as a paste/slurry “barium meal” this accumulates in stomach ulcers.

Ultrasound

Use of high frequency sound waves (approx. 3-10 million Hz, audible range = 16Hz – 20,000Hz) into an area being investigated. Reflected sound is converted into visual radiation (does not damage DNA)

Endoscopy

The insertion of a camera into the body

Blood pressure measurement

Measured by a sphygmomanometer

Sight and hearing tests

Tests for visual acuity. Also tests for colour-blindness (Ishihara test)

CT scanning

Advanced X-ray technique, low dosage. Examination of area in slices and computer analysis constructs internal picture.

MRI scanning

Uses a strong magnetic field which causes all the nuclei of the atoms that compose the body to line up and spin in the same direction. When a radio frequency wave is beamed into the magnetic field the nuclei move

out of alignment. When the radio wave is stopped they move back into alignment and release energy ~ this can be measured by a receiver

Hypertension

- Hypertension is where systolic and/or diastolic blood pressure is chronically elevated at rest. Their values must exceed 140 mm Hg and 90 mm Hg over several examinations.
- Clinical problems that are linked to high blood pressure include strokes and heart attacks.
- Hypertension is a multifactoral disease

NACL AND HYPERTENSION

- Physiological requirement for Na » 20mmol/day » 1g NaCl intake
- Average British NaCl intake » 9g/day

AVERAGE % OF NA FROM DIFFERENT SOURCES	
Discretionary	
Added at table	9.0
Used in cooking	6.0
Food	
Naturally occurring	18.5
Added salt in processing	58.7
Non-salt additives	7.2
Salt in water supply (average)	0.6
	100.0

ALCOHOL AND HYPERTENSION

Alcohol intake is associated with raised blood pressure. Heavy drinkers have higher blood pressures than light drinkers and abstainers. The effect begins at about 3 units of alcohol per day

Minerals

Mineral	Source	Function	Deficiency disease
Calcium	Dairy products, green vegetables	Calcium is a component of teeth and bone. Calcium ions are essential for nerve and muscle function as well as being involved in blood clotting	Rickets
Iron	Liver, meat (especially red meat), egg yolks, nuts and legumes (i.e.. Beans and pulses)	Iron is a component of haemoglobin and myoglobin. It is also part of the electron carriers involved in respiration	Anaemia (low haemocrit, which is the amount of haemoglobin in blood) – It is worth noting that there are many different forms of anaemia
Iodine	Seafood and vegetables grown in coastal areas (iodized salt in many countries)	Iodine is a component of the hormone thyroxin	Goitre which retards growth

Vitamins

Vitamins are: Organic substances found in some foods with a specific biochemical function in the body that

are required in very small amounts

Vitamin	Source	Physiological Function	Deficiency Disease
A (Retinol)	Fish liver oil, dairy products. [Carrots and some other vegetables provide beta-carotene, which the body can convert into vitamin A.]	precursor to retinal, the prosthetic group of all of the light-absorbing pigments in the eye.	night-blindness, xerophthalmia (dry cornea). [Excess: stored in the liver, but can be toxic in large doses, especially in children. High doses taken early in pregnancy have been linked to a greater risk of birth defects.]
C (Ascorbic acid)	All fresh fruit and vegetables contain some vitamin C. Citrus fruits, green peppers, tomatoes; destroyed by cooking.	coenzyme in the synthesis of collagen.	Scurvy. [Excess: Many people take huge amounts of vitamin C, hoping to ward off colds, cancer, etc. They seem to suffer no harm except, perhaps, to their wallets.]
D (Calciferol)	synthesized when ultraviolet light strikes the skin (forms vitamin D3). Present in fish liver oils, butter, and steroid-containing foods irradiated with ultraviolet light (vitamin D2).	absorption of calcium from the intestine and bone formation.	Deficiency: rickets in children; osteomalacia (softening of the bones) in adults. [Excess: However, this fat-soluble vitamin is dangerous in very high doses causing excessive calcium deposits and mental retardation.]

GUIDELINES FOR NUTRITION

NUTRIENT	GUIDELINES	UNDERLYING PRINCIPLES
Fat	Reduce total fat consumption and shift the balance in fat consumption from saturated to unsaturated fatty acids *revise from mod 1 (monounsaturated fats are best)	Our diets contain more than enough fat to supply the essential fatty acids/uses e.g. fuel for muscle respiration once glucose and stores of glycogen are used up. Excess fat is stored as fat reserves. A high intake of saturated fatty acids is associated with high levels of blood cholesterol and increases the risk of atherosclerosis. Plant fats - usually unsaturated. Animal fats usually saturated
Salt	reduce salt intake (more salt necessary eg if doing strenuous exercise in hot climate)	Modern diets tend to supply more than enough salt - eg salt in prepared foods and other packaged foods. NaCl is important in maintaining tendency of blood to take up water. Na+ & Cl- have major roles in nerve impulse transmission. Excess dietary salt can cause fluid retention (oedema) & may contribute to high blood pressure (hypertension) Salt loss from excessive sweating & inadequate intake can cause heat exhaustion
Sugar	Reduce sugar intake	Allows bacteria to grow on teeth, producing acids which dissolve the outer surface (enamel) causing tooth decay. Glucose can be obtained by breaking down carbohydrates. Glucose (the respiratory substrate) is stored as glycogen in the liver. Surplus glucose is converted to fat for long term storage in fat cells eg under the skin
Additives (none-nutrient)	A large proportion are safe and useful but some are unnecessary with potentially adverse side effects for sensitive people. e.g. one in a million are sensitive to E102 - (tartrazine)	
Fibre	Eat a high fibre diet	SOLUBLE FIBRE - binds CHOLESTEROL into a complex that cannot be absorbed from the intestine so it is passed out in stools. Important in small intestine - slows digestion and absorption; products are released over a longer time (important to diabetics). INSOLUBLE FIBRE - important in colon. Absorbs water and swells; stretches walls of intestine and stimulates peristalsis. Speeds up passage of food through colon and so reduces the time for possible carcinogens to be in contact with intestinal wall. Reduces the risk of constipation, piles and colon cancer.

The effects of exercise on the incidence of certain diseases

Heart disease

- Regular exercise increases heart efficiency, and makes heart contraction more efficient (i.e. more powerful).
- It increases blood HDL (high density lipoprotein) levels. HDLs carry cholesterol away from the tissues back to the liver, where they are secreted into bile. So HDLs are beneficial and reduce the risk of heart disease. LDLs carry most of the cholesterol in the blood. They deliver cholesterol to the cells. LDLs increase the incidence of atheroma. The ratio of plasma LDL cholesterol : plasma HDL cholesterol is important: the lower the ratio, the lower the risk of atheroma.
- Artery wall elasticity is maintained improved/improved by regular exercise.
- Resting heart rate is lowered; this decreases the 'loading' (strain) on the heart.
- Resting blood pressure is lowered, meaning that less effort is needed for the heart to pump.
- Exercise may lead to weight loss, which would decrease the loading on the heart.

Circulatory problems eg atheroma

- Exercise reduces stress.
- Regular exercise reduces the amount of adrenaline release (adrenaline promotes the breakdown of glycogen for respiration).
- Exercise increases the metabolism of fats.
- Exercise increases HDL and lowers LDL.
- The points above contribute to reducing the chance of atheroma being deposited on the inner lining of the arteries.

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