AQA AS Biology Unit 1
Contents

Biology Unit 1 Specification

Biochemistry
Biological Molecules
Biological molecules such as carbohydrates and proteins are often polymers and are based on a small number of chemical elements.

- Proteins have a variety of functions within all living organisms. The general structure of an amino acid. Condensation and the formation of peptide bonds linking together amino acids to form polypeptides. The relationship between primary, secondary, tertiary and quaternary structure, and protein function.
- Monosaccharides are the basic molecular units (monomers) of which carbohydrates are composed. The structure of α-glucose and the linking of α-glucose by glycosidic bonds formed by condensation to form maltose and starch. Sucrose is a disaccharide formed by condensation of glucose and fructose. Lactose is a disaccharide formed by condensation of glucose and galactose.

Glycerol and fatty acids combine by condensation to produce triglycerides. The R-group of a fatty acid may be saturated or unsaturated. In phospholipids, one of the fatty acids of a triglyceride is substituted by a phosphate group.

Biochemical Tests
Iodine/potassium iodide solution for starch. Benedict's reagent for reducing sugars and non-reducing sugars. The biuret test for proteins. The emulsion test for lipids.

Enzymes
Enzymes as catalysts lowering activation energy through the formation of enzyme-substrate complexes. The lock and key and induced fit models of enzyme action. Use the lock and key model to explain the properties of enzymes. Recognise its limitations and be able to explain why the induced fit model provides a better explanation of specific enzyme properties. The properties of enzymes relating to their tertiary structure.

Description and explanation of the effects of temperature, competitive and non-competitive inhibitors, pH and substrate concentration. Investigate the effect of a specific variable on the rate of reaction of an enzyme-controlled reaction.

Cell Biology
Cells
The appearance, ultrastructure and function of plasma membrane; microvilli; nucleus; mitochondria; lysosomes; ribosomes; endoplasmic reticulum and Golgi apparatus. Apply their knowledge of these features in explaining adaptations of other eukaryotic cells.

The structure of prokaryotic cells to include cell wall, plasma membrane, capsule, circular DNA, flagella and plasmid.

Microscopes and Cell Fractionation
The difference between magnification and resolution. The principles and limitations of transmission and scanning electron microscopes. Principles of cell fractionation and ultracentrifugation as used to separate cell components.

Plasma Membranes
The arrangement of phospholipids, proteins and carbohydrates in the fluid-mosaic model of membrane structure. Use the fluid mosaic model to explain appropriate properties of plasma membranes.

- The role of carrier proteins and protein channels in facilitated diffusion.
- Osmosis is a special case of diffusion in which water moves from a solution of higher water potential to a solution of lower water potential through a partially permeable membrane. Investigate the effect of solute concentration on the rate of uptake of water by plant issue.
- The role of carrier proteins and the transfer of energy in the active transport of substances against a concentration gradient.

Physiology
Exchange
Diffusion is the passive movement of substances down a concentration gradient. Surface area, difference in concentration and the thickness of the exchange surface affect the rate of diffusion.

Gas Exchange System
The gross structure of the human gas exchange system limited to the alveoli, bronchioles, bronchi, trachea and lungs. The essential features of the alveolar epithelium as a surface over which gas exchange takes place. The exchange of gases in the lungs. The mechanism of breathing. Pulmonary ventilation as the product of tidal volume and ventilation rate.

Lung Diseases
The course of infection, symptoms and transmission of pulmonary tuberculosis. The effects of fibrosis, asthma and emphysema on lung function. Explain the symptoms of diseases and conditions affecting the lungs in terms of gas exchange and respiration. Interpret data relating to the effects of pollution and smoking on the incidence of lung disease. Analyse and interpret data associated with specific risk factors and the incidence of lung disease.

Heart

Heart structure and function. The gross structure of the human heart and its associated blood vessels in relation to function. Myogenic stimulation of the heart and transmission of a subsequent wave of electrical activity. Roles of the sinoatrial node (SAN), atrioventricular node (AVN) and bundle of His. Pressure and volume changes and associated valve movements during the cardiac cycle. Candidates should be able to analyse and interpret data relating to pressure and volume changes during the cardiac cycle. Cardiac output as the product of heart rate and stroke volume. Investigate the effect of a specific variable on human heart rate or pulse rate.

Coronary Heart Disease

Atheroma as the presence of fatty material within the walls of arteries. The link between atheroma and the increased risk of aneurysm and thrombosis. Myocardial infarction and its cause in terms of an interruption to the blood flow to heart muscle. Risk factors associated with coronary heart disease: diet, blood cholesterol, cigarette smoking and high blood pressure. Describe and explain data relating to the relationship between specific risk factors and the incidence of coronary heart disease.

Digestive System

The gross structure of the human digestive system limited to oesophagus, stomach, small and large intestines and rectum. The glands associated with this system limited to the salivary glands and the pancreas. The structure of an epithelial cell from the small intestine as seen with an optical microscope.

Digestion is the process in which large molecules are hydrolysed by enzymes to produce smaller molecules that can be absorbed and assimilated. The role of salivary and pancreatic amylases in the digestion of starch and of maltase located in the intestinal epithelium. Digestion of disaccharides by sucrase and lactase. Absorption of the products of carbohydrate digestion. The roles of diffusion, active transport and co-transport involving sodium ions. The role of microvilli in increasing surface area. Lactose intolerance.

Cholera

The cholera bacterium as an example of a prokaryotic organism. Cholera bacteria produce toxins that increase secretion of chloride ions into the lumen of the intestine. This results in severe diarrhoea. The use of oral rehydration solutions (ORS) in the treatment of diarrhoeal diseases. Discuss the applications and implications of science in developing improved oral rehydration solutions; and ethical issues associated with trialling improved oral rehydration solutions on humans.

Disease

Lifestyle and Disease

Disease may be caused by infectious pathogens or may reflect the effects of lifestyle.

• Pathogens include bacteria, viruses and fungi. Disease can result from pathogenic microorganisms penetrating any of an organism's interfaces with the environment. These interfaces include the digestive and gas-exchange systems. Pathogens cause disease by damaging the cells of the host and by producing toxins.

• Lifestyle can affect human health. Specific risk factors are associated with cancer and coronary heart disease. Changes in lifestyle may also be associated with a reduced risk of contracting these conditions. Analyse and interpret data associated with specific risk factors and the incidence of disease. Recognise correlations and causal relationships.

Defence against Disease

Mammalian blood possesses a number of defensive functions. Phagocytosis and the role of lysosomes and lysosomal enzymes in the subsequent destruction of ingested pathogens.

Definition of antigen and antibody. Antibody structure and the formation of an antigen-antibody complex. The essential difference between humoral and cellular responses as shown by B cells and T cells. The role of plasma cells and memory cells in producing a secondary response. The effects of antigenic variabilty in the influenza virus and other pathogens on immunity.

Vaccines and monoclonal antibodies

The use of vaccines to provide protection for individuals and populations against disease. The use of monoclonal antibodies in enabling the targeting of specific substances and cells.

Evaluate methodology, evidence and data relating to the use of vaccines and monoclonal antibodies. Discuss ethical issues associated with the use of vaccines and monoclonal antibodies. Explain the role of the scientific community in validating new knowledge about vaccines and monoclonal antibodies thus ensuring integrity. Discuss the ways in which society uses scientific knowledge relating to vaccines and monoclonal antibodies to inform decision-making.

Biological Molecules

Living things are made up of thousands and thousands of different chemicals. These chemicals are called organic because they contain the element carbon. In science organic compounds contain carbon–carbon bonds, while inorganic compounds don't. There are four important types of organic molecules found in living organisms: carbohydrates, lipids, proteins, and nucleic acids (DNA). These molecules are mostly polymers, very large molecules made up from very many small molecules, called monomers. 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	Elements	Monomers	Polymers	% dry mass of a cell
Carbohydrates	CHO	monosaccharides	polysaccharides	15
Lipids	CHOP	fatty acids + glycerol*	triglycerides*	10
Proteins	CHONS	amino acids	polypeptides	50
Nucleic acids	CHONP	nucleotides	polynucleotides	18

* Triglycerides are not polymers, since they are formed from just four molecules, not many (see p8).

We'll study carbohydrates, lipids and proteins in detail now, and we'll look at nucleic acids (DNA) in unit 2.

Chemical Bonds

In biochemistry there are two important types of chemical bond: the covalent bond and the hydrogen bond.

Covalent bonds are strong. They are the main bonds holding the atoms together in the organic molecules in living organisms. Because they are strong, covalent bonds don't break or form spontaneously at the temperatures found in living cells. So in biology covalent bonds are always made or broken by the action of enzymes. Covalent bonds are represented by solid lines in chemical structures.
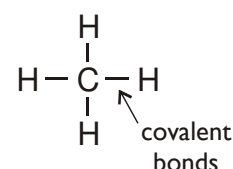
Hydrogen bonds are much weaker. They are formed between an atom (usually hydrogen) with a slight positive charge (denoted $\delta+$) and an atom (usually oxygen or nitrogen) with a slight negative charge (denoted $\delta-$). Because hydrogen bonds are weak they can break and form spontaneously at the temperatures found in living cells without needing enzymes. Hydrogen bonds are represented by dotted lines in chemical structures.
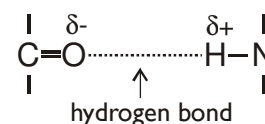
Water

Life on Earth evolved in the water, and all life still depends on water. At least 80% of the total mass of living organisms is water. Water molecules are charged, with the oxygen atom being slightly negative (δ-) and the hydrogen atoms being slightly positive (δ+). These opposite charges attract each other, forming hydrogen bonds that bind water molecules loosely together.
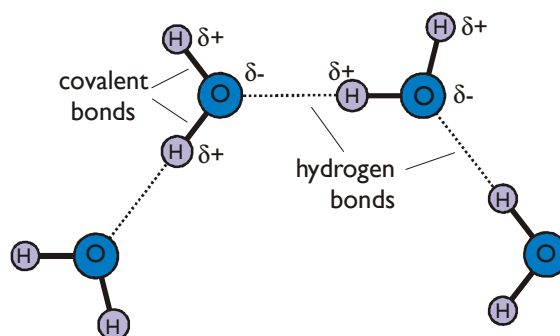
Because it is charged, water is a very good <u>solvent</u>, and almost all the chemical reactions of life take place in <u>aqueous solution</u>.
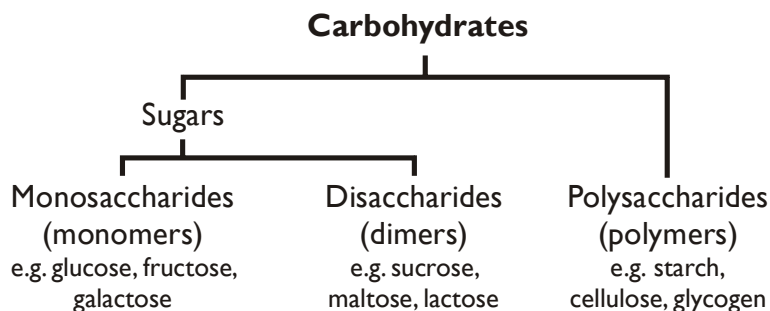
• Charged or polar molecules such as salts, sugars, amino acids dissolve readily in water and so are called <u>hydrophilic</u> ("water loving").

• Uncharged or non-polar molecules such as lipids do not dissolve so well in water and are called <u>hydrophobic</u> ("water hating").

Many important biological molecules ionise when they dissolve (e.g. acetic acid $\rightleftharpoons$ acetate$^-$ + H$^+$), so the names of the acid and ionised 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 ionised form is the one found in living cells.

Water molecules "stick together" due to their hydrogen bonds, so water has high <u>cohesion</u>. 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.

Carbohydrates

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

Carbohydrates

Sugars

Monosaccharides (monomers)	Disaccharides (dimers)	Polysaccharides (polymers)
e.g. glucose, fructose, galactose	e.g. sucrose, maltose, lactose	e.g. starch, cellulose, glycogen

Monosaccharides

These all have the formula $(CH_2O)_n$, where n can be 3-7. The most common and important monosaccharide is <u>glucose</u>, which is a six-carbon or <u>hexose</u> sugar, so has the formula $C_6H_{12}O_6$. Its structure is:

or more simply
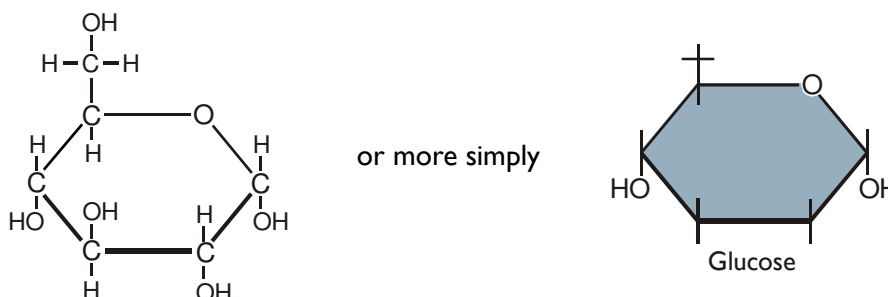
Glucose

Glucose forms a six-sided ring, although in three-dimensions it forms a structure that looks a bit like a chair. In animals glucose is the main transport sugar in the blood, and its concentration in the blood is carefully controlled. There are many <u>isomers</u> of glucose, with the same chemical formula ($C_6H_{12}O_6$), but different structural formulae. These isomers include <u>galactose</u> and <u>fructose</u>:

Galactose

Fructose

Common five-carbon, or pentose sugars (where n = 5, $C_5H_{10}O_5$) include <u>ribose</u> and <u>deoxyribose</u> (found in nucleic acids and ATP, see unit 2) and <u>ribulose</u> (which occurs in photosynthesis). Three-carbon, or <u>triose</u> sugars (where n = 3, $C_3H_6O_3$) are also found in respiration and photosynthesis (see unit 4).

Disaccharides

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


glycosidic bond
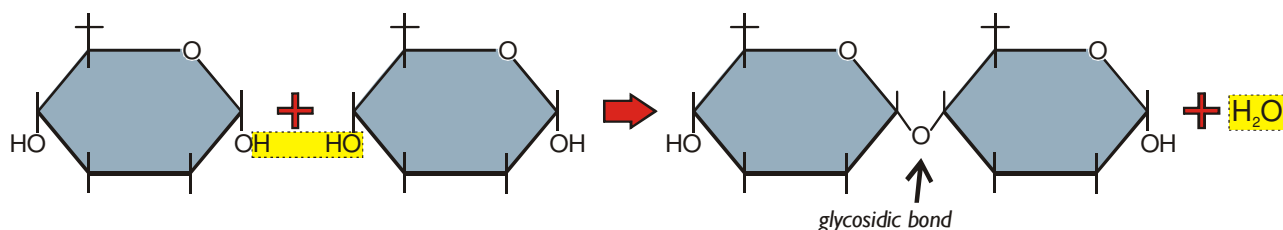
This shows two glucose molecules joining together to form the disaccharide maltose. This kind of reaction, where two molecules combine into one bigger molecule, is called a condensation reaction. The reverse process, where a large molecule is broken into smaller ones by reacting with water, is called a hydrolysis reaction.

In general:
- polymerisation reactions are condensations
- breakdown reactions are hydrolyses

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.
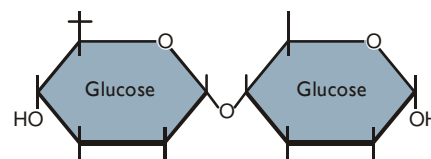
Sucrose (or cane sugar) is glucose–fructose. It is common in plants because it is less reactive than glucose, and it is their main transport sugar. It is the common table sugar that you put in your tea.
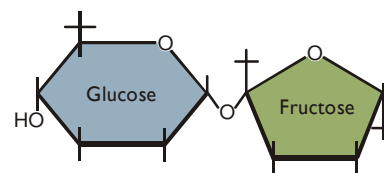
Lactose (or milk sugar) is galactose–glucose. It is found only in mammalian milk, and is the main source of energy for infant mammals.
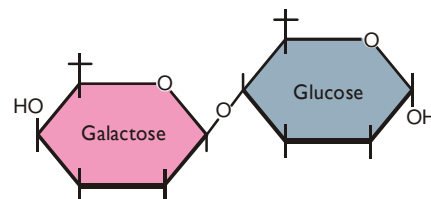
Polysaccharides

Polysaccharides are chains of many glucose monomers (often 1000s) joined together by glycosidic bonds. Starch, glycogen and cellulose are polysaccharides. They will be studied in unit 2.

Lipids

Lipids are a mixed group of hydrophobic compounds composed of the elements carbon, hydrogen, oxygen and sometime phosphorus (CHOP). The most common lipids are triglycerides and phospholipids.
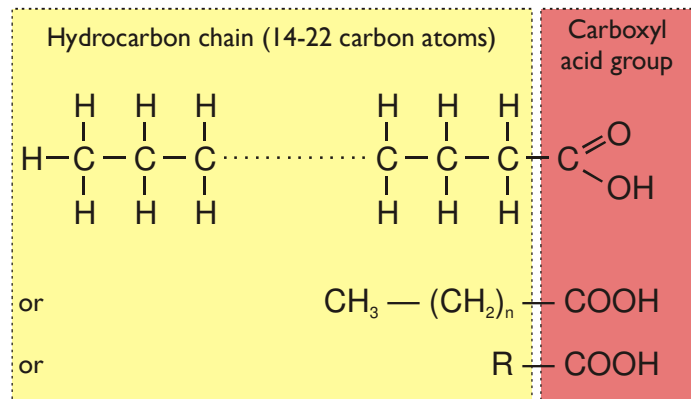
Triglycerides

Triglycerides, or triacylglycerols, are made of glycerol and fatty acids.

Glycerol is a small, 3-carbon molecule with three alcohol (OH) groups.
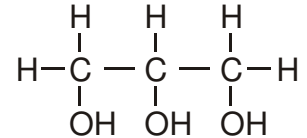
Fatty acids are long molecules made of a non-polar hydrocarbon chain with a polar carboxyl acid group at one end. The hydrocarbon chain can be from 14 to 22 CH_2 units long. Because the length of the hydrocarbon chain can vary it is sometimes called an R group, so the formula of a fatty acid can be written as R-COOH.

One molecule of glycerol joins together with three fatty acid molecules by ester bonds to form a triglyceride molecule, in another condensation polymerisation reaction:
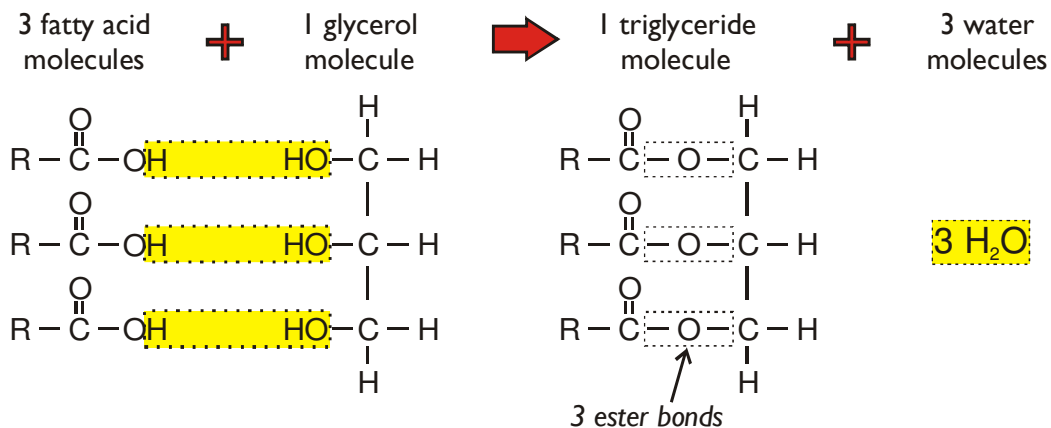
Triglycerides are commonly known as fats or oils, and are insoluble in water. They are used for storage, insulation and protection in fatty tissue (or adipose tissue) found under the skin (sub-cutaneous) or surrounding organs. When oxidised triglycerides yield more energy per unit mass than other compounds so are good for energy storage. However, triglycerides can't be mobilised quickly since they are so insoluble, so are no good for quick energy requirements. Tissues that need energy quickly (like muscles) instead store carbohydrates like glycogen.

- If the fatty acid chains in a triglyceride have no C=C double bonds, then they are called <u>saturated fatty acids</u> (i.e. saturated with hydrogen). Triglycerides with 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.

- If the fatty acid chains in a triglyceride do have C=C double bonds they are called <u>unsaturated fatty acids</u> (i.e. unsaturated with hydrogen). Fatty acids with more than one double bond are called poly-unsaturated fatty acids (PUFAs). Triglycerides with 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. An "omega number" is sometimes used to denote the position of a double bond, e.g. omega-3 fatty acids.
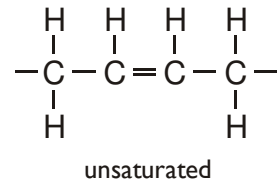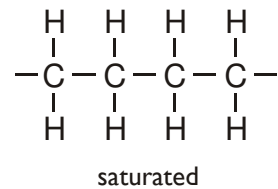
Phospholipids

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).
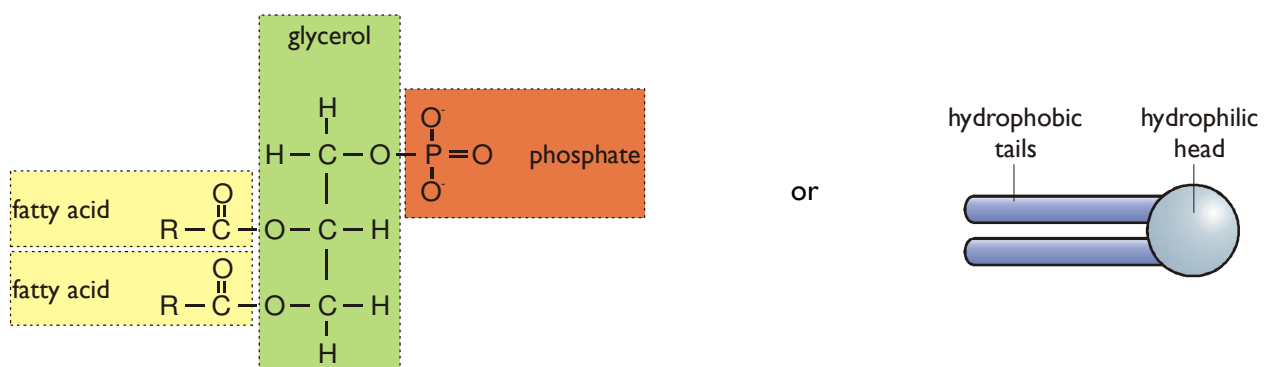
This mixture of properties is fundamental to biology, for phospholipids are the main components of cell membranes. When mixed with water, phospholipids form droplet spheres with a double-layered <u>phospholipid bilayer</u>. The hydrophilic heads facing the water and the hydrophobic tails facing each other. 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 (see p35).
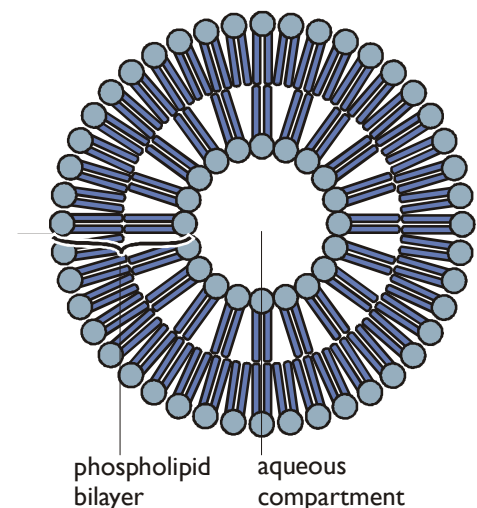
Proteins

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

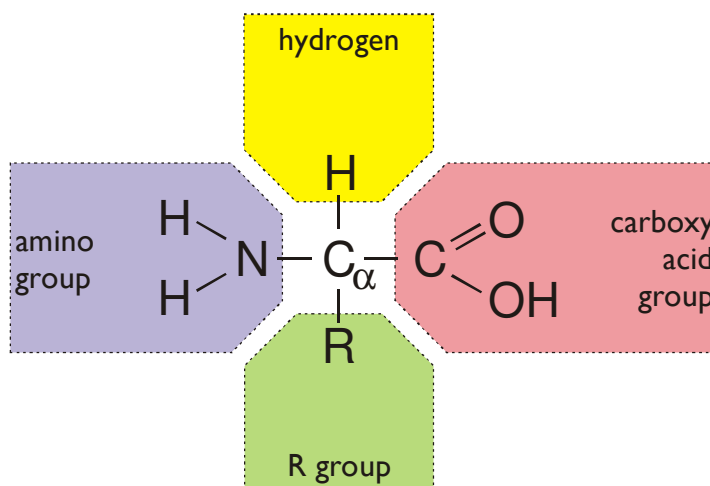|	
structure	e.g. collagen (bone, cartilage, tendon), keratin (hair), actin (muscle)
enzymes	e.g. amylase, pepsin, catalase, etc (>10,000 others)
transport	e.g. haemoglobin (oxygen), transferrin (iron)
pumps	e.g. Na^+K^+ pump in cell membranes
motors	e.g. myosin (muscle), kinesin (cilia)
hormones	e.g. 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. cholera toxin
antifreeze	e.g. glycoproteins in arctic flea
and many more!	

Amino Acids

Proteins are made of <u>amino acids</u>. Amino acids are made of the five elements C H O N S. Amino acids are so-called because they contain both an amino group and an acid group. The general structure of an amino acid molecule is shown on the right. There is a central carbon atom (called the "alpha carbon", C_α), with four different chemical groups attached to it:

1. a hydrogen atom

2. a basic amino group (NH_2 or NH_3^+)

3. an acidic carboxyl group (COOH or COO⁻)

4. a variable "R" group (or side chain)

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 table on page xx 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. There are 3-letter and 1-letter abbreviations for each amino acid.

Polypeptides

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

peptide bond

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

N-terminus   C-terminus

H_2N-Gly — Pro — His — Leu — Tyr — Ser — Trp — Asp — Lys — Cys-COOH

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

In a protein the polypeptide chain may be many hundreds of amino acids long. Amino acid polymerisation to form polypeptides is part of protein synthesis. It takes place in ribosomes, and is special because it requires an RNA template. The sequence of amino acids in a polypeptide chain is determined by the sequence of the bases in DNA. Protein synthesis is studied in detail in unit 5.

Protein Structure

Polypeptides are just strings of amino acids, but they fold up and combine 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:
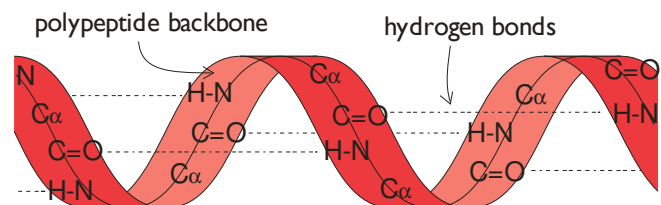
1. Primary Structure

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.

2. Secondary Structure

This is the most basic level of protein folding, and consists of a few basic motifs that are found in almost 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 most common secondary structure motifs are the α-helix and the β-sheet.

$$C=O \cdots\cdots\cdots\cdots H-N$$
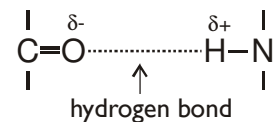
hydrogen bond

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. Do not confuse the α-helix of proteins with the famous double helix of DNA – helices are common structures throughout biology.

The β-sheet. The polypeptide chain zig-zags back and forward forming a sheet of antiparallel strands. Once again it is held together by hydrogen bonds.
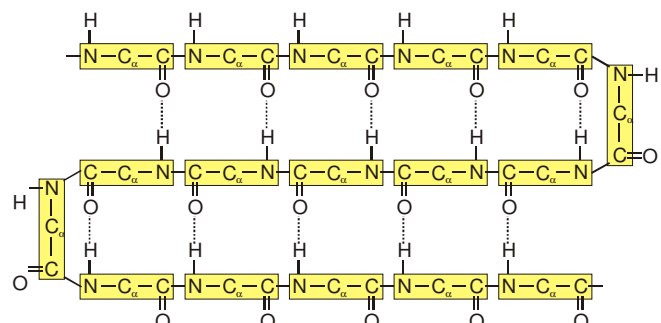
3. Tertiary Structure

This is the compact globular 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. These bonds include weak hydrogen bonds and <u>sulphur bridges</u> - covalent S–S bonds between two cysteine amino acids, which are much stronger.
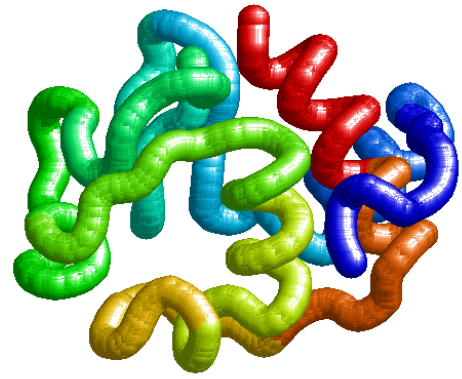
So the secondary structure is due to backbone interactions and is thus largely independent of primary sequence, while tertiary structure is due to side chain interactions and thus depends on the amino acid sequence.

4. Quaternary Structure

Almost all working proteins are actually composed of more than one polypeptide chain, and the quaternary structure is the arrangement of the different chains. There are a huge variety of quaternary structures e.g.:
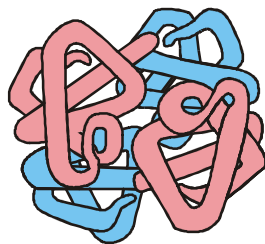
Haemoglobin consists of four chains arranged in a tetrahedral (pyramid) structure.
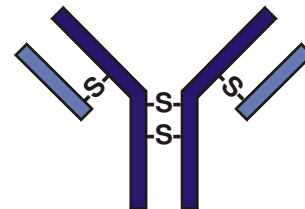
Antibodies comprise four chains arranged in a Y-shape.
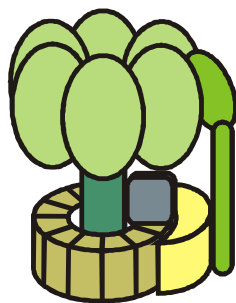
The enzyme ATP synthase is composed of 22 chains forming a rotating motor.

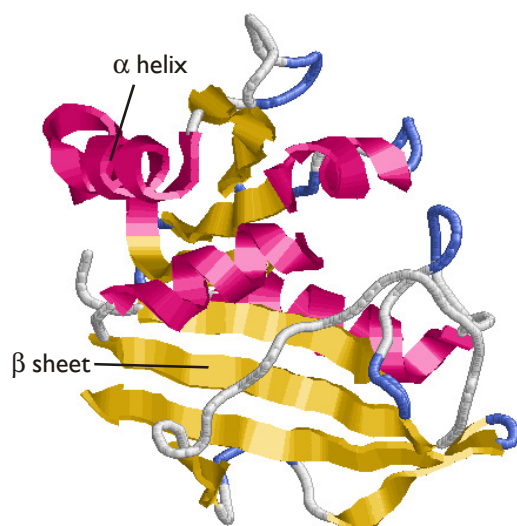Collagen consists of three chains in a triple helix structure.

Actin consists of hundreds of globular chains arranged in a long double helix.

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 <u>globular</u> or <u>fibrous</u>.

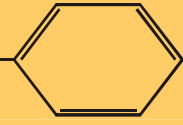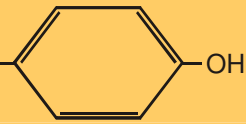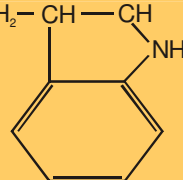| **Globular Proteins**	**Fibrous (or Filamentous) Proteins**
The vast majority of proteins are globular, i.e. they have a compact, ball-shaped structure. This group includes enzymes, membrane proteins, receptors and storage proteins. The diagram below shows a typical globular enzyme molecule. It has been drawn to highlight the different secondary structures.	Fibrous proteins are long and thin, like ropes. They tend to have structural roles, such as collagen (bone), keratin (hair), tubulin (cytoskeleton) and actin (muscle). They are always composed of many polypeptide chains. This diagram shows part of a molecule of collagen, which is found in bone and cartilage.

α helix

β sheet
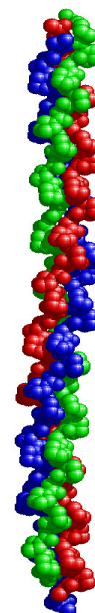
A few proteins have both structures: for example the muscle protein myosin has a long fibrous tail and a globular head, which acts as an enzyme (see unit 4).

Protein Denaturing

Since the secondary, tertiary and quaternary structures are largely held together by hydrogen bonds, the three-dimensional structure of proteins is lost if the hydrogen bonds break. The polypeptide chain just folds up into a random coil and the protein loses its function. This is called <u>denaturing</u>, and happens at temperatures above about 50°C or at very low or high pH. Covalent bonds are not broken under these conditions, so the primary structure is maintained (as are sulphur bridges).

The Twenty Amino Acid R-Groups

Simple R groups

Glycine
Gly G

$— H$

Alanine
Ala A

$— CH_3$

Valine
Val V

$— CH \big\langle{}^{CH_3}_{CH_3}$

Leucine
Leu L

$— CH_2 — CH \big\langle{}^{CH_3}_{CH_3}$

Isoleucine
Ile I

$— CH — CH_2 — CH_3$
$\quad\; |$
$\quad\; CH_3$

Basic R groups

Lysine
Lys K

$— CH_2 — CH_2 — CH_2 — CH_2 — NH_3^+$

Arginine
Arg R

$— CH_2 — CH_2 — CH_2 — NH — C \big\langle{}^{NH_2}_{NH_2^+}$

Histidine
His H

$— CH_2 — C$ (imidazole ring with CH, N, NH, CH)

Asparagine
Asn N

$— CH_2 — C \big\langle{}^{O}_{NH_2}$

Glutamine
Gln Q

$— CH_2 — CH_2 — C \big\langle{}^{O}_{NH_2}$

Hydroxyl R groups

Serine
Ser S

$— CH_2 — OH$

Threonine
Thr T

$— CH — OH$
$\quad\; |$
$\quad\; CH_3$

Acidic R groups

Aspartate
Asp D

$— CH_2 — CH_2 — C \big\langle{}^{O}_{OH}$

Glutamate
Glu E

$— CH_2 — C \big\langle{}^{O}_{OH}$

Sulphur R groups

Cysteine
Cys C

$— CH_2 — SH$

Methionine
Met M

$— CH_2 — CH_2 — S — CH_3$

Ringed R groups

Phenylalanine
Phe F

$— CH_2 —$ (benzene ring)

Tyrosine
Tyr Y

$— CH_2 —$ (benzene ring) $— OH$

Tryptophan
Trp W

$— CH_2 — CH — CH$ (indole ring with NH)

Cyclic R group

Proline
Pro P

$H — C_\alpha — CH_2$ with COOH, NH, CH_2, CH_2 forming ring

Biochemical Tests

These five tests identify the main biologically-important chemical compounds. For each test take a small sample of the substance to test and, if it isn't already a solution, grind it with some water 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.

1. **Starch** (iodine test). Add a few drops of iodine/potassium iodide solution to the sample. A blue-black colour indicates the presence of starch as a starch-polyiodide complex is formed.

2. **Reducing Sugars** (Benedict's test). All monosaccharides and most disaccharides (except sucrose) are called <u>reducing sugars</u> because they will reduce ions like Cu^{2+}. Add a few mL of <u>Benedict's reagent</u> to the sample. Shake, and heat for a few minutes at 95°C in a water bath. A coloured 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 <u>semi-quantitative</u>. 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.

3. **Non-reducing Sugars** (Benedict's test). Sucrose is a <u>non-reducing sugar</u>, so there is no direct test for sucrose. However, if it is first hydrolysed 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 before 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.

4. **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 vigorously shake some of the test sample with about 4 mL of ethanol. Decant the liquid into a second 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.

5. **Protein** (biuret test). Add a few mL of biuret solution to the sample. Shake, and the solution turns lilac-purple, indicating protein.
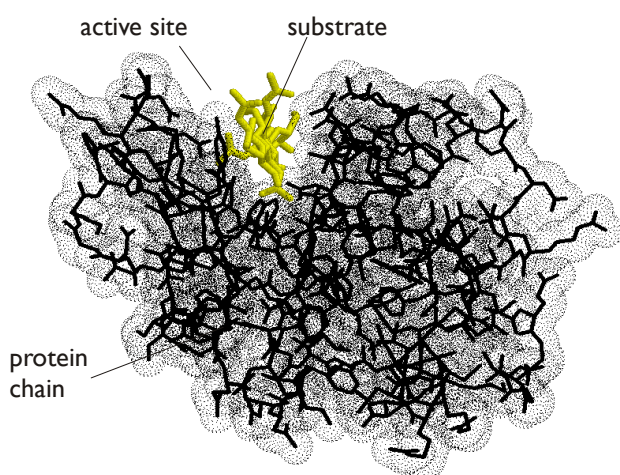
Enzymes

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.

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. Enzymes Manipulate the Substrate in the Active Site

Enzymes are proteins, and their function is determined by their complex 3-dimentional 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". The substrate molecule binds to the active site and the product is released.

Lysozyme – whole molecule
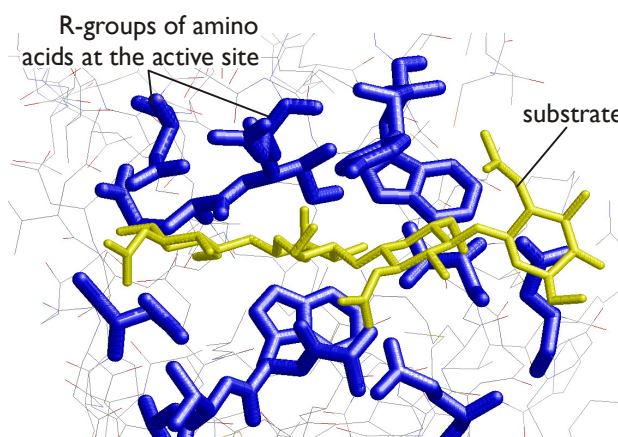
Close-up of substrate binding to amino acids in the active site

There are two models for the action of enzyme active sites:

- The lock and key model states that the enzyme's active site is complementary to the substrate molecule. The active site is like a lock and the substrate is like a key fitting perfectly into the lock. The shape and properties of the active site are given by the amino acids around it. These amino acids form weak hydrogen and ionic bonds with the substrate molecule, so the active site binds one substrate only.

- The Induced fit model states that the enzyme is flexible and so the active site can change shape. The active site isn't exactly complementary to the substrate, but as the substrate starts to bind, the active site changes shape to fit the substrate more closely. This change in turn distorts the substrate molecule in the active site, 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 if a bond is to be made between two molecules, the two molecules can be held in exactly the right position and orientation and "pushed" together, making the bond more likely to form. The enzyme can also 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. The induced fit model explains the action of enzymes more fully than the lock and key model.

Many enzymes also have small non-protein molecules called <u>coenzymes</u> at their active sites to help bind to the substrate. Many of these are derived from dietary vitamins, which is why vitamins are so important.

2. Enzymes Take an Alternative Reaction Pathway

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

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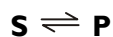
(There may be more than one substrate and more than one product, but that doesn't matter here.) In an enzyme-catalysed reaction, the substrate first binds to the active site of the enzyme to form an <u>enzyme-substrate (ES) complex</u>, then the substrate is converted into product while attached to the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

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$
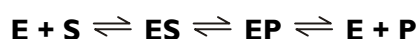
The enzyme is then free to start again. The end result is the same ($S \rightleftharpoons P$), but a different route is taken, so that the $S \rightleftharpoons P$ reaction as such never takes place. In by-passing this step, and splitting the reaction up into many small steps rather than one big step, the reaction can be made to happen much more quickly.

3. Enzymes Lower the Activation Energy

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,
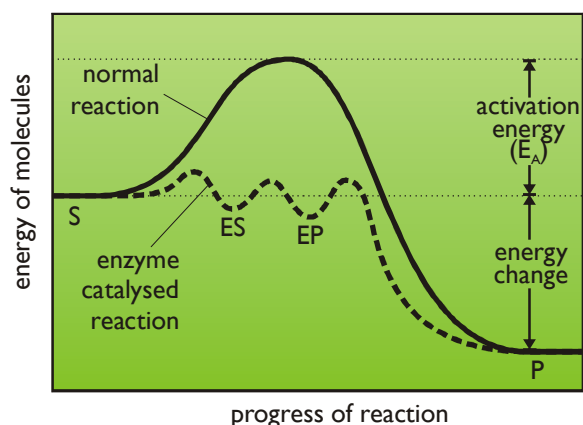
the slower the reaction will be because only a few substrate molecules will by chance have sufficient energy to overcome the activation energy barrier. Imagine pushing boulders over a hump before they can roll down hill, and you have the idea. Most physiological reactions have large activation energies, 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 breakdown of hydrogen peroxide ($2H_2O_2 \rightleftharpoons 2H_2O + O_2$):

- $E_A = $ 86 kJ mol^{-1} with no catalyst
- $E_A = $ 62 kJ mol^{-1} with an inorganic catalyst of iron filings
- $E_A = $ 1 kJ mol^{-1} in the presence of the enzyme peroxidase (catalase).

Factors that Affect the Rate of Enzyme Reactions

1. Temperature

All chemical reactions get faster as the temperature increases, but with enzyme reactions this is only true up to a certain temperature, above which the rate slows down again. This <u>optimum temperature</u> is about 40°C for mammalian enzymes 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.
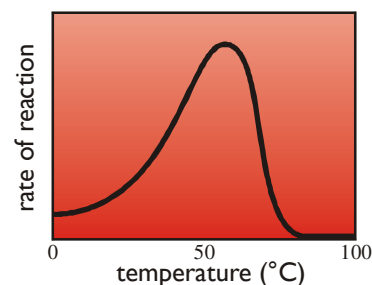

Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the enzyme and substrate molecules both have more kinetic energy so collide more often, and also because more molecules have sufficient energy to overcome the (greatly reduced) activation energy. The 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.

This increase in rate with temperature would continue indefinitely except that the enzyme molecule itself is affected by temperature. Above about 40°C there is enough thermal energy to break the weak hydrogen bonds holding the secondary, tertiary and quaternary structures of the enzyme together, so the enzyme (and especially the active site) loses its specific shape to become a random coil. The substrate can no longer bind, and the reaction is no longer catalysed. This denaturation is usually irreversible. The optimum temperature of enzymes is normally about 40°C because that is the temperature at which hydrogen bonds break. This is also the reason why mammals and birds maintain their body temperature at around 40°C. Remember that only the weak hydrogen bonds not peptide 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 R-groups of the amino acids at the active site. For example carboxyl R-groups are uncharged (COOH) in acid pH but negatively charged (COO⁻)
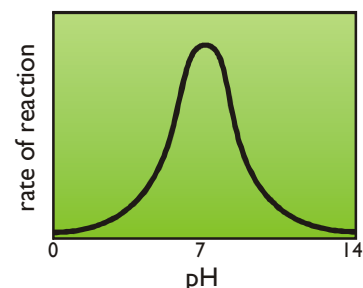
in alkali pH. Similarly amino R-groups are positively charged (NH_3^+) in acidic pH but uncharged (NH_2) in alkali pH. These changes can affect the shape as well as the charge of the active site, so the substrate can no longer bind and the reaction isn't catalysed.

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.
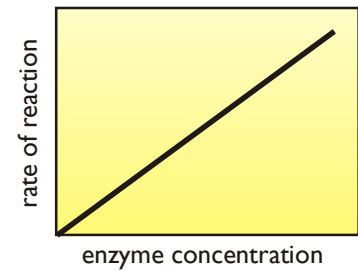
4. Substrate concentration

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 active sites become <u>saturated</u> 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).
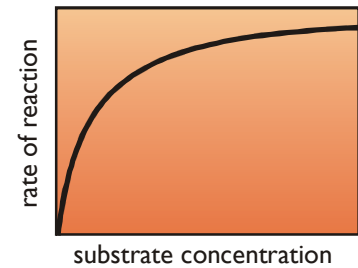
5. Inhibitors

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. Inhibitors that bind fairly weakly and can be washed out are called reversible inhibitors, while those that bind tightly and cannot be washed out are called irreversible inhibitors.
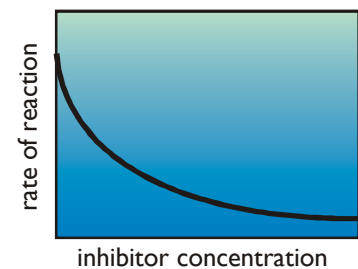
There are two kinds of inhibitors:

- <u>Competitive Inhibitors</u> are molecules with a similar structure to the normal substrate molecule, and can fit into the active site of the enzyme. They therefore compete with the substrate for the active site, so the reaction is slower. However, if the substrate concentration is increased high enough the substrate will out-compete the inhibitor and the rate can approach a normal rate. The sulphonamide anti-bacterial drugs are competitive inhibitors.
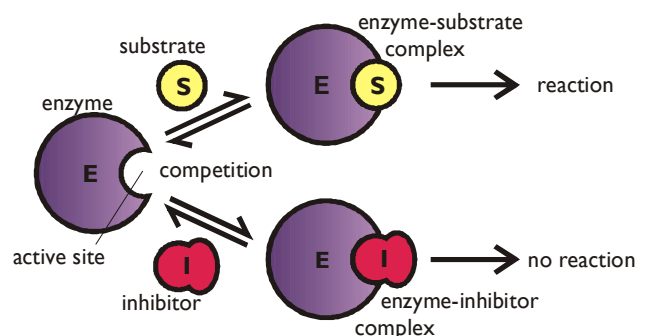
- <u>Non-competitive Inhibitors</u> are molecules with a quite different in structure from the substrate molecule and do not fit into the active site. They bind to another part of the enzyme molecule, changing

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). Poisons like cyanide, heavy metal ions and some insecticides are all non-competitive inhibitors.
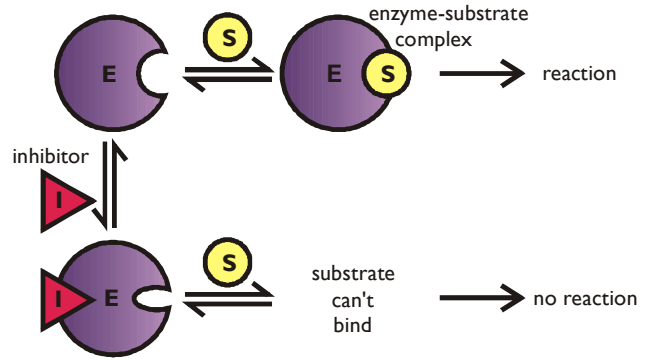
The two types of inhibitor can be distinguished experimentally by carrying out a substrate vs. rate experiment in the presence and absence of the inhibitor. If the inhibition is reduced at high substrate concentration then the inhibitor is a competitive one.
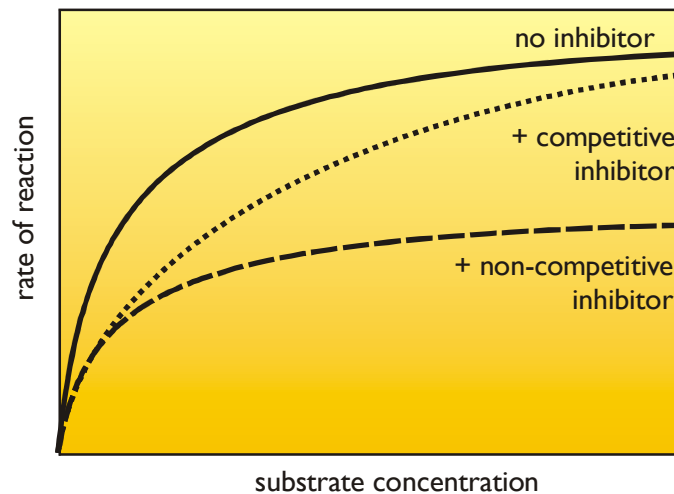
Active sites and binding sites

Enzymes and receptors are both protein molecules that work in similar ways. They have specific three-dimensional shapes with a site where another molecule can bind.

Enzymes have an active site. The molecule that binds (the substrate) is changed and released as a different molecule (the product).

molecule binds tightly and specifically to active site — released as different molecule
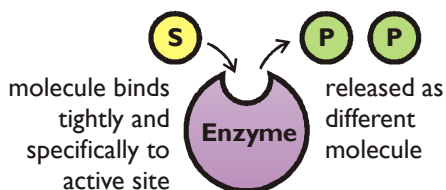
Receptors have a binding site. The molecule that binds (the ligand) is released unchanged.

molecule binds tightly and specifically to binding site — released as same molecule
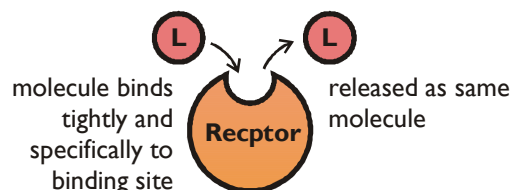
Measuring the Rate of Enzyme Reactions

1. Firstly you need a <u>signal</u> 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 that does generate one of these changes.
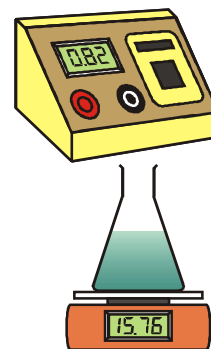
2. If you mix the substrate with enzyme and measure the signal, you will obtain a <u>time-course</u>. If the signal is proportional to substrate concentration it will start high and decrease, while if the signal is proportional to product it will start low and increase. In both cases the time-course will be curved (actually an exponential curve).

3. 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 actually ends: it just gradually approaches the end-point. The rate is in fact the <u>slope</u> (or gradient) 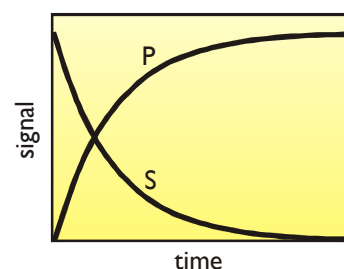of the time-course, so we can see that the rate (and slope) decreases as the reaction proceeds. The best measurement is the <u>initial rate</u> - that is the initial slope of the time-course. This also means you don't need to record the whole time-course, but simply take one measurement a short time after mixing.
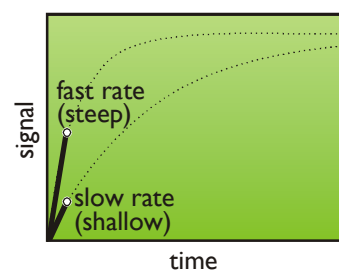
4. Repeat this initial rate measurement under different conditions (such as different temperatures or substrate concentrations) and then plot a graph of rate vs. the factor. Each point on this second graph is taken from a separate initial rate measurement (or better still is an average of several initial rate measurements under the same conditions). Draw a smooth curve through the points.
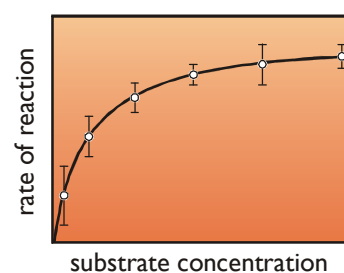
Be careful not to confuse the two kinds of graph (the time-course and rate graphs) when interpreting data.

Cells

All living things are made of cells, and cells are the smallest units that can be alive. There are thousands of different kinds of cell, but the biggest division is between the cells of the <u>prokaryote</u> kingdom (the bacteria) and those of the other four kingdoms (animals, plants, fungi and protoctista), which are all <u>eukaryotic</u> 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. These show the individual organelles inside a cell.

Euakryotic Cells

cell wall — small vacuole — Golgi body — cytoskeleton — rough endoplasmic reticulum — nucleus — nucleoplasm — nucleolus — nuclear envelope — nuclear pore — lysosome

cell membrane — large vacuole — chloroplast — mitochondrion — 80S ribosomes — smooth endoplasmic reticulum — centriole — undulipodium

Not all eukaryotic cells have all the parts shown here

10 µm
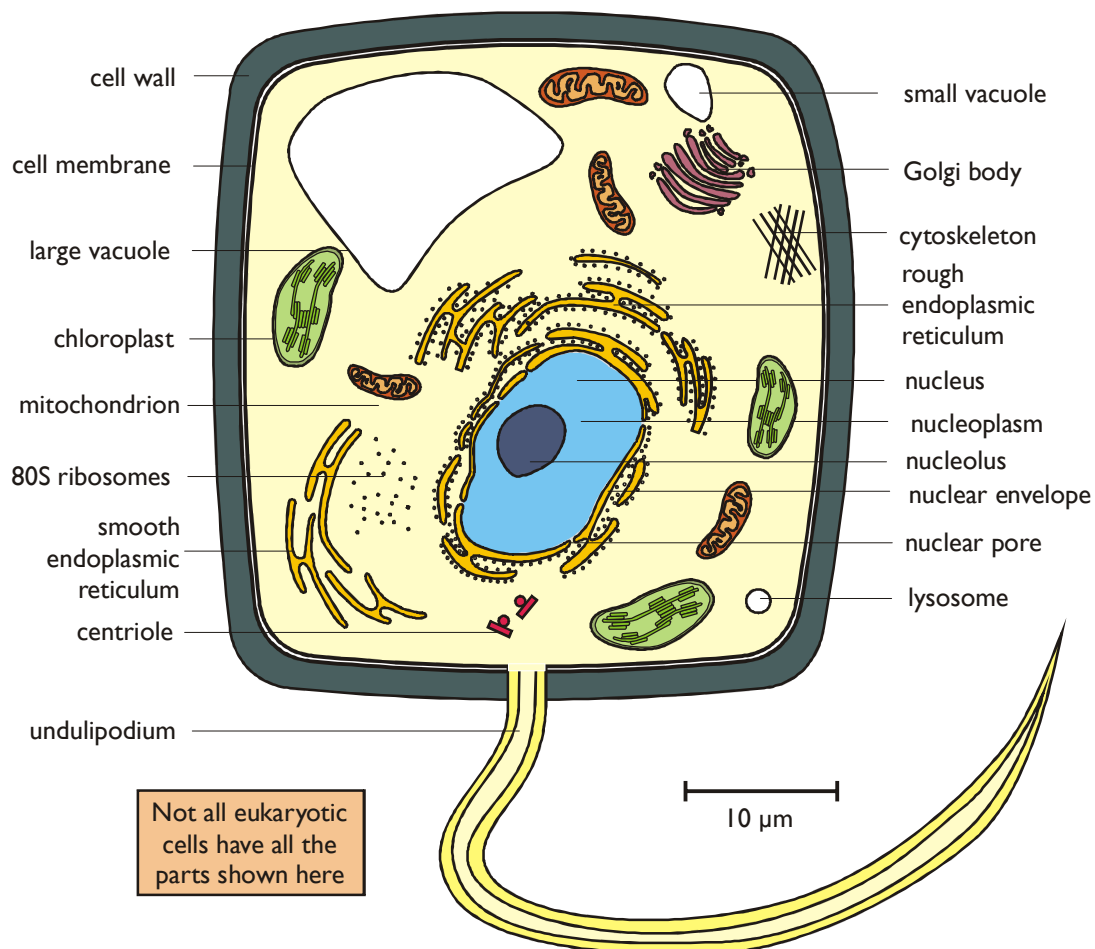
- **Cytoplasm (or Cytosol).** This is the solution within the cell membrane. It contains enzymes for glycolysis (part of respiration) and other metabolic reactions together with sugars, salts, amino acids, nucleotides and everything else needed for the cell to function.

- **Nucleus**. This is the largest organelle. It is surrounded by a nuclear envelope, which is a double membrane with nuclear pores – large holes containing proteins that control the exit of substances from the nucleus. The interior is called the nucleoplasm, which is full of chromatin – the DNA/protein complex (see unit 2). 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 (anaerobic respiration takes place in the cytoplasm). Mitochondria release energy (in the form of the molecule ATP) from carbohydrates, lipids and other energy-rich molecules. Cells that use a lot of energy (like muscle cells) have many mitochondria.
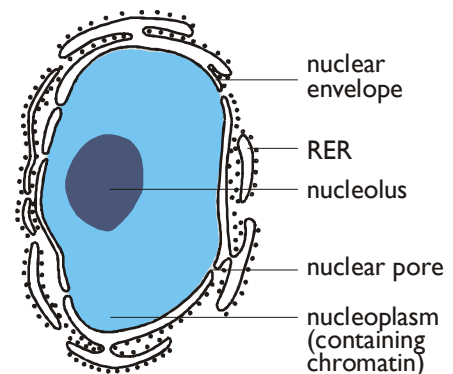
  Mitochondria are surrounded by a double membrane: the outer membrane is simple and quite permeable, while the inner membrane is highly folded into cristae, which give it a large surface area. The space enclosed by the inner membrane is called the mitochondrial matrix, and contains small circular strands of DNA. The inner membrane is studded with stalked particles, which are the enzymes that make ATP.
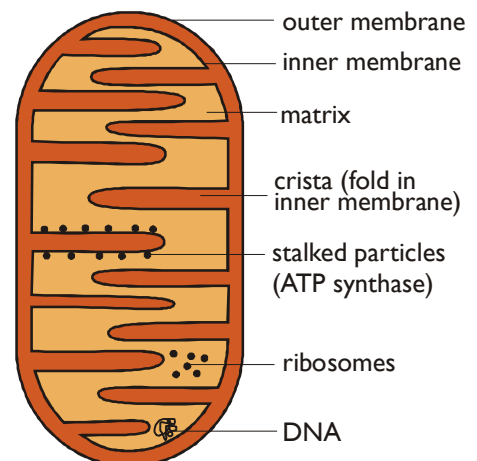
- **Ribosomes**. These are the smallest and most numerous of the cell organelles, and are the sites of protein synthesis. Ribosomes are either found free in the cytoplasm, where they make proteins for the cell's own use, or they are found attached to the rough endoplasmic reticulum, where they make proteins for export from the cell. All eukaryotic ribosomes are of the larger, "80S", type.
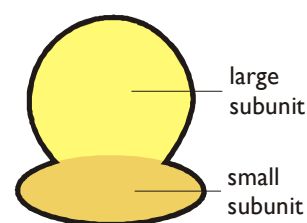
- **Endoplasmic Reticulum (ER).** This is a series of membrane channels involved in synthesising and transporting materials. Rough Endoplasmic Reticulum (RER) is studded with numerous ribosomes, which give it its rough appearance. The ribosomes synthesise proteins, which are processed in the RER (e.g. by enzymatically modifying the polypeptide chain, or adding carbohydrates), before being exported from the cell via the Golgi Body. Smooth Endoplasmic Reticulum (SER) does not have ribosomes and is used to process materials, mainly lipids, needed by the cell.

cisternae
ribosomes
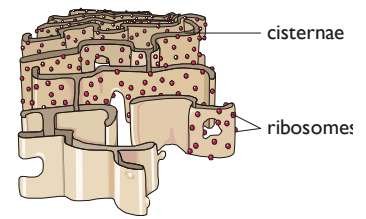
- **Golgi Body (or Golgi Apparatus).** Another series of flattened membrane vesicles, formed from the endoplasmic reticulum. Its job is to transport proteins from the RER to the cell membrane for export. Parts of the RER containing proteins fuse with one side of the Golgi body membranes, while at the other side small vesicles bud off and move towards the cell membrane, where they fuse, releasing their contents by exocytosis.
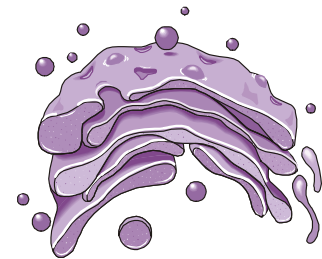
- **Lysosomes**. These are small membrane-bound vesicles formed from the RER containing a cocktail of digestive enzymes. They are used to break down unwanted chemicals, toxins, organelles or even whole cells, so that the materials may be recycled. They can also fuse with a feeding vacuole to digest its contents.
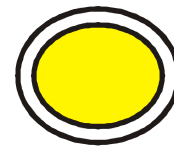
- **Cytoskeleton**. This is a network of protein fibres extending throughout all eukaryotic cells, used for support, transport and motility. The cytoskeleton is attached to the cell membrane and gives the cell its shape, as well as holding all the organelles in position. The cytoskeleton is also responsible for all cell movements, such as cell division, cilia and flagella, cell crawling and muscle contraction in animals.

Protein filaments
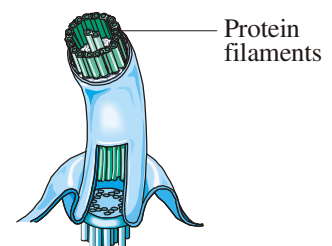
- **Undulipodium (Cilium or Flagellum).** This is a long flexible tail present in some cells used for motility. It is an extension of the cell membrane, and is full of microtubules and motor proteins so is capable of complex swimming movements. There are two kinds: cilia are short and numerous (e.g. trachea, ciliates), while flagella are longer than the cell, and there are usually only one or two of them (e.g. sperm).

cilia

Flagellum
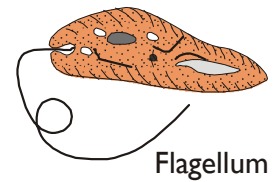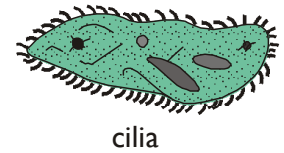
- **Microvilli**. These are small finger-like extensions of the cell membrane found in certain cells such as in the epithelial cells of the intestine and kidney, where they increase the surface area for absorption of materials. They are just visible under the light microscope as a brush border. Don't confuse microvilli (sub-cellular structures) with villi (much bigger multi-cellular structures).

Microvilli
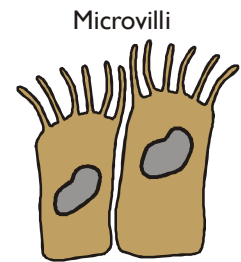
- **Cell Membrane (or Plasma Membrane).** This is a thin, flexible layer round the outside of all cells made of phospholipids and proteins. It separates the contents of the cell from the outside environment, and controls the entry and exit of materials. The membrane is examined in detail later.
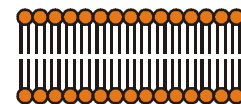
- Plant Cells also contain chloroplasts, permanent vacuoles and cell walls. These will be studied in unit 2.

Comparison of different types of Eukaryotic Cell

	Fungi	**Plants**	**Animals**
Nucleus	✓	✓	✓
Mitochondria	✓	✓	✓
Chloroplast	✗	✓	✗
80S ribosome	✓	✓	✓
Vacuoles	✓	✓	✗
Cytoskeleton	✓	✓	✓
Undulipodium	✗	✗	✓
Plasma membrane	✓	✓	✓
Cell Wall	✓ (chitin)	✓ (cellulose)	✗

Prokaryotic Cells

Prokaryotic cells are smaller than eukaryotic cells and do not have a nucleus or indeed any membrane-bound organelles. All prokaryotes are bacteria. Prokaryotic cells are much older than eukaryotic cells and they are far more abundant (there are ten times as many bacteria cells in a human than there are human cells). The main features of prokaryotic cells are:

- **Cytoplasm**. Contains all the enzymes needed for all metabolic reactions, since there are no organelles

- **Ribosomes**. The smaller "70S" type, all free in the cytoplasm and never attached to membranes. Used for protein synthesis.

- **Nuclear Zone** (or **Nucleoid**). The region of the cytoplasm that contains DNA. It is not surrounded by a nuclear membrane.

- **DNA**. Always circular (i.e. a closed loop), and not associated with any proteins to form chromatin. Sometimes referred to as the bacterial chromosome to distinguish it from plasmid DNA.

Labels on diagram: 70S ribosomes, plasmid, flagellum, capsule, cell wall, cell membrane, nucleoid, DNA

Not all prokaryotic cells have all the parts shown here

1 µm

- **Plasmid**. Small circles of DNA, separate from the main DNA loop. Used to exchange DNA between bacterial cells, and also very useful for genetic engineering (see unit 5).

- **Plasma membrane**. Made of phospholipids and proteins, like eukaryotic membranes.

- **Cell Wall**. Made of <u>murein</u> (not cellulose), which is a glycoprotein (i.e. a protein/carbohydrate complex, also called <u>peptidoglycan</u>).

- **Capsule**. 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. In some species the capsules of many cells fuse together forming a mass of sticky cells called a biofilm. Dental plaque is an example of a <u>biofilm</u>.

- **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. The bacterial flagellum is quite different from the eukaryotic flagellum.
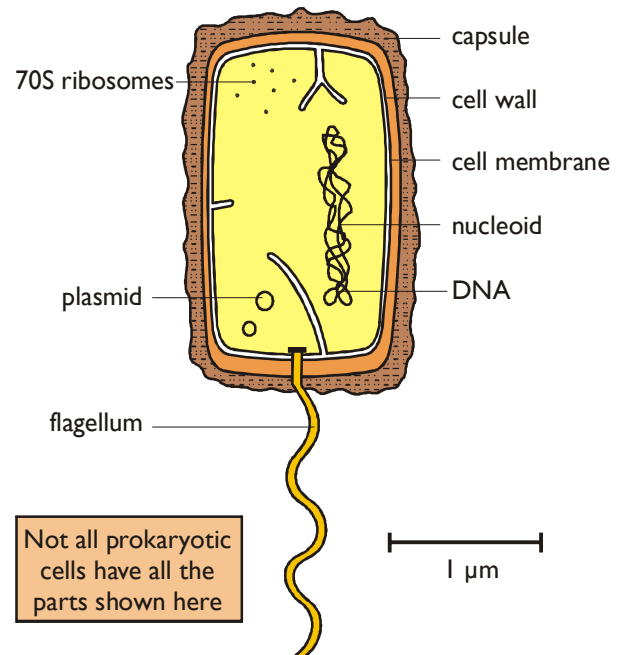
Summary of the Differences Between Prokaryotic and Eukaryotic Cells

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
motility by rigid rotating flagellum, made of flagellin	motility by flexible waving undulipodium, made of tubulin
cell division is by binary fission	cell division is by mitosis or meiosis
reproduction is always asexual	reproduction is asexual or sexual
huge variety of metabolic pathways	common metabolic pathways

Endosymbiosis

Prokaryotic cells are far older and more diverse than eukaryotic cells. Prokaryotic cells have probably been around for 3.5 billion years, while eukaryotic cells arose only about 1 billion years ago. It is thought that eukaryotic cell organelles like nuclei, 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.
- organelles reproduce by binary fission, like bacteria.
- organelles are very like some bacteria that are alive today.

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 <u>differential centrifugation</u>:

1. Cut tissue (eg liver, heart, leaf, etc) in ice-cold isotonic buffer.
   • Cold to slow enzyme reactions
   • Isotonic to stop osmosis, so organelles don't burst
   • Buffer to stop pH changes
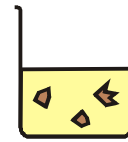
2. Grind tissue in a blender to break open cells.

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

4. Centrifuge filtrate at low speed (1 000 x g for 10 min).
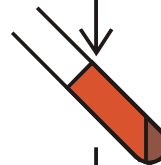
This pellets nuclei, which can be resuspended

5. Centrifuge supernatant at medium speed (10 000 x g for 30 min).
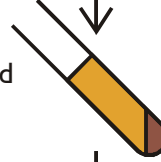
This pellets mitochondria and chloroplasts, which can be resuspended

6. Centrifuge supernatant at high speed (100 000 x g for 1 h)
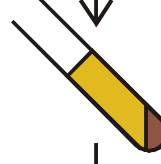
This pellets ER, golgi and other membrane fragments, which can be resuspended

7. Centrifuge supernatant at very high speed (300 000 x g for 3 h)
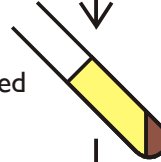
This pellets ribososmes, which can be resuspended

8. Supernatant is now organelle-free cytoplasm.

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 (never use cm!)
micrometre	μm	$= 10^{-6}$ m
nanometre	nm	$= 10^{-9}$ m
picometre	pm	$= 10^{-12}$ m
angstrom	Å	$= 10^{-10}$ m (obsolete)

Magnification and Resolution

- <u>Magnification</u> simply indicates how much bigger the image is that the original object. It is usually given as a magnification factor, e.g. x100. By using more lenses microscopes can magnify by a larger amount, but the image may get more blurred, so this doesn't always mean that more detail can be seen.

- <u>Resolution</u> is the smallest separation at which two separate objects can be distinguished (or resolved), and is therefore a distance (usually in nm). The resolution of a microscope is ultimately limited by the wavelength of light used (400-600nm for visible light). To improve the resolution 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 Microscopes

These are the oldest, simplest and most widely-used form of microscopy. Specimens are illuminated with light, which is focused using glass lenses and viewed using the eye or photographic film. Specimens can be living or dead, but often need to be coloured with a coloured <u>stain</u> to make them visible. Many different stains are available that stain specific parts of the cell such as DNA, lipids, cytoskeleton, etc. There are different kinds of light microscope:

- <u>Transmission microscopes</u> are the most common kind, where the light transmitted through the specimen is focussed to form an image. Transmission microscopes have a resolution of about 200nm, which is good enough to see tissues and cells, but not the details of cell organelles.

- <u>Fluorescence microscopes</u> use a fluorescent dye to stain specimens. The specimen is illumined with invisible ultraviolet radiation, and the stained objects emit visible light, so they can be seen even if the object is smaller than the wavelength of light. Fluorescence microscopy has a resolution of about 10nm.

- Interference microscopes use the interference pattern produced by combing two light beams that have passed through different object to produce an image. These microscopes have a resolution of about 1nm.

- Confocal microscopes use lasers to scan a thin layer of a thick specimen. By combining scan of different layers in a computer, a three-dimensional image an be built up.

Electron Microscopes

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), focused (using electromagnets) and detected (using a phosphor screen or photographic film). A beam of electrons has an effective wavelength of less than 1nm, 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.

There are several problems with electron microscopy:

- there must be a vacuum inside an electron microscope (so the electron beam isn't scattered by air molecules), so it can't be used for living organisms.

- specimens must be very thin, so are embedded in plastic for support, so can't be manipulated under the microscope.

- specimens can be damaged by the electron beam, so delicate structures and molecules can be destroyed.

- specimens are usually transparent to electrons, so 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.

- Transmission electron microscopes (TEM) work much like a light microscope, transmitting a beam of electrons through a thin specimen and then focusing 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 (<1nm).

- Scanning electron microscopes (SEM) scan a fine beam of electron onto a specimen and collect the electrons scattered by the surface. This has poorer resolution, but gives excellent 3-dimentional images of surfaces.

Comparison of Different Microscopes

	Light Microscope	**TEM**	**SEM**
Pros	good magnification (1000x) can use living specimens colour images video images possible simple and cheap	high magnification (5000 000x) very good resolution (1nm) good for sections good for organelles and prokaryotes	gives 3-dimentional images good for surfaces don't need thin sections don't need stain
Cons	poor resolution (200nm) can't see organelles specimens must be stained	can't use living specimens needs very thin sections specimens often need stains no colour very expensive	resolution not as good as TEM (10nm) can't see internal structures very expensive
Uses	tissues, cells and small organisms	cell organelles, microbes and viruses	surfaces of living and non-living specimens

Magnification Calculations

Microscope drawings and photographs (micrographs) are usually magnified, and you have to be able to calculate the actual size of the object from the drawing. There are two ways of doing this:

1. Using a Magnification Factor

Sometimes the image has a magnification factor on it. The formula for the magnification is:

$$\text{magnification} = \frac{\text{image length}}{\text{actual length}}, \text{ or}$$

For example if this drawing of an object is 40mm long and the magnification is ×1000, then the object's actual length is: $\frac{I}{M} = \frac{40}{1000} = 0.04\text{mm} = 40\mu m$. Always convert your answer to appropriate units, usually µm for cells and organelles.

× 1000
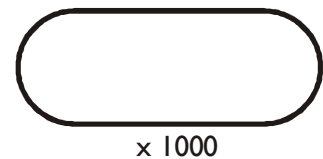
Sometimes you have to calculate the magnification. For example if this drawing of an object is 40mm long and its actual length is 25µm, the magnification of the drawing is: $\frac{I}{A} = \frac{40}{0.025} = \times 1600$. Remember, the image and actual length must be in the same units. Magnifications can also be less than one (e.g. x 0.1), which means that the drawing is smaller than the actual object.

2. Using a Scale Bar

Sometimes the picture has a scale bar on it. The formula for calculating the actual length is: $\text{actual size} = \frac{\text{image length}}{\text{bar length}} \times \text{bar scale}$. The image size and bar length must be measured in the same units (usually mm), and the actual size will come out in the same units as the bar scale.

For example if this drawing of an object is 40mm long and the 5µm scale bar is 10mm long, then the object's actual size is: $\frac{40}{10} \times 5\mu m = 20\mu m$.

5µm
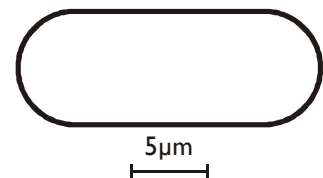
It's good to have a rough idea of the size of objects, to avoid silly mistakes. A mitochondrion is not 30mm long! Scale bars make this much easier than magnification factors.

The Cell Membrane

The cell membrane (or <u>plasma membrane</u>) surrounds all living cells, and is the cell's most important organelle. 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 as shown in this diagram.
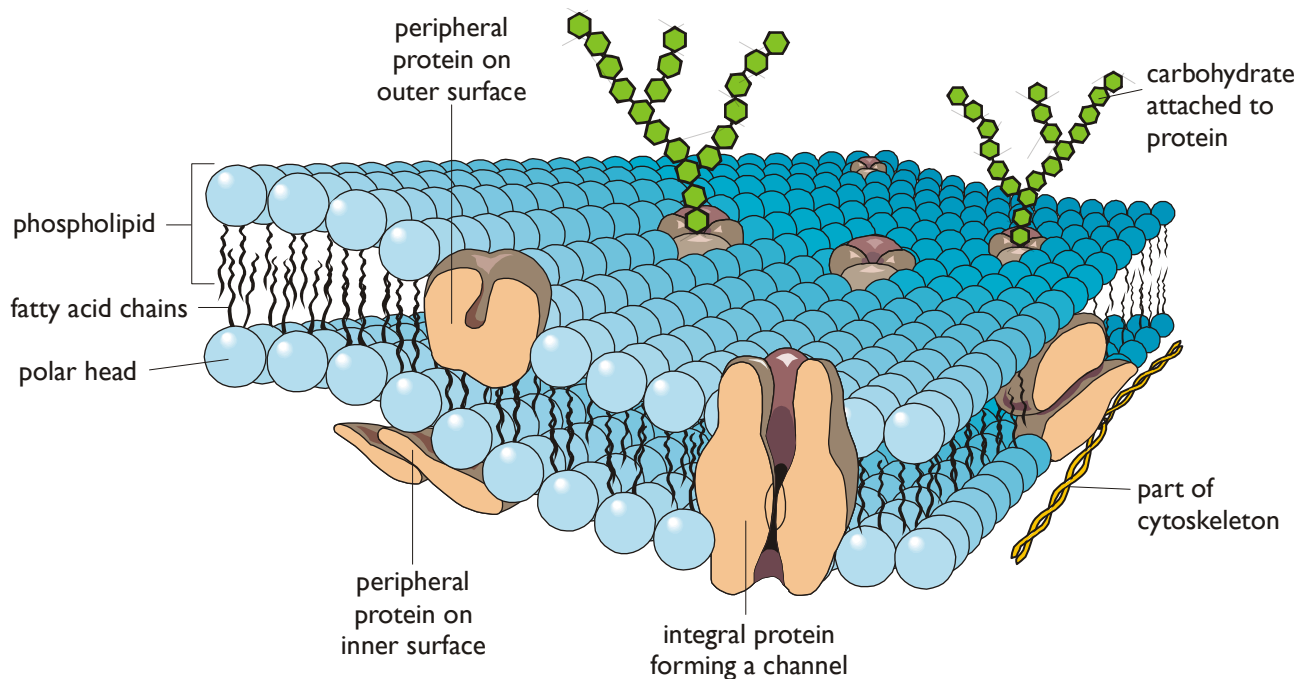
The phospholipids form a thin, flexible sheet, while the proteins "float" in the phospholipid sheet like icebergs, and the carbohydrates extend out from the proteins. This structure is called a <u>fluid mosaic structure</u> because all the components can move around (it's fluid) and the many different components all fit together, like a mosaic.

The phospholipids are arranged in a <u>bilayer</u> (i.e. a double layer), with their polar, hydrophilic phosphate heads facing out towards water, 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 most 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 (<u>integral proteins</u>), but can also sit on one of the surfaces (<u>peripheral proteins</u>). 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.

- **Transport proteins**. Most transport of small molecules across the membrane take place through integral proteins. This transport includes facilitated diffusion and active transport (more details below).

- **Receptor proteins**. Receptor proteins must be on the outside surface of cell membranes and have a specific binding site where hormones or other chemicals can bind to form a hormone-receptor complex (like an enzyme-substrate complex). This binding then triggers other events in the cell membrane or inside the cell.

- **Enzymes**. Enzyme proteins catalyse reactions in the cytoplasm or outside the cell, such as maltase in the small intestine (more in digestion).

- **Recognition proteins**. Some proteins are involved in cell recognition. These are often glycoproteins, such as the A and B antigens on red blood cell membranes.

- **Structural proteins**. Structural proteins on the inside surface of cell membranes and are attached to the cytoskeleton. They are involved in maintaining the cell's shape, or in changing the cell's shape for cell motility. Structural proteins on the outside surface can be used in cell adhesion – sticking cells together temporarily or permanently.
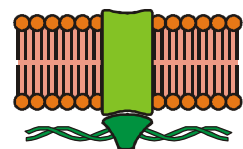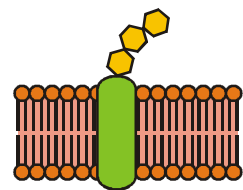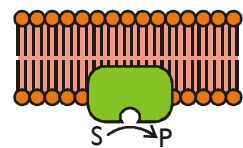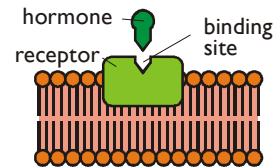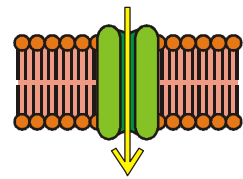
The carbohydrates are found on the outer surface of all eukaryotic cell membranes, and are attached to the membrane proteins or sometimes to the phospholipids. Proteins with carbohydrates attached are called glycoproteins, while phospholipids with carbohydrates attached are called glycolipids.

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

Movement across Cell Membranes

Substances move around inside cells by <u>diffusion</u>, which is the random movement of particles due to thermal motion. Diffusion does not require any energy (other than the thermal energy of the surroundings), so it is referred to as a <u>passive process</u>. If there is a concentration difference between two places then the random movement results in the substance diffusing down its concentration gradient from a high to a low concentration:
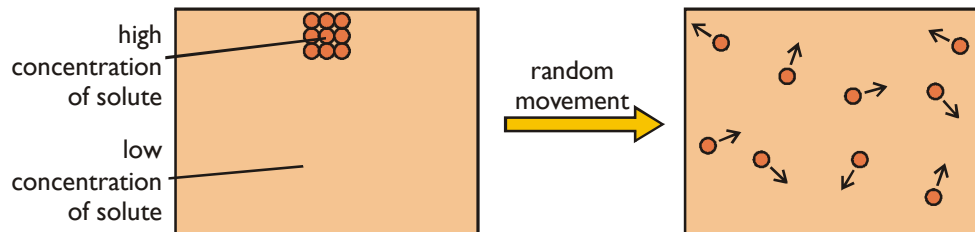
Cell membranes are a barrier to most substances, so we say that membranes are selectively permeable. This means that cell membranes can allow some substances through but not others. This selective permeability allows materials to be concentrated inside cells, excluded from cells, or simply separated from the outside environment. This is <u>compartmentalisation</u> is essential for life, as it enables reactions to take place that would otherwise be impossible. Eukaryotic cells can also compartmentalise materials inside organelles.

Obviously materials need to be able to enter and leave cells, and there are four main methods by which substances can move across a cell membrane:

1. Lipid Diffusion
2. Osmosis (Water Diffusion)

3. Facilitated Diffusion
4. Active Transport

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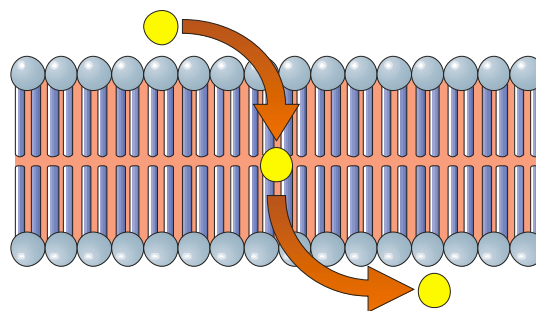
A few substances can diffuse directly through the lipid bilayer part of the membrane. The only substances that can do this are hydrophobic (lipid-soluble) molecules such as steroids, and a few extremely small hydrophilic molecules, such as H_2O, O_2 and CO_2. For these molecules the membrane is no barrier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

2. Osmosis (Water Diffusion)

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 50mol L^{-1}), the diffusion of water has its own name – osmosis. The contents of cells are essentially solutions of numerous different solutes, and each solute molecule attracts a <u>hydration shell</u> of water molecules attached to it. The more concentrated the solution, the more solute molecules there are in a given volume, and the more water molecules are tied up in hydration shells, so the fewer free water molecules there are. Free water molecules can diffuse easily across a membrane in both directions, but the net movement is always down their concentration gradient, so water therefore diffuses <u>from a more dilute solution to a more concentrated solution</u>.

water molecules   membrane   solute molecules

hydration shell

dilute solution	concentrated solution
low concentration of solute	high concentration of solute
high concentration of free water	low concentration of free water
high water potential (ψ)	low water potential (ψ)

net movement of water
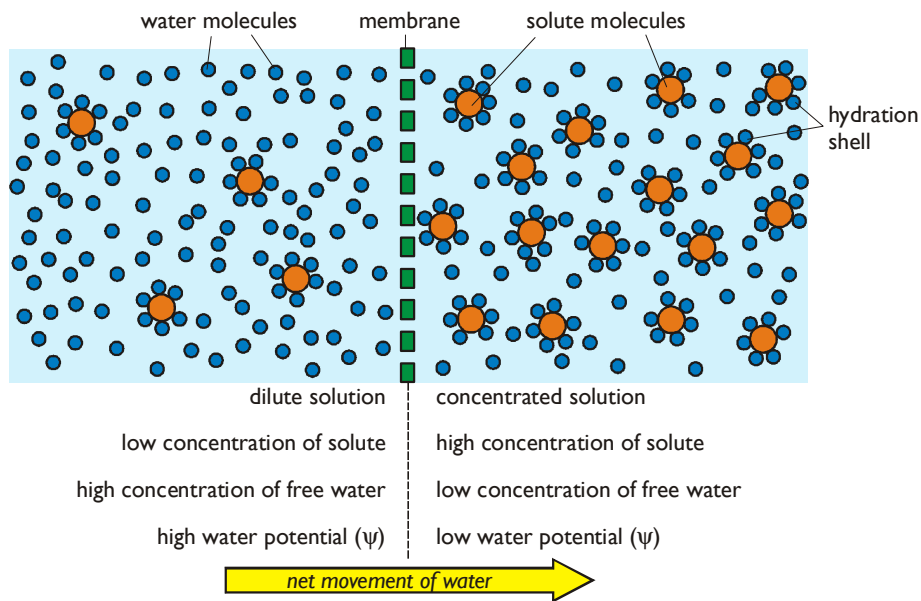
Water Potential. Osmosis can be quantified using <u>water potential</u>, so we can calculate which way water will move, and how fast. Water potential (Ψ, the Greek letter psi, pronounced "sy") is simply the effective concentration of free water. It is measured in units of pressure (Pa, or usually kPa), and the rule is that <u>water always "falls" from a high to a low water potential</u> (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 $\Psi < 0$, and you cannot get $\Psi > 0$. An example of water potentials is shown in this diagram:

pure water
ψ = 0 kPa

dilute solution
ψ = -200 kPa

concentrated solution
ψ = -500 kPa

water diffuses
from 0kPa to -200kPa

water diffuses
from -200kPa to -500kPa
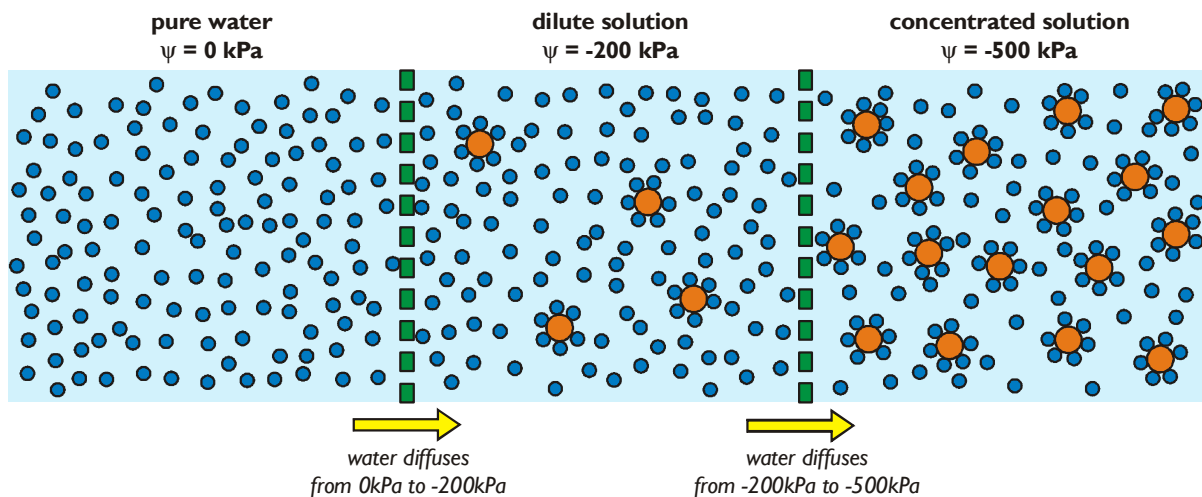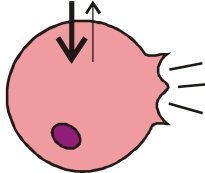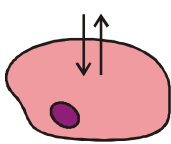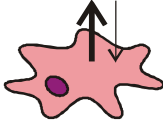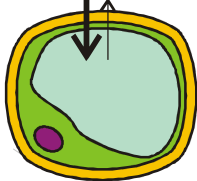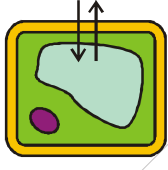
Cells and Osmosis

The water potential of the solution that surrounds a cell affects the state of the cell, due to osmosis. There are three possible concentrations of solution to consider (the word "tonic" means strength i.e. solute concentration):

- Isotonic solution   a solution of equal water potential to a cell ("same strength")
- Hypertonic solution   a solution of lower water potential than a cell ("high strength")
- Hypotonic solution   a solution of higher water potential than a cell ("low strength")

The effects of these solutions on cells are shown in this diagram:

	Surrounding solution hypotonic or high ψ (e.g. fresh water)	Surrounding solution isotonic or equal ψ	Surrounding solution hypertonic or low ψ (e.g. sea water)
Animal cell	Net diffusion of water into cell, so cell swells and bursts (lysis)	No net diffusion of water, so cell is normal size	Net diffusion of water out of cell, so cell shrinks and crenates.
Plant cell	Net diffusion of water into cell, so cell swells a bit and becomes turgid.	No net diffusion of water, so cell is normal size	Net diffusion of water out of cell, so cytoplasm shrinks from cell wall and cell plasmolyses.

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 (high so water tends to diffuse in by osmosis. These cells constantly need to expel water using contractile vacuoles to prevent swelling and lysis.
- Cells in marine environments are surrounded by a hypertonic solution (low Ψ, so water tends to diffuse out by osmosis. These cells 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 (or Passive Transport).
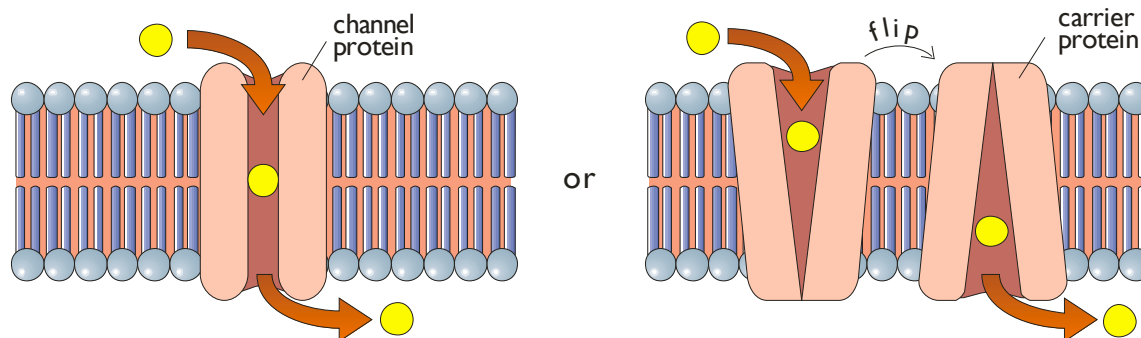
Facilitated Diffusion is the diffusion of substances across a membrane through a trans-membrane protein molecule. The transport proteins tend to be specific for one molecule, so substances can only cross a membrane that contains an appropriate protein. This is a passive diffusion process, so no energy 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 to diffuse across membranes. Most channels can be gated (opened or closed), allowing the cell to control the entry and exit of ions. In this way cells can change their permeability to certain ions. Ions like Na^+, K^+, Ca^{2+} and Cl^- diffuse across membranes through specific ion channels.

- 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. Important solutes like glucose and amino acids diffuse across membranes through specific carriers. Sometimes carrier proteins have two binding sites and so carry two molecules at once. This is called cotransport, and a common example is the sodium/glucose cotransporter found in the small intestine (see next page). Both molecules must be present for transport to take place.

4. Active Transport.
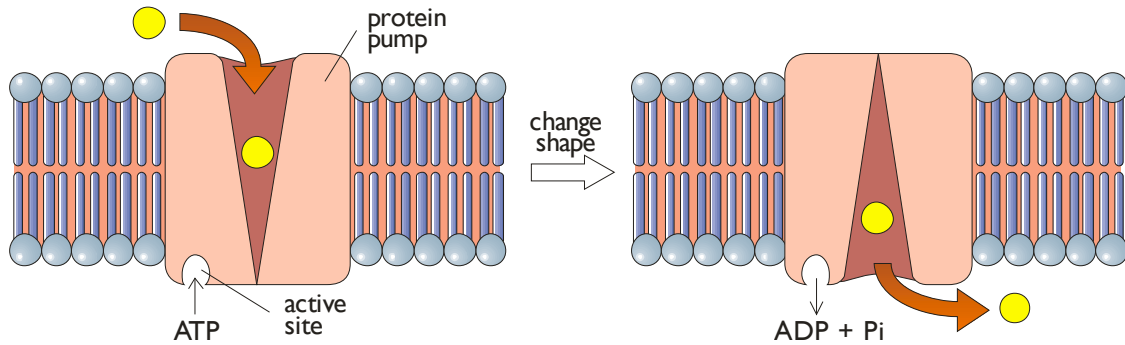
Active transport is the pumping of substances across a membrane by a trans-membrane protein pump molecule, using energy. The protein binds a molecule of the substance to be transported on one side of the membrane, changes shape, and releases it on the other side. The proteins are highly specific, so there is a different protein pump for each molecule to be transported. Since active transport uses energy it is called an active process (unlike diffusion, which is passive), and is the only transport mechanism that can transport substances up their concentration gradient.

ATP in active transport

All the processes that need energy in a cell (including active transport) use a molecule called adenosine triphosphate (ATP) as their immediate source of energy. ATP is synthesised from ADP and phosphate (P_i) using energy released from glucose in respiration in mitochondria (see p24).

Active transport pumps hydrolyse (split) the ATP back to ADP and P_i, and use the energy released to change shape and pump substances across membranes. They are therefore ATPase enzymes, since they have an active site that catalyses the hydrolysis of ATP to ADP + P_i.

A common active transport pump is the sodium/potassium ATPase (Na/K pump), found in all animal cell membranes. This pump continually uses ATP to actively pump sodium ions out of the cell and potassium ions into the cell. This creates ion gradients across the cell membrane, which can be used to regulate water potential and drive other process (such as absorption in the gut, see p65).
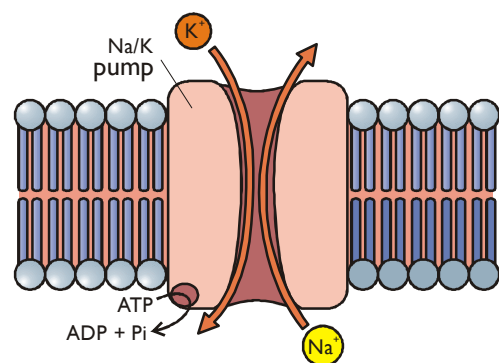
Effect of concentration difference on rate of transport

The three kinds of transport can be distinguished experimentally by the effect of solute concentration on its rate of transport:

- Lipid diffusion shows a linear relationship. The greater then concentration difference the great the rate of diffusion (see Fisk's law p44).

- Facilitated diffusion has a curved relationship with a maximum rate. At high concentrations the rate is limited by the number of transport proteins.

- Active transport has a high rate even when there is no concentration difference across the membrane. Active transport stops if cellular respiration stops, since there is no energy.
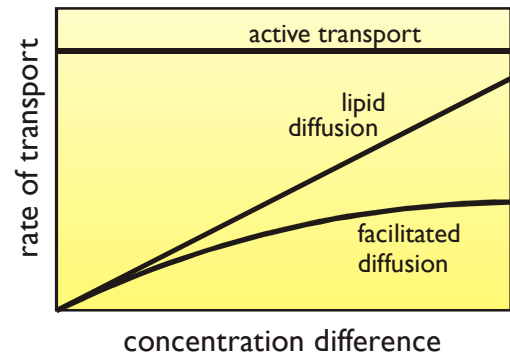
Summary of Membrane Transport

method	uses energy?	which part of membrane?	specific?	concentration gradient
Lipid Diffusion	✖	phospholipid bilayer	✖	↓
Osmosis	✖	phospholipid bilayer	✔	↓
Facilitated Diffusion	✖	proteins	✔	↓
Active Transport	✔	proteins	✔	↑

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Physiology

Exchange

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

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

From Fick's law we can predict that, in order to support a fast rate of diffusion, exchange surfaces must have:

- a large surface area
- a small distance between the source and the destination
- a mechanism to maintain a high concentration gradient across the gas exchange surface.

This table summarises how these requirements are met in the human digestive and gas exchange systems.

system	large surface area	small distance	high concentration gradient
Human small intestine	7m long, folds, villi and microvilli give surface area of 2000m²	blood capillaries close to surface of villus	stirred by peristalsis and by microvilli
Human circulatory system	100m of capillaries with a surface area of 6000m²	capillary walls are only one flattened cell thick	constant blood flow replenishes the blood
Human lungs	600 million alveoli with a total area of 100m²	alveoli walls are only one flattened cell thick	constant ventilation replaces the air

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

Epithelial Tissue

Epithelial tissue is the name given to the layer of cells covering all the external and internal surfaces of the body. Exchange therefore takes place through epithelial tissue and the cells are adapted for exchange. There are many different kinds of epithelial tissue:

- Squamous epithelium is found surrounding the alveoli (see p46). The cells are extremely flattened, like pancakes, and are often so thin that the nucleus makes a bulge.

- Endothelium is found lining capillaries and other blood vessels (see unit 2). These are also flat squamous cells, but on an internal surface (endo=inside).

- Columnar epithelium is found lining the alimentary canal (see p62). The cells are thick, but are lined with microvilli to give a large surface area.

- Ciliated epithelium is found on the trachea and bronchi (see p46). These cells are not adapted for exchange, but for lubrication and protection.

- Epidermis is found on the outer surface of the skin. It forms a tough, impermeable barrier preventing desiccation (water loss) and infection.

The Gas Exchange System

This diagram shows the gas exchange system in humans:
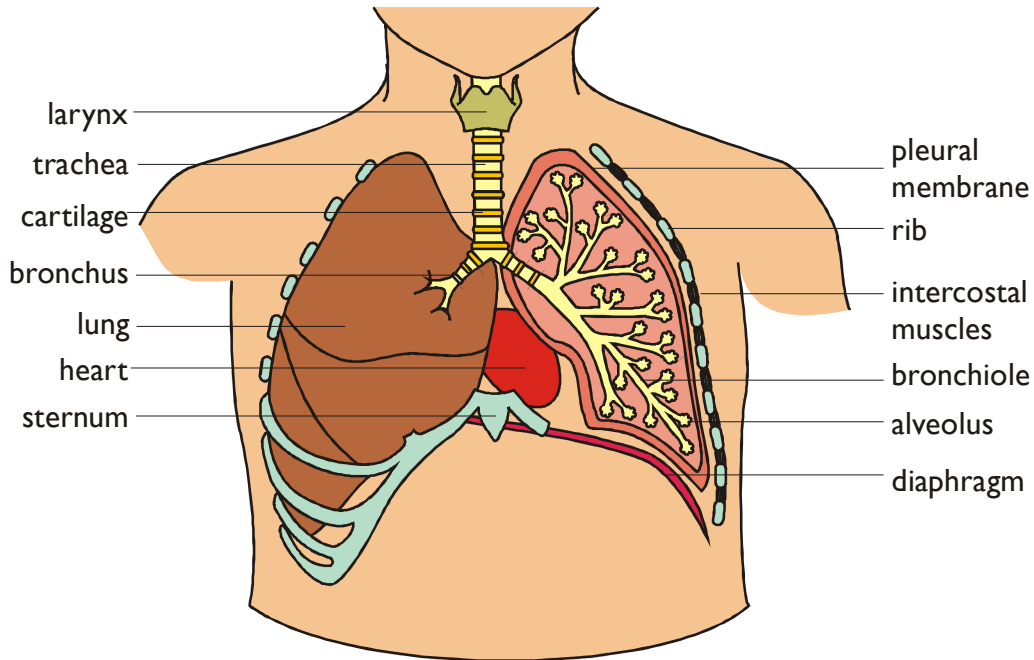
The gas exchange system is also referred to as the respiratory system, but this can be confusing as respiration takes place in all cells, and is quite distinct from gas exchange. The actual gas exchange surface is on the alveoli inside the lungs.
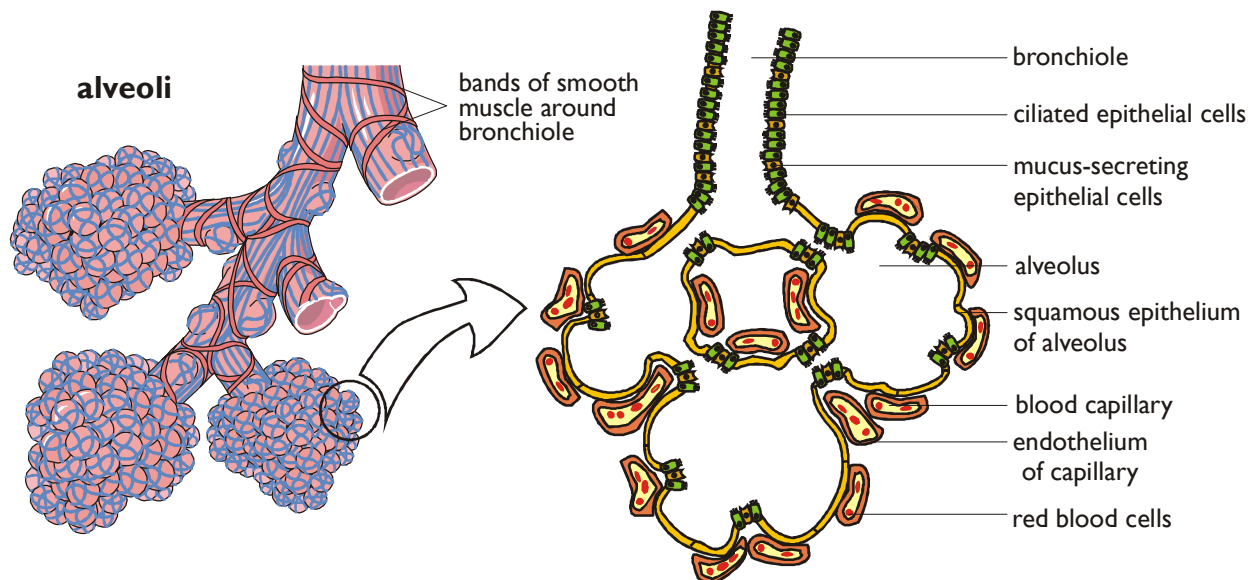
This surface meets the three requirements of Fick's law:

- A large surface area. Although each alveolus is tiny, an average adult has about 600 million alveoli, giving a total surface area of about 100m², so the area is huge.

- A small distance between the source and the destination. The walls of the alveoli are composed of a single layer of flattened underline epithelial cells, as are the walls of the capillaries, so gases need to diffuse through just two thin cells.

- A mechanism to maintain a high concentration gradient across the gas exchange surface. The steep concentration gradient across the gas exchange surface is maintained in two ways: by blood flow on one side and ventilation 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

TEM of Human Lungs

Alveolar air space

Alveolar squamous epithelium

capillary endothelium

Blood capillary

red blood cell
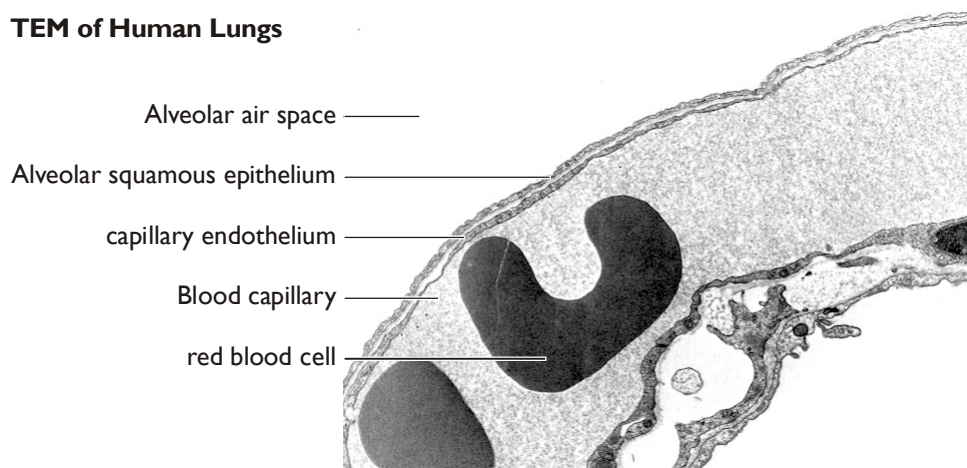
The large surface area and short distance that are ideal for gas exchange also cause a problem: water loss. Water inevitably diffuses down its concentration gradient from the tissue fluid and alveoli cells into the air in the alveoli, so the air in the alveoli is constantly moist. This is why exhaled air contains more water than normal, inhaled air, and this represents a significant loss of water from the body. However, by having the gas exchange surface deep inside the body at the end of long narrow bronchioles, the water loss is minimised. The moist alveolar air means that there is less of a diffusion gradient (and so less water is lost) than if the alveoli were exposed to outside dry air. The epithelial cells secrete a soapy surfactant to reduce the surface tension of the water (due to hydrogen bonds) and make it less "sticky". Without this surfactant the alveoli would collapse, and this can be a problem in premature babies.

Some of the epithelial cells of the bronchioles secrete mucus, which traps bacteria and other microscopic particles that enter the lungs. This mucus is constantly swept upwards by the cilia of the ciliated epithelial cells to the throat, where it is swallowed and any bacteria in it are killed by the acid in the stomach. Phagocyte cells migrate from the blood capillaries to the alveolar air space to kill any bacteria that have not been trapped by the mucus.

Ventilation

Ventilation means the movement of air over the gas exchange surface (also known as breathing). 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. 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.

The muscle contractions change the volume of the thorax, which in turn changes the pressure in the lungs (by Boyle's law), which in turn causes air to move. Ventilation in humans is tidal, which means the air flows in and out by the same route. The rule is that *air always flows from a high pressure to a low pressure*. These volume and pressure changes are shown in this graph:

Inspiration	• The diaphragm contracts and flattens downwards and the external intercostal muscles contract, pulling the ribs up and out.
	• This increases the volume of the thorax and the lungs, and stretches the elastic-walled alveoli.
	• This decreases the pressure of air in the alveoli below atmospheric.
	• Air flows in from high pressure to low pressure.
Normal expiration	• The diaphragm relaxes and curves upwards and the external intercostal muscles relax, allowing the ribs to fall.
	• This decreases the volume of the thorax and the lungs, and allows the alveoli and bronchioles to shrink by elastic recoil.
	• This increases the pressure of air in the alveoli above atmospheric.
	• Air flows out from high pressure to low 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

Pulmonary Ventilation

<u>Pulmonary Ventilation</u> is the volume air ventilating the lungs each minute. It is calculated as the product of the ventilation rate and the tidal volume.

$$\text{pulmonary ventilation}\ =\ \text{ventilation rate}\ \times\ \text{tidal volume}$$

- The <u>ventilation rate</u> can be calculated from the pressure graph by measuring the time taken for one ventilation cycle and using the formula:

$$\text{ventilation rate (breaths per minute)} = \frac{60}{\text{cycle time (s)}}$$

- The <u>tidal volume</u> is the normal volume of air breathed in each breath (also called the breathing depth). It can be measured from the volume graph.

Both the ventilation rate and the tidal volume can be varied by the body. When the body exercises the pulmonary ventilation can increase so that

- oxygen can diffuse from the air to the blood faster
- carbon dioxide can diffuse from the blood to the air faster

These changes allow aerobic respiration in muscle cells to continue for longer.

	ventilation rate (breaths min⁻¹)	tidal volume (cm³ breath⁻¹)	pulmonary ventilation (cm³ min⁻¹)
at rest	12	500	6 000
at exercise	18	1000	18 000

Gas Exchange and Ventilation

It important to be clear about the meaning of the terms gas exchange and ventilation. Gas exchange is when certain gases (usually oxygen and carbon dioxide) are moved between the environment and the blood. Ventilation is a muscular movement that helps to speed up gas exchange. Ventilation increases the rate of gas exchange by increasing the concentration difference across the respiratory surface, which increases the rate of diffusion by Fick's law (p44). This table summarises the differences:

Gas Exchange	Ventilation
uses diffusion	uses mass flow
passive (no energy needed)	active (thorax muscles use ATP energy)
gases move down their own concentration gradients (so can be in different directions)	all gases in air move together in one direction
slow	quick

Lung Diseases

The features of the lungs that make them so good at gas exchange also makes them susceptible to disease. The large volumes of air passing through the lungs may carry infectious pathogens or other microscopic particles that cause disease. We shall look at five diseases of the lungs: asthma, tuberculosis, emphysema, lung cancer and fibrosis.

1. Asthma

Asthma is an <u>allergic response</u> that causes difficulty breathing, wheezing, tight chest and coughing. It is thought to affect 10% of the world's population and is responsible for 2000 deaths per year in the UK.

Course of Disease

1. Asthma is caused by physical factors called <u>allergens</u> in the environment. These allergens include pollen, dust mites faeces and fur.

2. These allergens trigger an <u>inflammatory response</u> by the immune system (see p73). White blood cells called mast cells release <u>histamines</u> (see unit 5), which cause the smooth circular muscles of the bronchioles to contract, narrowing the airways – <u>bronchoconstriction</u>.

3. The epithelial cells also secrete more mucus, which further blocks the airways.

4. The constricted bronchioles stimulate wheezing and coughing as the lungs try to loosen the mucus.

5. The constrictions reduce the tidal volume, so alveolar air is only replaced slowly. The oxygen concentration gradient across the alveolar epithelium is reduced, so the rate of diffusion in the alveoli is reduced by Fick's law. Less oxygen diffuses into the blood, so less oxygen is available for cellular respiration throughout the body.
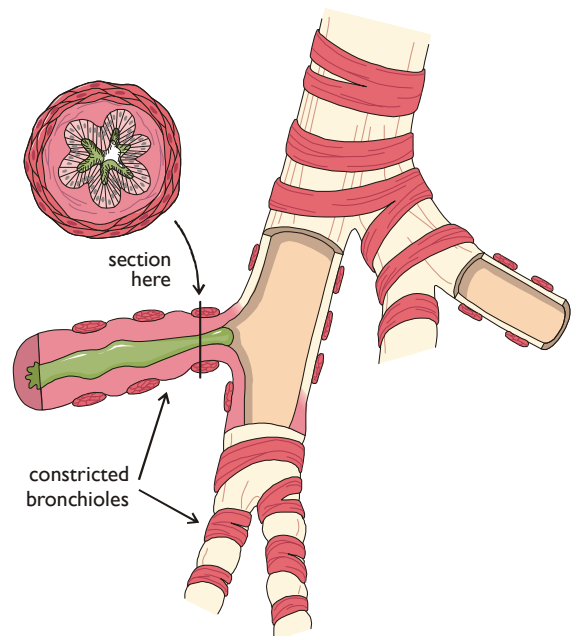
Risk Factors

The risk factor for asthma is exposure to allergens. As well as pollen, faeces of dust mites and animal fur, other factors that can contribute to asthma include polluting gases like sulphur dioxide, exercise, cold weather, infection and stress. Asthma can be treated by inhaling drugs that relax the smooth muscles and by anti-inflammatory drugs.

2. Pulmonary Tuberculosis

Pulmonary Tuberculosis (or TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. In 19th-century England one in five died of TB, and although the disease has been almost eradicated in the developed world, it is still a major killer in the developing nations, responsible for 1.5 million deaths in 2006. The symptoms are a persistent cough with chest pains, tiredness, a loss of appetite and weight loss, and in serious cases coughing up blood, wasting away and death.

Course of Disease

1. TB is transmitted by aerosol droplets from coughs and sneezes of infected persons. Infection is most likely to result from prolonged exposure.

2. The bacterial cells are breathed in and invade the epithelial cells of the alveoli and bronchioles. Here they multiply to form lumps called tubercles, in which the bacteria remain alive but dormant.

chest x-ray

scar tissue

3. The tubercles stimulate an inflammatory response by the white blood cells of the immune system, resulting in the formation of fibrous scar tissue. This scar tissue reduces the elasticity of the alveoli and thickens their walls, so reducing the rate of oxygen diffusion.

4. After a delay of months to years the bacteria emerge from the tubercles and start reproducing inside the lung epithelial cells, killing them. The damaged alveoli have a smaller surface area, so further reducing the rate of gas exchange.

lung tissue

tubercle

damaged alveoli

5. The TB bacteria can also spread through the bloodstream to other organs, like the kidney, bone and nervous tissue, which are destroyed as well. This causes weakness as the body wastes away and the bacteria appear to "consume" the body – hence the old name for TB: consumption.
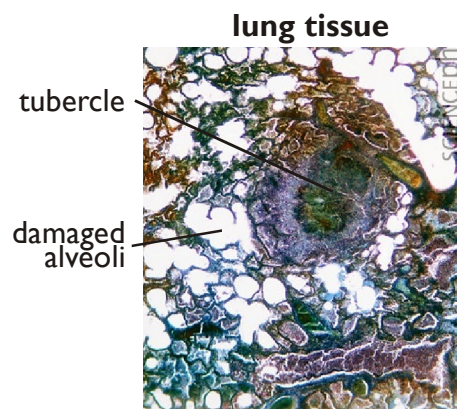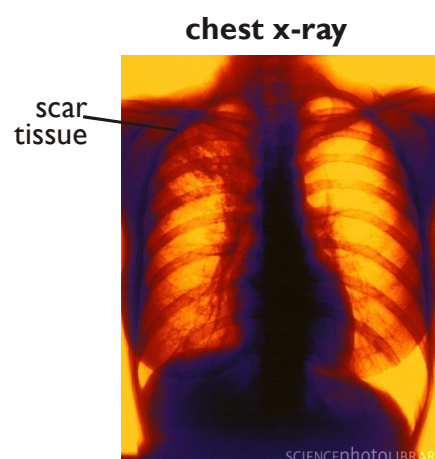
Risk Factors

The main risk factor for TB is overcrowding, such as in crowded slums or hospitals, as this allows TB to spread rapidly between hosts. Other factors include poor diet and AIDS, as these both impair the immune system. Since it is a bacterial disease, TB can be treated by antibiotics, and can also be prevented by the BCG vaccine. Unfortunately, the incidence of TB is currently rising due to resistance of the bacterium to the BCG vaccine and to the increase in AIDS.

3. Emphysema

Emphysema is a lung disease characterised by severe breathing difficulties resulting in an inability to do any exercise. It caused almost exclusively by smoking and 20% of all smokers suffer from emphysema; 10% of absence from work in the UK is due to emphysema and it kills 20 000 per year in the UK.

Course of Disease

1. The tar in cigarette smoke stimulates the white blood cells to release inflammatory protease enzymes in the lungs.

2. These protease enzymes digest the protein elastin, which forms the elastic tissue in the epithelial cells of the alveoli. The alveoli can no longer expand and recoil, reducing the tidal volume in ventilation. This reduces the oxygen diffusion gradient, so less oxygen diffuses into the blood.

3. In more severe cases the epithelial cells are completely destroyed, so alveoli merge to form large air sacs with a much smaller surface area and thicker walls. These all reduce the rate of oxygen diffusion, so less oxygen is available for cellular respiration and muscular activity is very difficult.
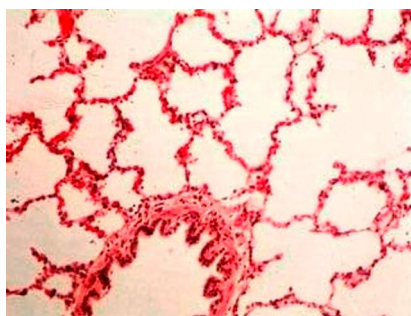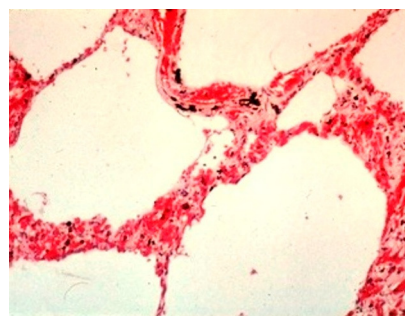
normal alveoli   alveoli with emphysema

Risk Factors

By far the most important risk factor for emphysema is smoking, and 20% of all smokers suffer from emphysema. 10% of absence from work in the UK is due to emphysema and it kills 20 000 per year in the UK. Emphysema is incurable; though giving up smoking prevents the symptoms getting any worse. Breathing pure oxygen compensates for the poor efficiency of gas exchange, so allowing more respiration.

4. Lung Cancer

Lung cancer is the growth of excess tissue in the lungs due to uncontrolled cell division of the epithelial cells. Mutagenic agents in the environment cause epithelial cells to mutate and start to divide continuously and uncontrollably, forming a tumour. As the tumour grows it can constrict the bronchioles and alveoli, so slowing the rate of gas exchange. Lung cancers often spread to other parts of the body and are a major cause of death in the developed world.

Risk Factors

The risk factor for lung cancer is exposure to the mutagenic agents. These agents include tobacco smoke, asbestos and radon gas, which is present in the air of some locations.

5. Pulmonary Fibrosis

Pulmonary fibrosis is a severe shortness of breath caused by inhalation of fine dust particles or chemicals.

Course of Disease

1. The particles stimulate an inflammatory response in the lungs, which results in the growth of fibrous scar tissue around the alveoli.

2. This scar tissue thickens the alveolar walls so that there is a longer diffusion pathway and a smaller surface area for oxygen diffusion.

3. The scar tissue also reduces the elasticity of the alveoli so normal passive exhalation is reduced. This means there is a smaller oxygen diffusion gradient, so less oxygen reaches the blood.

Risk Factors

There are hundreds of different causes of pulmonary fibrosis, and since these are usually found in work-place environments, pulmonary fibrosis is known as an <u>occupational disease</u>. Some of the main causes are shown in this table:

Disease	Cause	Risk occupation
pneumoconiosis	coal dust	coal mining
asbestosis	asbestos	demolition workers
silicosis	silica dust	quarrying, mining
berylliosis	beryllium	electronics, nuclear power industries
farmer's lung	mould spores in hay	farming
bird fancier's lung	proteins in bird faeces	bird breeders, poultry farmers

Lung Diseases Summary

Disease	Cause	Symptoms	How Gas Exchange is Slowed	Risk Factors
asthma	allergens	temporary breathing difficulties; wheezing	bronchoconstriction reduces tidal volume	pollen, dust, SO_2, cold air.
TB	bacterial infection	chest pain, coughing blood, fever, death	inflammation and scar tissue make fewer, thicker alveoli and reduce elasticity.	overcrowding, poor diet, AIDS
emphysema	smoking	permanent breathing difficulty	reduced elasticity prevents exhalation.	smoking
lung cancer	smoking	breathing difficulty, weight loss	constrictions reduce tidal volume.	smoking, asbestos, radon gas.
pulmonary fibrosis	dust	breathing difficulty	inflammation and scar tissue make fewer, thicker alveoli and reduce elasticity.	coal dust, silica dust, mould spores.

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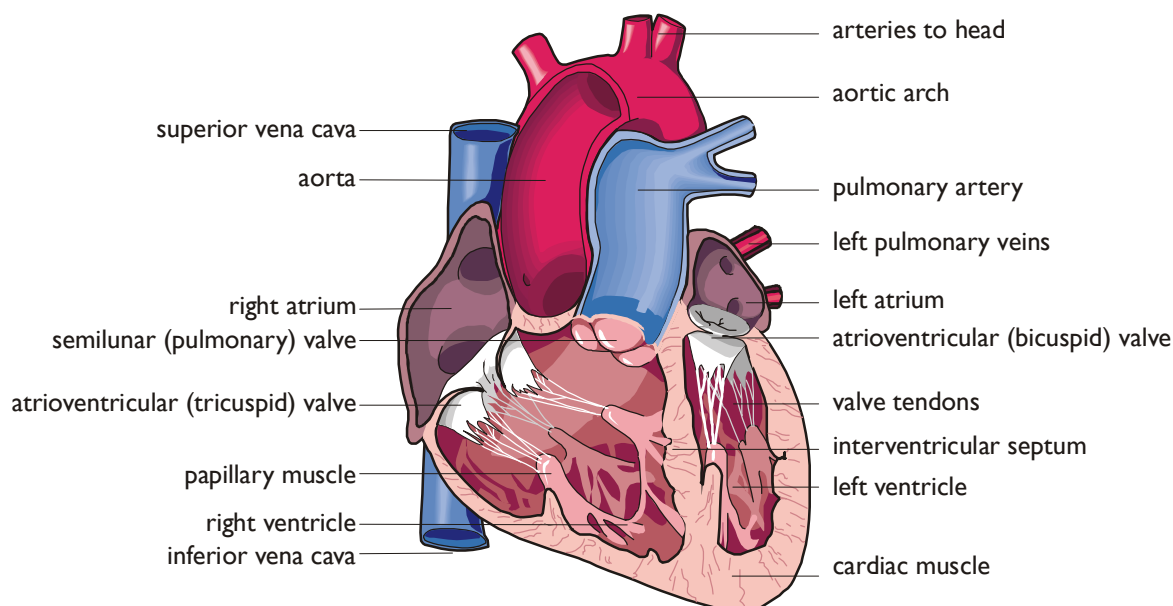
The human heart has four chambers: two thin-walled <u>atria</u> on top, which receive blood, and two thick-walled <u>ventricles</u> underneath, which pump blood. <u>Veins</u> carry blood into the atria and <u>arteries</u> carry blood away from the ventricles. Between the atria and the ventricles are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

The left and right halves of the heart are separated by the <u>inter-ventricular septum</u>. 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 internal volume of the left and right ventricles is the same.

The heart is made of <u>cardiac muscle</u>, composed of cells called <u>myocytes</u>. 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 <u>coronary arteries</u>. These arise from the aorta as it leaves the heart. Blood returns via the <u>coronary sinus</u>, which drains directly into the right atrium.

The Cardiac Cycle

Cardiac muscle contracts about 75 times per minute, pumping around 75 cm³ of blood from each ventricle each beat (the stroke volume). It does this continuously for up to 100 years.

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.

sino-atrial node (SAN)
atrio-ventricular node (AVN)
Bundle of His
Purkinje fibres
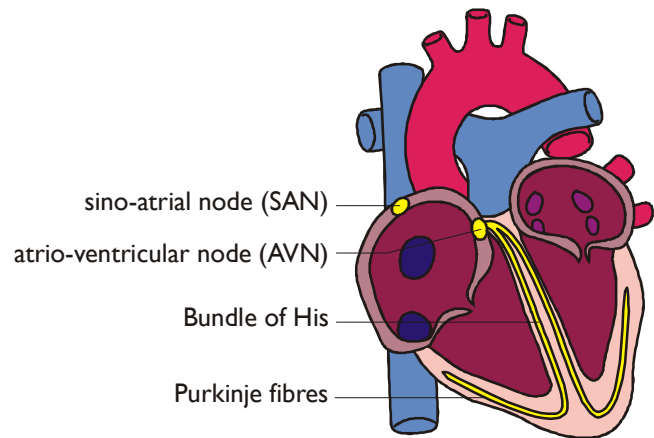
There is a complicated sequence of events at each heartbeat called the cardiac cycle. The cardiac cycle has three stages:

1. **Atrial Systole**. 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. The blood can't flow back into the veins because of the valves in the veins.

2. **Ventricular Systole**. The electrical impulse passes from the atrioventricular node (AVN) to the Purkinje (or Purkyne) fibres, with a short but important delay of about 0.1s. The Purkinje fibres pass down through the interventricular septum as the bundle of His, which is insulated from the surrounding muscle cells, so the ventricles do not contract yet. At the base of the ventricles the Purkinje fibres spread out and initiate ventricular contraction. 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 "lub" sound.

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 chart below. 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 are opened by blood flowing through them the right way and are forced closed when blood tries to flow through them the wrong way. Whenever lines cross in the pressure graph it means that a valve opens or closes.
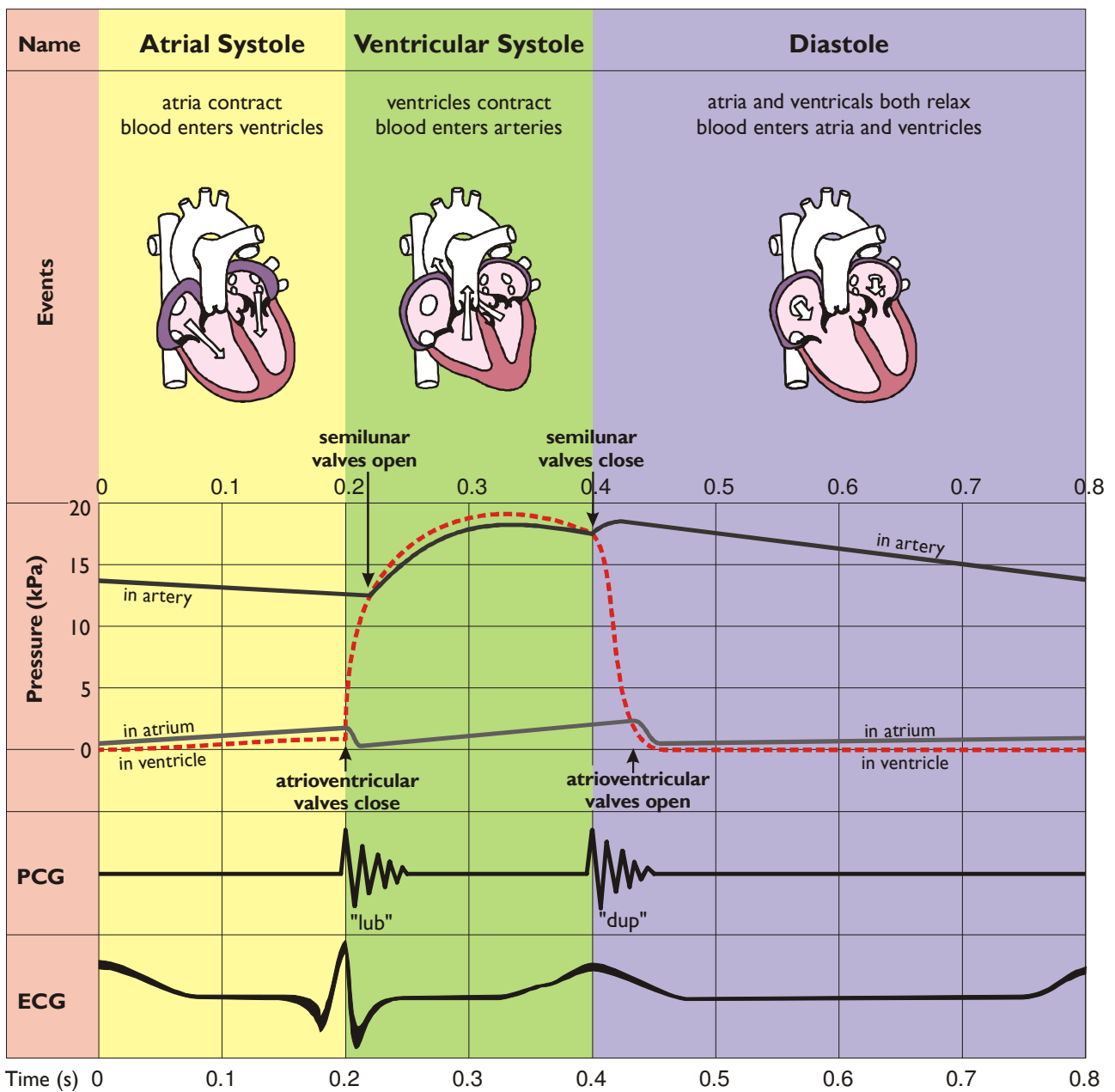
This diagram just shows one side of the heart. The two sides have identical traces except that the pressures in the right side are lower than those in the left side.

- 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 due to the atrioventricular valves closing and the second (dup) is due to 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.

Cardiac Output

Cardiac Output is the amount of blood flowing through the heart each minute. It is calculated as the product of the heart rate and the stroke volume:

$$\text{cardiac output} \; = \; \text{heart rate} \; \times \; \text{stroke volume}$$

- The heart rate can be calculated from the pressure graph by measuring the time taken for one cardiac cycle and using the formula:

$$\text{heart rate (beats per minute)} = \frac{60}{\text{cycle time (s)}}$$

- The stroke volume is the volume of blood pumped in each beat.

Both the heart rate and the stroke volume can be varied by the body. When the body exercises the cardiac output can increase dramatically so that
- oxygen and glucose can get to the muscles faster
- carbon dioxide and lactate can be carried away from the muscles faster
- heat can be carried away from the muscles faster

	heart rate (beats min^{-1})	stroke volume (cm^3 beat^{-1})	cardiac output (cm^3 min^{-1})
at rest	75	75	5 600
at exercise	180	120	22 000

Coronary Heart Disease

Coronary heart disease (CHD) is caused by a blockage in the coronary blood system. This is the system that feeds the cardiac muscle cells so that they can respire and contract. Cardiac muscle works constantly throughout life and is incapable of anaerobic respiration, so it has a great demand for oxygen and glucose. There are two coronary arteries that arise directly from the aorta, and these split into numerous smaller arteries (arterioles) and then a network of capillaries, where exchange with the cardiac cells actually takes place.   A blockage in a coronary artery can restrict the supply of oxygen to the cardiac cells, killing them and causing a heart attack. The steps are as follows:
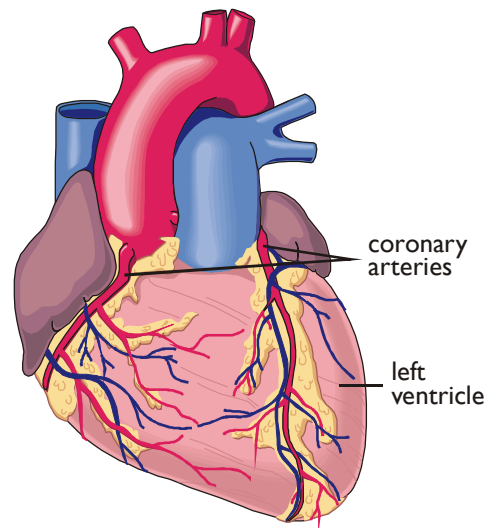
1. Cholesterol and other insoluble lipids collect on the inside of a coronary artery. This deposit is called an atheroma, and it narrows the lumen of the artery, restricting blood flow – atherosclerosis.

2. The atheroma can collect minerals and become hardened to form a rough plaque.

3. The plaque weakens the wall of the artery, so the pressure of blood causes a local swelling called an aneurism. If the wall is particularly weak the aneurism may burst causing blood loss and probable death.

4. The plaque can also encourage the formation of a blood clot called a thrombus. Alternatively, a mobile clot from elsewhere in the blood stream (an embolism) can become lodged in the atheroma. The clot grows until it completely blocks the artery, forming a coronary thrombosis.

5. The thrombosis prevents oxygen reaching the cardiac cells "downstream" of the blockage, so they cannot respire and so die. This death of myocytes is a myocardial infarction, more commonly known as a heart attack. The severity of the heart attack depends on how far along the coronary artery the thrombosis is. If only a small part of one ventricle is killed then the patient will recover, but a thrombosis early in the coronary artery will always be fatal.
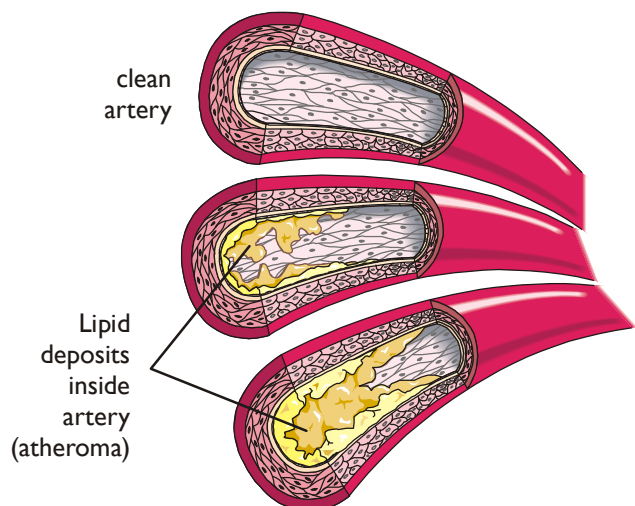
The five stages in a heart attack are summarised in this diagram

①
Atheroma
(fat deposits)

↓

②
Plaque
(hardened atheroma)

③
Aneurism
(swelling due to weak wall)

④
Coronary Thrombosis
(blockage due to blood clot)

⑤
Myocardial Infarction
(death of cardiac cells)
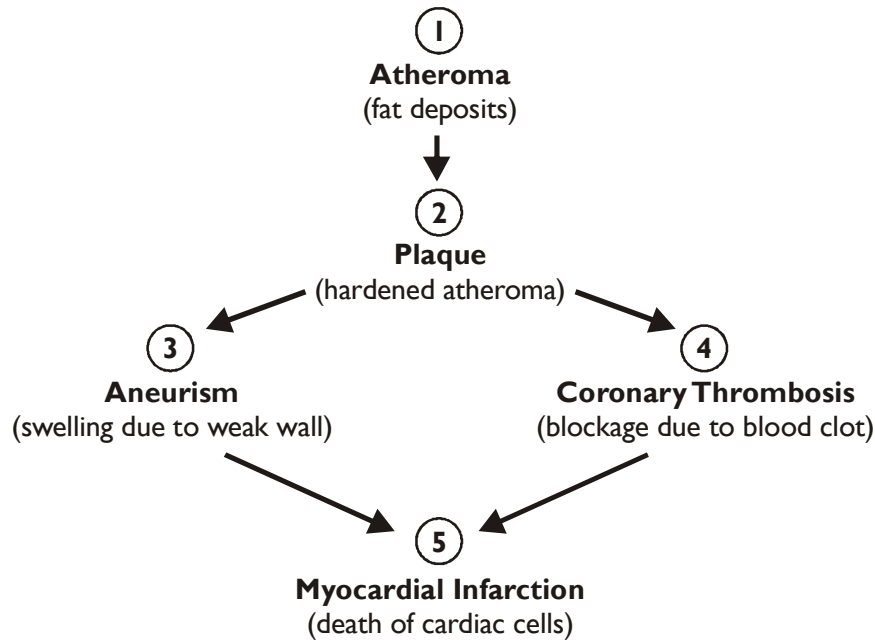
Risk Factors for Coronary Heart Disease

There are a number of risk factors that are associated with coronary heart disease. The more of the factors that apply, the greater the risk of a heart attack. Some of the main factors are:

- **Blood Cholesterol**. Cholesterol in the blood comes from the diet and from the liver, where it is synthesised. Cholesterol is carried in large complexes with proteins, called <u>lipoproteins</u>. <u>High-density lipoproteins</u> (HDLs) remove cholesterol from tissues, so decrease the risk of atheromas, while <u>Low-density lipoproteins</u> (LDLs) deliver cholesterol to tissues, so increase the risk of atheromas.

- **Blood Pressure**. High blood pressure increases the risk of an aneurism and stimulates thickening of artery wall, increasing the risk of thrombosis. Stress, diet and lack of exercise can all increase blood pressure.

- **Genetics**. Both blood pressure and fat metabolism are affected by genes, so genes undoubtedly affect the chance of a coronary thrombosis. This doesn't mean that, for some people, a heart attack is inevitable; it just means some people have to be even more careful about their lifestyle risk factors.

- **Diet**. High levels of saturated fat increase the amount of cholesterol carried in the blood and so increase the risk of atherosclerosis. High levels of salt increase blood pressure and so increase the risk of aneurism. However, dietary fibre and vitamin C reduce the risk of heart disease.

- **Smoking**. Smokers are between two and six times more likely to suffer from coronary heart disease than non-smokers. The carbon monoxide and nicotine in cigarette smoke both cause an increase in blood pressure.

The Digestive System

Humans, like all animals, use <u>holozoic nutrition</u>, which consists of these five 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. (Do not confuse <u>egestion</u>, which is the elimination of material from a body cavity, with <u>excretion</u>, which is the elimination of waste material produced from within the body's cells.)

The Alimentary Canal

The human digestive system comprises a long tube, the <u>alimentary canal</u> or <u>digestive tract</u> (or simply <u>gut</u>) which extends from the mouth to the anus, together with a number of associated <u>glands</u>: the salivary glands, the pancreas and the liver.
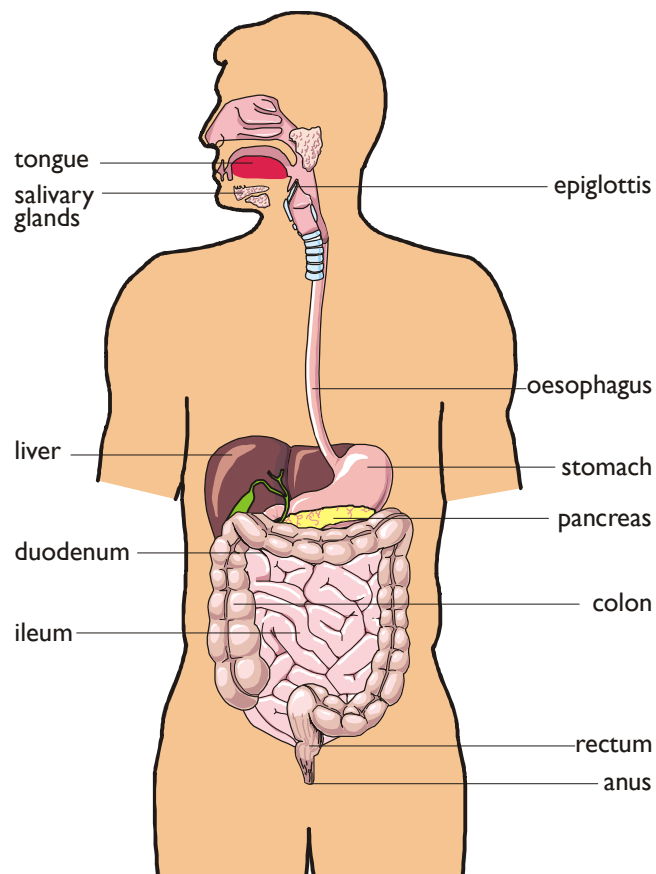
The digestive system is made up of different tissues doing different jobs. The lining wall of the alimentary canal appears different in different parts of the gut, reflecting their different roles, but always has these four basic layers:
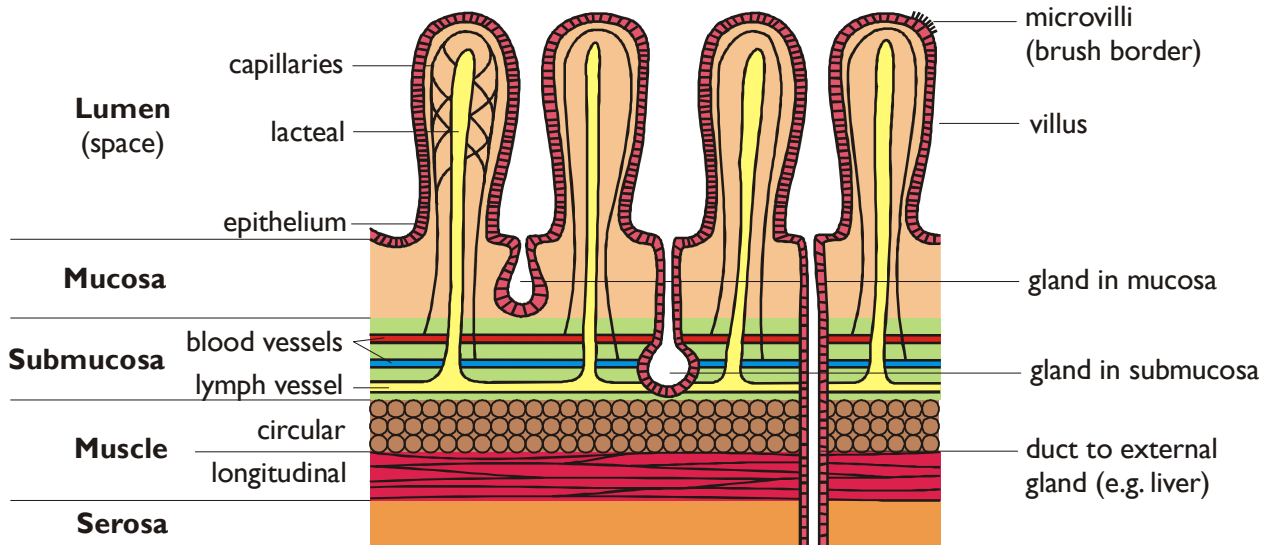
- The <u>mucosa</u>, which secretes digestive juices and absorbs digested food. It is often folded to increase its surface area. There is a layer of <u>columnar epithelial</u> cells lining the mucosa. These epithelial cells contain microvilli, membrane proteins for facilitated diffusion and active transport, mitochondria, and membrane-bound enzymes. Epithelial cells are constantly worn away by friction with food moving through the gut, so are constantly being replaced.

- The <u>submucosa</u>, which contains blood vessels, lymph vessels and nerves to control the muscles. It may also contain secretory glands.

- The <u>muscle layer</u>, which is made of smooth muscle, under involuntary control. It can be subdivided into <u>circular muscle</u> (which squeezes the gut when it contracts) and <u>longitudinal muscle</u> (which shortens the gut when it contracts). These two muscles therefore have opposite effects and so are <u>antagonistic</u>. The combination of these two muscles allows food to be pushed along the gut by peristalsis.

- The <u>serosa</u>, which is a thin, tough layer of connective tissue that holds the gut together, and attaches it to the abdomen.

Parts of the Alimentary Canal

1. **Mouth (Buccal cavity)**. The teeth and tongue physically break up the food into small pieces with a larger surface area, and form it into a ball or <u>bolus</u>. The <u>salivary glands</u> secrete <u>saliva</u>, which contains water to dissolve soluble substances, mucus for lubrication, lysozymes to kill bacteria and <u>salivary amylase</u> 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 here. There is a epithelium, no villi, a few glands secreting mucus, and a thick layer of circular and longitudinal muscle to propel the food by peristalsis. Peristalsis is a wave of circular muscle contraction, which passes down the gut 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.
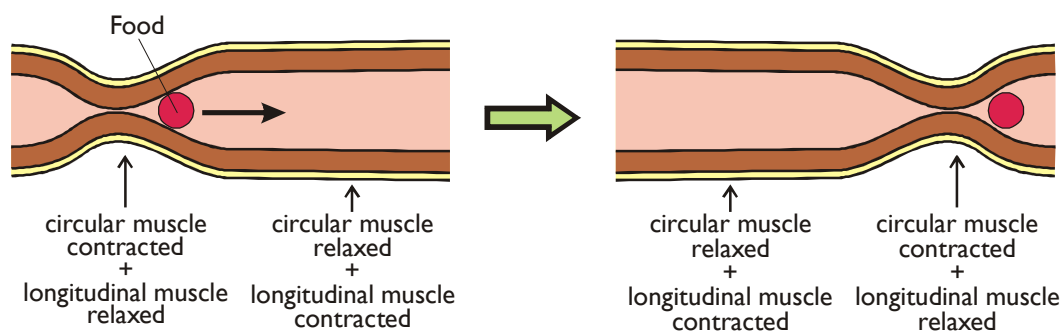
3. **Stomach**. This is an expandable bag where the food is stored for up to a few hours. There are three layers of muscle to churn the food into a liquid called <u>chyme</u>. This chime is gradually released in to the small intestine by a <u>sphincter</u>, a region of thick circular muscle that acts as a valve. The mucosa of the stomach wall has no villi, but does have numerous <u>gastric pits</u> (10^4 cm^{-2}) leading to <u>gastric glands</u> in the mucosa layer. These glands secrete <u>gastric juice</u>, 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 some protease enzymes. No other digestion takes place in the stomach.

4. **Small Intestine**. The first 30cm of the small intestine is called the <u>duodenum</u>. Although this is short, almost all the digestion takes place here, due to two secretions: <u>pancreatic juice</u> and <u>bile</u>. Pancreatic juice is secreted by the pancreas into the duodenum through the <u>pancreatic duct</u>. Pancreatic juice contains numerous amylase, protease and lipase enzymes. Bile is secreted by the liver, stored in the gall bladder, and released into the duodenum through the bile duct. Bile doesn't contain any enzymes, but it does contain bile salts to aid lipid digestion, and the alkali sodium hydrogen carbonate to neutralise the

stomach acid. This alkali gives chyme in the duodenum a pH of around 7.5, so the pancreatic enzymes can work at their optimum pH. 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 rest of the small intestine is called the <u>Ileum</u>. This is the site of final digestion and absorption. There are numerous glands in the mucosa and submucosa secreting enzymes, mucus and sodium hydrogen carbonate. To maximise the rate of absorption the ileum has the three features dictated by Fick's law:

- The ileum has a huge surface area. It is over 6m long; the mucosa has large circular folds, <u>villi</u> and the epithelial cells have <u>microvilli</u>. Don't confuse these two: villi are large structures composed of hundreds of cells that can easily be seen with a light microscope, while microvilli are small sub-cellular structures formed by the folding of the plasma membrane of individual epithelial cells. Microvilli can only be seen clearly with an electron microscope, and appear as a fuzzy <u>brush border</u> under the light microscope. The total internal surface area of the ileum is over 2000m².

circular folds in mucosa   **villi**   **microvilli**
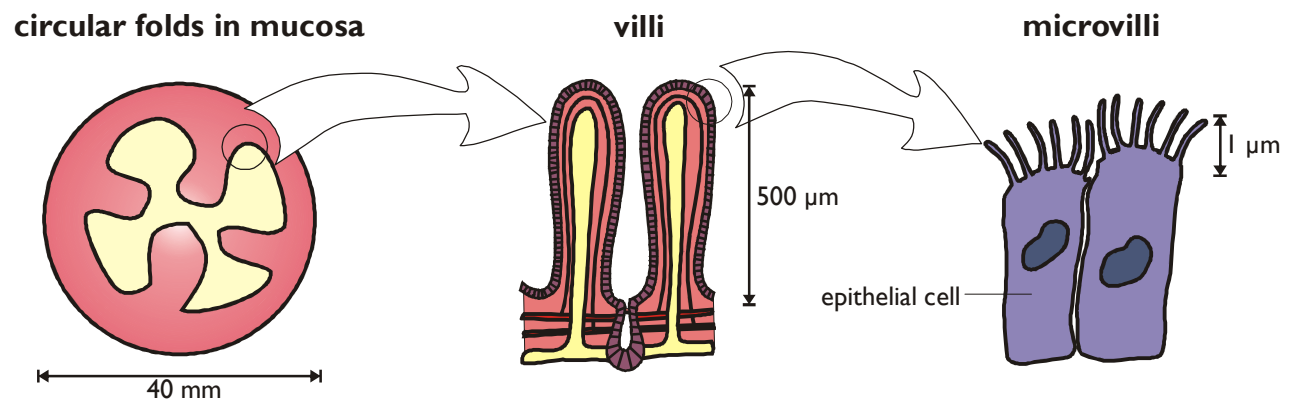
- There is a short diffusion distance. There is a network of blood capillaries in the submucosa of each villus, so between the lumen of the gut and the blood there is just one layer of epithelium lining the mucosa and one layer of endothelium lining the capillaries.
- A high concentration gradient is maintained by mixing the fluids on either side of the exchange surface. On the lumen side, the circular and longitudinal muscles propel the chyme by peristalsis, and mix the contents by <u>pendular movements</u> (bi-directional peristalsis). The microvilli can also wave to stir the contents near the epithelial cells. On the blood side, the blood flow ensures there is always a low concentration of nutrients.

5. **Large Intestine**. The large intestine comprises the caecum, appendix, colon and rectum. Food can spend 36 hours in the large intestine, 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 plant fibre (cellulose mainly), cholesterol, bile, mucus, mucosa cells (250 g 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).

Digestion of carbohydrates

By far the most abundant carbohydrate in the human diet is starch (in bread, potatoes, cereal, rice, pasta, biscuits, cake, etc). Starch is digested to glucose in two stages:

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

1. **Salivary amylase** starts the digestion of starch in the mouth. Very little digestion actually takes place, since amylase is quickly denatured in the stomach, but is does help to clean the mouth of starch and reduce bacterial infection.

2. **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.

3. **Disaccharidases** in the membrane of the ileum 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:

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

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

$$\text{lactose} \xrightarrow{\text{lactase}} \text{glucose} + \text{galactose}$$
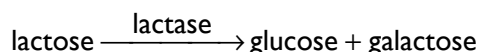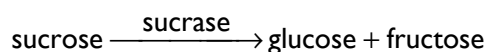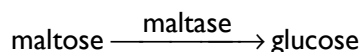
The disaccharidase enzymes are unusual in that they are located in the membrane of the ileum epithelial cells (see step "D" in the diagram below). This means the glucose is produced at the cells where it needs to be absorbed into the body.

Absorption of Glucose

Glucose and the other monosaccharides are absorbed from the gut by a special kind of active transport called <u>coupled active transport</u>. In coupled active transport the monosaccharides are transported by a facilitated diffusion protein, which is coupled to an active transport protein.
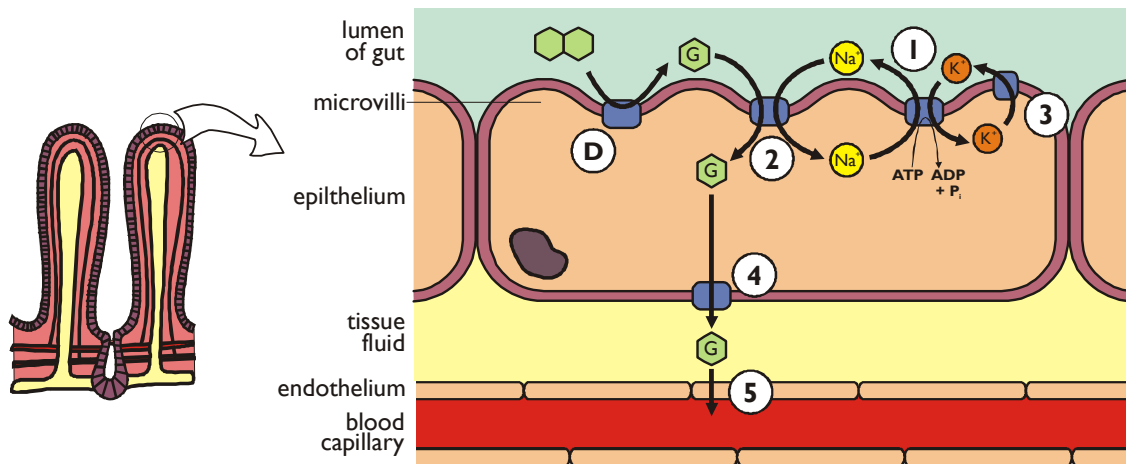
1. The active transport protein is the <u>sodium-potassium ATPase</u> (see p41), which is present in all animal cell membranes. This protein continuously pumps sodium ions out of the epithelial cell and potassium ions into the cell, using the energy from the hydrolysis of ATP to do so. Because this is active transport, the ions are pumped up their concentration gradients, so there is a large build-up of sodium ions in the lumen of the gut.

2. The facilitated diffusion protein is the <u>sodium-glucose co-transporter</u> protein, which is only found in the membrane of the epithelial cells of the ileum. This carrier protein has two binding sites: one for glucose and one for sodium ions, and both molecules must be carried together. Since this process is diffusion, the molecules are only carried down their concentration gradients. But the very large concentration gradient of sodium ions across the epithelial cell membrane drives the sodium-glucose co-transporter in one direction only – carrying both molecules <u>into</u> the cell. So while the sodium ions are diffusing <u>down</u> their concentration gradient, the glucose molecules can be carried <u>up</u> their concentration gradient.

3. The sodium ions are pumped out again by the Na/K ATPase to restore the sodium gradient. The potassium ions diffuse out through a potassium ion channel, doing no work in the process. So both ions constantly cycle in and out of the epithelial cell.

4. There is now a high concentration of glucose inside the epithelial cell. The glucose diffuses through the epithelial cell and diffuses into the tissue fluid of the villus by facilitated diffusion, through a glucose carrier protein that is only found on the inner surface of the epithelial cell.

5. The glucose enters the blood capillary by diffusing through gaps between the capillary endothelial cells. The glucose is carried in the blood to every cell in the body, where it is used for respiration.

The combination of steps 1 and 2 is the transport of glucose up its concentration gradient into the epithelial cell, together with the hydrolysis of ATP. So in effect the energy released by splitting ATP is used to pump glucose into the cell, though indirectly. This is coupled active transport.

Lactose Intolerance

Lactose is the sugar found in mammalian milk, and, as we've seen, lactose is digested by the disaccharidase enzyme <u>lactase</u> (in the membrane of the ileum epithelial cells) to glucose and galactose:

$$\text{lactose} \xrightarrow{\text{lactase}} \text{glucose} + \text{galactose}$$
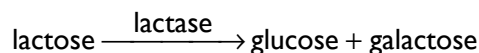
Some people are <u>lactose intolerant</u>, which means they feel ill if they eat foods containing milk. The symptoms include flatulence and explosive diarrhoea. This happens because they don't have the lactase enzyme, so they can't digest lactose. Since lactose can't be absorbed it remains in the gut, passing on to the large intestine, where there wouldn't normally be any sugars. This is where the problems are caused:

- The presence of lactose lowers the water potential of the colon lumen, so water cannot be absorbed from the faeces by osmosis, and in fact water often diffuses out of the colon mucosa cells in to the lumen down the water potential gradient. All this excess water in the gut lumen causes diarrhoea

- The colon is home to large numbers of bacteria (the <u>commensal flora</u>), who can respire the sudden bounty of lactose and increase in number. Their fermentation produces acids and gases like methane and carbon dioxide, which cause flatulence.

In fact most adult humans (like all other adult mammals) are lactose intolerant, and this is the "normal" state. Lactose is only found in milk, which is produced by the mammary glands of female mammals to feed their young. Suckling juvenile mammals all synthesise lactase in order to digest the lactose in milk, but when they are weaned (eat solid food) they stop drinking milk and the gene for lactase production is switched off. Humans are unique in that some continue to drink animal milk even as adults, at least in some human societies. These humans generally have a mutation that causes lactase to be produced throughout life, so these people are <u>lactose tolerant</u> and can drink milk without any ill effects. Societies that adopted a pastoral lifestyle (farming animals), such as most Europeans, northern Indians and some Africans, are generally lactose tolerant today. The rest (most Asians, Africans, Native Americans and Australians) remain lactose intolerant as adults.

Cholera

Cholera is an infectious disease caused by the bacterium *Vibrio cholerae*. *V. cholerae* is a typical prokaryotic cell with a slightly curved rod shape and a single flagellum (right). The symptoms of cholera include stomach cramps, vomiting, fever and severe diarrhoea. In severe cases up to 20 litres of water can be lost per day, and if untreated, leads to death in 75% of cholera patients. There were several serious outbreaks of cholera in the UK in the 19th century and cholera remains a major killer of small children in developing countries (several million deaths each year). However cholera can be treated simply and cheaply.

How *V.cholerae* causes Diarrhoea

1. The cholera bacterium adheres to the epithelium and secretes the cholera toxin CT. CT enters the epithelial cells and activates a chloride ion channel in the cell membrane.

2. This causes chloride ions to diffuse out of the cells into the lumen.

3. This lowers the water potential in the lumen of the gut.

4. So water is lost from cells to the lumen by osmosis, producing diarrhoea and dehydration.
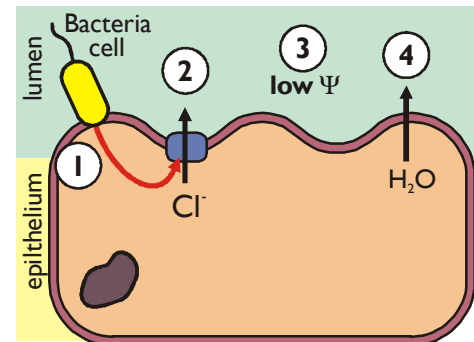
Treatment for Diarrhoea

The treatment for diarrhoea was revolutionised in the 1960s, with the development of oral rehydration therapy (ORT). This simple and cheap treatment consists of drinking an oral rehydration solution (ORS) of glucose and salt (NaCl), and sometimes other ions like potassium and bicarbonate. ORT makes use of the sodium-glucose co-transporter protein that normally absorbs glucose into the ileum epithelial cells.

1. If both Na+ and glucose are present in the lumen, they bind to the sodium-glucose co-transporter protein. Transport only works if both molecules are present, which is why salt alone is not an effective treatment. ORS contain equimolar concentrations of glucose and salt.

2. The transporter protein carries the Na+ and glucose into the cell, down their concentration gradients.

3. This lowers the water potential inside the epithelial cells. So water diffuses from the lumen into the epithelial cells by osmosis, rehydrating cells and reducing diarrhoea.
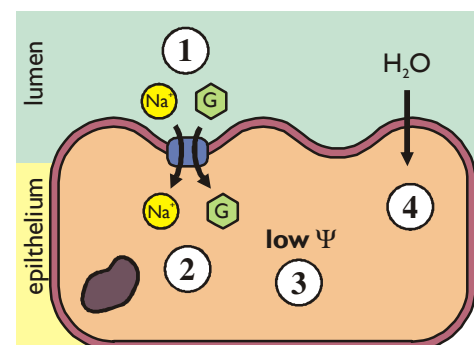
Disease

<u>Disease</u> is a general term meaning a disorder of the body. Diseases can be caused by many different factors:

- **Infectious Diseases** are caused by pathogenic organisms (usually microbes) living in or on the body and so causing harm (e.g. cold, TB, AIDS).

- **Dietary Deficiency Diseases** are caused by a lack of specific nutrients in the diet, e.g. kwashiorkor (protein), scurvy (vitamin C), rickets (vitamin D).

- **Environmental Diseases** are caused by non-living factors in the environment. They include skin cancer (caused by radiation), lung cancer (caused by smoking), asthma (caused by dust), pulmonary fibrosis (caused by dust or pollution), and Creutzfeldt-Jakob disease (caused by prions).

- **Social Diseases** are caused by human activities and lifestyle. They include alcoholism, emphysema, coronary heart disease, anorexia, drug addiction and even accidents.

- **Ageing Diseases** are caused by degeneration of body tissues and include arthritis, arteriosclerosis and cataracts.

- **Genetic Diseases** are caused by genes inherited from parents. These are really characteristics that are unusual in the population and are life-threatening (e.g. muscular dystrophy, cystic fibrosis, haemophilia). In fact all diseases are affected by genetics, but these "single gene disorders" are governed entirely by the action of a single allele and are not influenced by any other factor.

Infectious Disease

To most people "disease" means an infectious disease, and these are the diseases you can "catch". Infectious diseases are caused by a variety of pathogens, including viruses, bacteria, fungi and proctoctists. A few of the common pathogens are shown in this table:

	Disease	Pathogen
Viral Diseases	common cold	Rhinovirus
	influenza	Myovirus
	measles	Paramyxovirus
	mumps	paramyxovirus
	chickenpox	Varicella zoster virus
	AIDS	HIV
Bacterial Diseases	tuberculosis	*Mycobacterium tuberculosis*
	typhoid	*Salmonella typhi*
	cholera	*Vibrio cholerae*
	tetanus	*Clostridium tetani*
	whooping cough	*Bordetella pertussis*
	pneumonia	*Streptococcus pneumoniae*
Fungal Diseases	thrush	*Candida albicans*
	athletes foot	*Tinea pedis*
	ringworm	*Tinea capititis*
Protoctist Diseases	malaria	*Plasmodium vivax*
	amoebic dysentery	*Entamoeba histolytica*
	sleeping sickness	*Trypanosoma spp.*

For a pathogen to cause a disease these steps must take place:

1. The pathogen must be transmitted to the human host. Pathogens can be transmitted through drinking water, eating food, breathing aerosol droplets, animal bites, or direct contact.

2. The pathogen must gain entry inside the human body. The human body is protected by a tough layer of endodermis (skin), but pathogens can enter via cuts in the skin (e.g. malaria); or the thinner epithelium exchange interfaces, such as the digestive system (e.g. cholera) or lungs (e.g. tuberculosis).

3. The pathogen must evade the defences of the host. Humans have a range of defences, such as stomach acid, lysozyme enzymes and the immune system, and these defences are usually very effective at preventing disease. But it only takes a few pathogen cells resisting the defences to multiply and cause a disease.

4. The pathogen must harm the host. Pathogens harm their hosts in two ways.

   • First, by reproducing inside host cells, using up cellular resources and preventing the cell from carrying out its normal reactions. The microbes then usually burst out of the host cell, rupturing the cell membrane and killing the cell in the process.

   • Second, by producing toxins – chemicals that interfere with the body's reactions. These chemicals may inhibit enzymes, bind to receptors, bind to DNA causing mutations, interfere with synapses and so on.

Lifestyle and Disease

A person's lifestyle affects their chances of suffering from any of the diseases listed on the previous page, except the single gene disorders. Lifestyle factors can include diet, exercise, work environment, sexual habits, smoking, drinking and drug-taking. Some of these factors have obvious association with disease (like smoking), while others are less obvious (like occupation), but all the factors have an associated risk.

Different diseasse have specific <u>risk factors</u>, i.e. factors that specifically increase the risk of getting that disease. A few examples are:

Disease	Risk Factors
lung cancer	smoking and cleanliness of the environment.
skin cancer	exposure to sunlight and colour of skin.
coronary heart disease	diet, age, genetics and exercise.
diabetes	genetics, diet and exercise.
AIDs	sexual habits, drug habits and genetics.

Some of these risk factors are beyond our control, e.g. genes and age. But the others are lifestyle factors and so within our power to change.

Correlation and Causation

How do we know what risk factors are associated with a disease? By epidemiological studies. Epidemiology is the study of the incidence, distribution and associations of diseases with a view to identifying their causes and so effect their prevention.
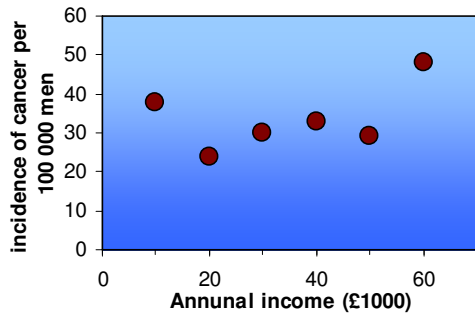
The first step is to look for a correlation (or association) between the incidence of the disease and some factor. This scatter chart plots the incidence of lung cancer in a sample of several thousand men against their annual income. There is clearly no pattern (and this can be confirmed with a statistical test), so income is not a risk factor.
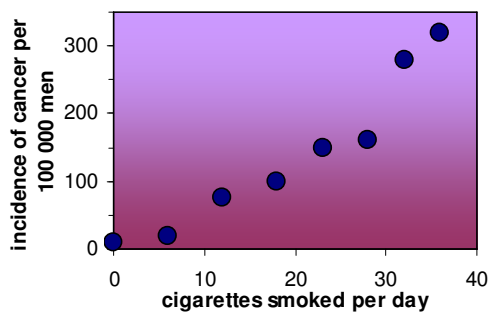
This scatter chart plots the incidence of lung cancer against number of cigarettes smoked. Here there is a correlation: as one variable goes up, so does the other. But this correlation is **not** evidence of causation (i.e. that smoking causes lung cancer). The correlation may be coincidence or it may be due to a third factor.
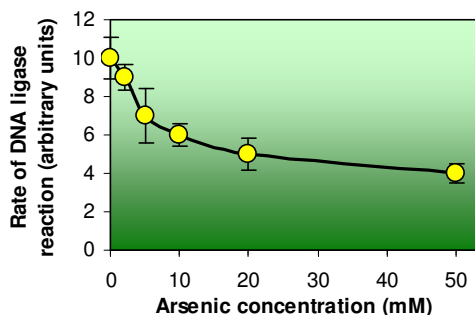
To demonstrate a causal relationship we need to carry out controlled laboratory experiments. This chart shows the effect of arsenic (a component of cigarette smoke) on DNA ligase (an enzyme that repairs DNA). The results show that arsenic inhibits DNA ligase, and in cells that would cause damage to DNA and cancer. So now we have a mechanism to explain the previous correlation, so we have evidence for a causal relationship, and we can say that smoking is a risk factor in lung cancer.
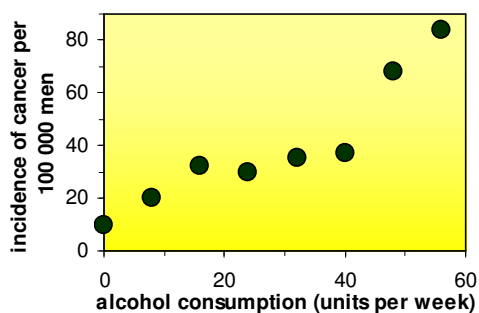
This scatter chart plots the incidence of lung cancer against alcohol consumption. There is a definite correlation, but laboratory studies have failed to show any causal link between alcohol and lung cancer – alcohol is not a risk factor. Instead, the correlation is indirect: heavy drinkers tend also to be heavy smokers and the smoking causes lung cancer.

The Immune System

The Immune System is the body's defence system against disease. It is made up of white blood cells (or leukocytes), which are found in the blood, lymph, tissue fluid and body cavities (such as alveoli). There are dozens of different kinds of leukocytes, which fall into four categories:

White Blood Cells
(**Leukocytes**)

| **Phagocytes**

for
phagocytosis | **Granulocytes**

for
inflamation | **T Lymphocytes**
for
cell-mediated
immunity | **B Lymphocytes**
for
antibody-mediated
immunity |

Non-Specific Immune System   **Specific Immune System**
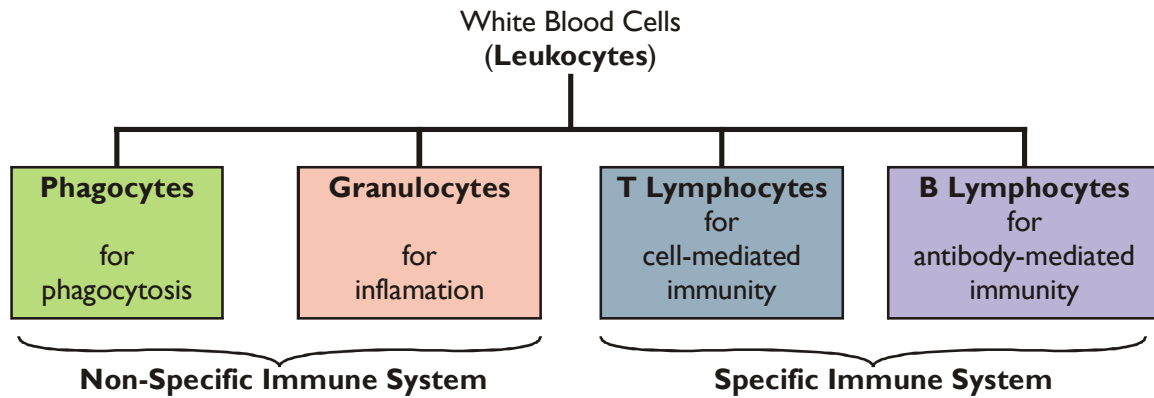
As this diagram shows there are two branches to the immune system: the non-specific immune system and the specific immune system.

The Non-Specific Immune System

The non-specific immune system is a collection of non-specific methods of destroying foreign bodies that have entered the body. The methods include phagocytosis and inflammation.

Phagocytosis

Phagocytosis is the digestion of microbes and other foreign bodies by phagocytes: large, irregularly-shaped white blood cells. Phagocytes have a complex cytoskeleton that allows them to move and change shape.

1. Phagocytes crawl through the blood and tissue fluid in response to chemicals released by microbes or other white blood cells.

2. When they reach the microbe they surround and engulf it through the process of phagocytosis. The microbe is now trapped in a membrane sac called a phagosome.

3. The phagosome then fuses with lysosomes - small vesicles containing lysozymes, which are released into the phagosome. These lysozymes are digestive enzymes (proteases, carbohydrases and lipases) that hydrolyse the proteins, carbohydrates and lipids that make up the microbe, so killing it.
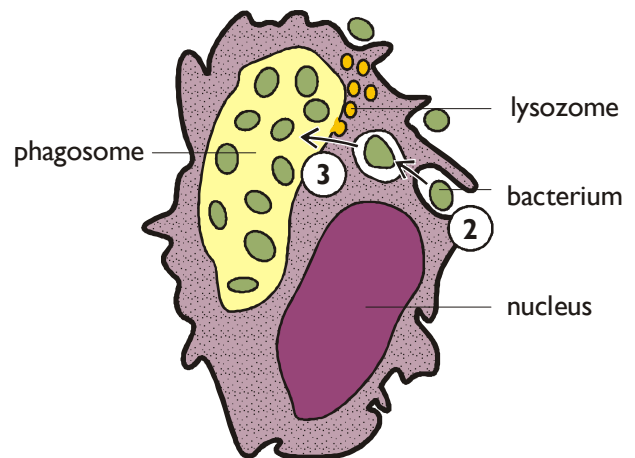
Different phagocyte cells work in different locations: neutrophils circulate in the blood, while macrophages are found in lymph, tissue fluid, lungs and other spaces, where they kill microbes before they enter the blood. Macrophages are also important as antigen-presenting cells (see p77).

Inflammation

Inflammation is a localised response to an injury or infection, driven by granulocyte cells. Granulocytes release chemicals, including histamines and prostaglandins (see unit 5), which stimulate:

- Vasodilation to increase the flow of blood to the area, so the area turns red.

- Capillary leakage so that phagocytes and granulocytes can enter the local tissue fluid. The area swells and the dead pathogens and phagocytes, together with excess tissue fluid, are released as pus.

- Sensory neurone impulses, so the area is tender or painful.

- Blood clotting to seal a wound, so a scab is formed.

On a slower timescale the inflammatory response also repairs the wound by depositing collagen and stimulating the growth of new cells, called scar tissue. This scar tissue is less specialised than the original tissue, so it leaves a visible scar or loss of function (for example see lung diseases).

The Specific Immune System

The specific immune system is a more complex and sophisticated collection of reactions that not only kills invading pathogens, but also leaves a "memory" of the pathogen so that it can be killed quickly on subsequent infections. While all animals have a non-specific immune system, only vertebrates have a specific immune system, so it must be a later evolutionary advance. The specific immune system involves the lymphocyte cells. There are two kinds of lymphocyte – B-lymphocytes (or just B-cells) and T-lymphocytes (or just T-cells). The key feature of the specific immune system is that it is capable of recognising foreign cells as distinct from its own cells, an ability called self/nonself recognition. It does this by making use of antigens.

Antigens

An antigen is a large molecule (protein, glycoprotein, lipoprotein or polysaccharide) on the outer surface of a cell. All living cells have these antigens as part of their cell membrane or cell wall. The capsid proteins of viruses and even individual protein molecules (such as toxins) can also be classed as antigens. Their purpose is for cell communication, and cells from different individuals have different antigens, while all the cells of the same individual have the same antigens. Antigens are genetically controlled, so close relative have more similar antigens than unrelated individuals. Blood groups are an example of antigens on red blood cells, but all cells have them.

B-Lymphocytes and antibodies

B-lymphocytes are white blood cells that make antibodies. An antibody (also called an immunoglobulin) is a protein molecule that can bind specifically to an antigen. Antibodies all have a similar structure composed of 4 polypeptide chains (2 heavy chains and 2 light chains) joined together by strong disulphide bonds to form a Y-shaped structure. The stem of the Y is called the constant region because in all immunoglobulins it has the same amino acid sequence, and therefore same structure. The ends of the arms of the Y are called the variable regions of the molecule because different immunoglobulin molecules have a different amino acid sequence and therefore different structures. These variable regions are where the antigens bind to form a highly specific antigen-antibody complex, much like an enzyme-substrate complex.

variable region

constant region

disulphide bridges

antigen-binding site

stylised diagram

Rasmol molecular model
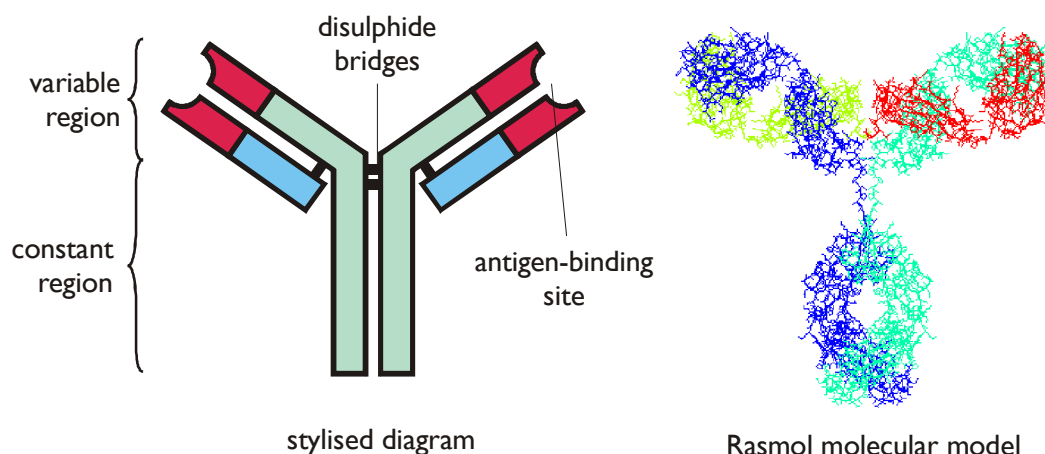
Each B-cell has around 10^5 membrane-bound antibody molecules on its surface and can also secrete soluble antibodies into its surroundings.

antigens

membrane-bound antibodies

soluble antibodies

antibody proteins are not to scale
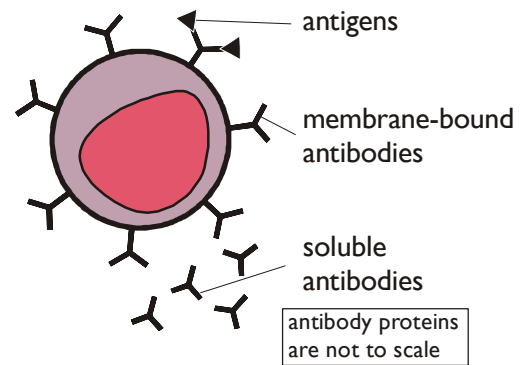
T-Lymphocytes and receptors

T-lymphocytes are white blood cells that have receptor proteins on their surfaces. Receptor proteins are very similar to antibodies, but receptor proteins have only one binding site, and are only found on the surface of T-cells, never free in solution. Receptors proteins bind specifically to antigens to form antigen-receptor complexes. Each T-cell has around 10^5 receptor proteins. T-cells do not secrete soluble proteins.

antigen

membrane receptor proteins

receptor proteins are not to scale
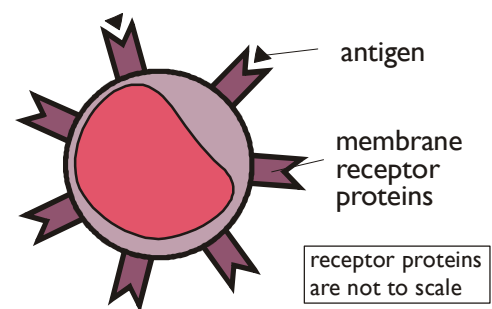
Every human has around 10^8 different types of B and T cell, each making antibodies or receptor proteins with slightly different binding sites. Between them, these antibodies and receptor proteins can therefore bind specifically to 10^8 different antigens, so there will be an antibody or receptor proteins to match almost every conceivable antigen that might enter the body. At birth we have less than 100 copies of each type of B or T lymphocyte. The B and T cells are exposed to so many "self" antigens on every normal cell they come across, that they quickly "learn" to recognise them very early in life. From then on self antigens are ignored, but any non-self antigens are recognised and stimulate an immune response as described below.

The actions of the specific immune system are summarised in this diagram:
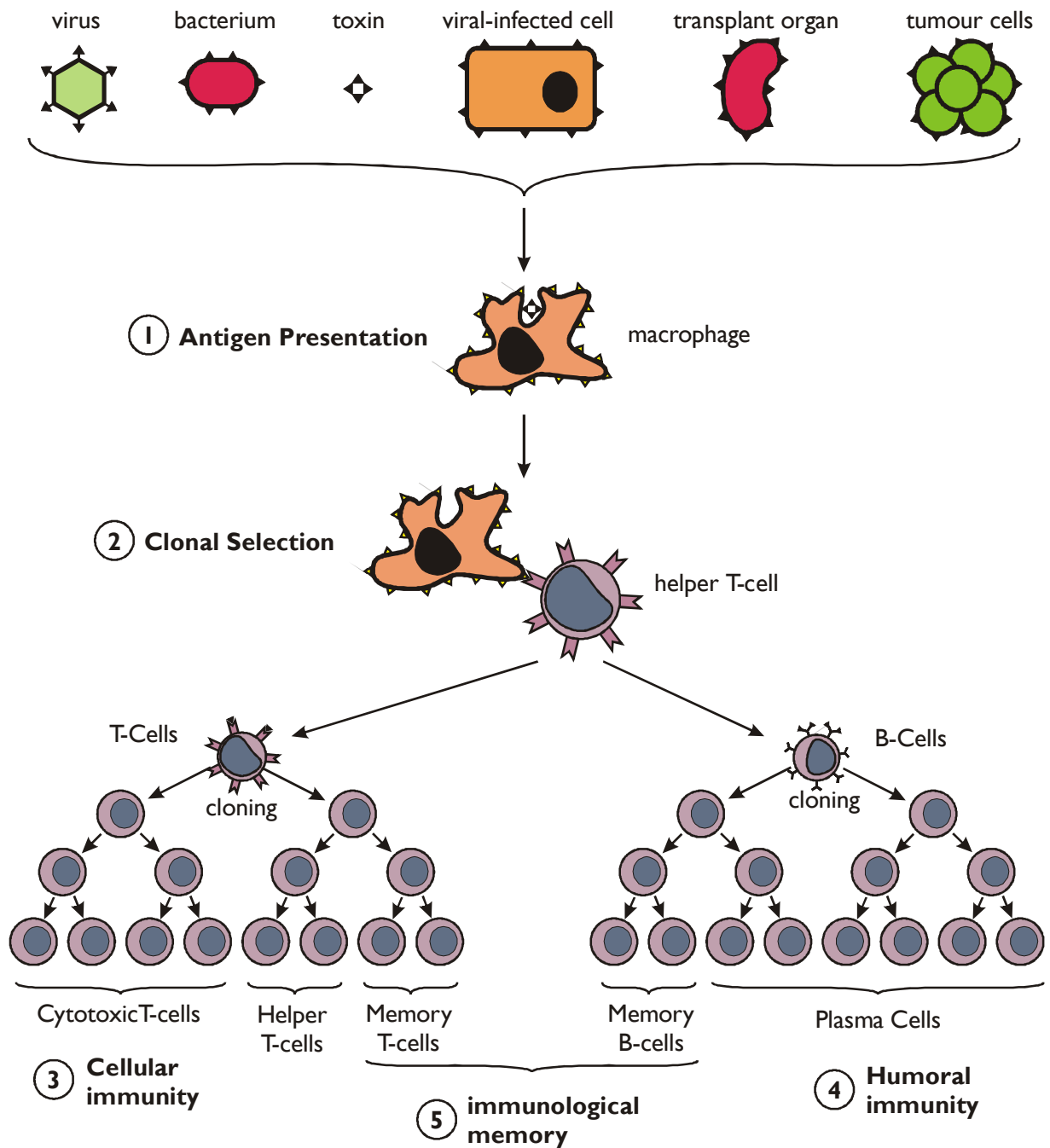
1. Macrophages and Antigen Presentation

Infection is started when cells with non-self antigens enter the blood or tissue fluid. The antigens can be from a variety of sources: e.g. a virus; a bacterial cell; a toxin released from a bacterium; on a cell infected with a virus so that it has viral proteins on its surface; on a transplanted cell; on a cancerous cell.

These foreign antigens need to be presented on antigen-presenting cells in order to initiate the specific immune response. Many body cells can act as antigen-presenting cells, but the most important are the macrophages, because they are the most numerous phagocytes. Whenever they find a non-self antigen, the macrophage ingests the antigen and its cell by phagocytosis. Some of the antigens pass to the surface of the macrophage, which thus becomes an antigen-presenting cell. This method of presenting antigens amplifies the number of foreign antigens in the blood without increasing the number of pathogens. The macrophage also secretes chemicals to stimulate the next stage of the immune response – clonal selection.

2. Helper T-cells and Clonal Selection

The antigen-presenting cells interact with the helper T-cells cells in the blood. Sooner or later the antigen-presenting cell will encounter a helper T-cell with a matching receptor molecule, so the two cells bind tightly to each other. It's a bit like Prince Charming trying to fit the glass slipper (the antigen) onto all the girls in the kingdom (the receptors on the different T-cells) until eventually he finds Cinderella, who is an exact fit.

As soon as a match is found, the tight binding of the antigen-presenting cell to the helper T-cell stimulates the helper T-cell to release chemicals called cytokines. These cytokines stimulate immature T and B lymphocyte cells to activate, proliferate and differentiate. When activated, the lymphocyte cells divide repeatedly by mitosis, making a clone army of about 10^6 genetically-identical cloned lymphocyte cells. This is called clonal selection, because only the selected cells are cloned. There is now a clone army of B and T lymphocytes with identical binding sites on their cell-surface proteins – binding sites that specifically complement the foreign antigen. The clone army can now destroy the infecting microbe, as described below.

## 3. T-Cells and Cellular Immunity

The activated T-lymphocytes differentiate into cytotoxic T-cells (or killer T-cells). These cytotoxic cells bind to antigens on infecting pathogen cells and kill them by making pores in their cell membrane, which allows water to diffuse in so that the cell lyses (bursts). This is called cellular immunity because the foreign cells are killed directly by the lymphocyte cells.

4. B-Cells and Humoral Immunity

The activated B-lymphocytes differentiate into <u>plasma cells</u>. Plasma cells contain large amounts of rough endoplasmic reticulum and are protein factories, synthesising and secreting large numbers of soluble antibodies. A single B-cell can divide to form 10^6 plasma cells, each of which can release 10^3 antibodies each second for 4 days. These antibodies are carried around the blood, lymph and tissue fluid binding to any antigens they come into contact with and forming antibody-antigen complexes. This binding kills cells in various ways:

1. By binding to antigens on viruses and bacteria they prevent the viruses or bacteria attaching to cells and so infecting them.

2. By binding to free toxin proteins they change the shape of the active region so that these proteins can no longer take part in the reactions that caused disease.

3. By linking cells together. Because each antibody molecule has two antigen-binding sites (one on each arm of the Y), antibodies can stick cells together into large clumps. This process, called <u>agglutination</u>, immobilises viruses and cells, and precipitates soluble toxins so that they can easily be destroyed by phagocytes or cytotoxic T-cells. Large antigen-antibody complexes also stimulate the various activities of the non-specific immune response, such as phagocytosis and inflammation.
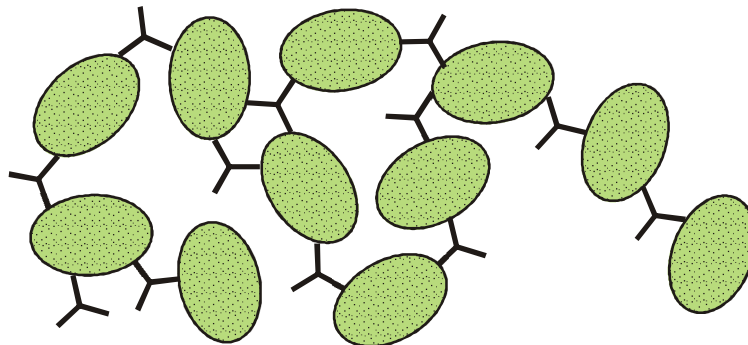
This is called humoral immunity because the foreign cells are killed by soluble antibodies dissolved in the blood plasma (body fluids were called humours in ancient terminology).

5. Memory Cells and Immunological Memory

The clone army of B and T cells only lasts for a few days, after which the cells are destroyed and recycled by phagocytes. However, some of the activated B and T cells differentiate into <u>memory cells</u>. These memory cells remain in the blood for many years after the infection and the memory B cells continue to secrete antibodies in small quantities. This means that the same antigen will be identified much more quickly in a subsequent infection, and the memory cells will quickly divide to form a new clone army. This is called the <u>secondary immune response</u> (see below).

Primary and Secondary Immune Responses

The first time a new antigen is encountered there are only a few lymphocyte cells of each kind (<100) for the antigen to encounter, so it can take several days for clonal selection to take place and the clone army to be assembled. Furthermore the clone army tends to be fairly small. This slow and weak response to a first infection is called the primary immune response. It is during this period that the symptoms of the disease are shown, partly due to toxins and cell death due to the pathogen, and partly due to the immune response itself (e.g. fever, inflammation).

After a primary response memory cells (both T and B lymphocytes) remain in the blood. This means that after a subsequent infection by the same antigen the clonal selection stage can be by-passed and the specific immune response is much faster and much greater (i.e. more clone B and T lymphocytes and antibodies are produced). This is called the secondary immune response, and is so fast that the pathogen is destroyed before it reproduces enough to cause disease. In other words the individual is immune to that disease. Note that the non-specific immune response is the same in all infections.
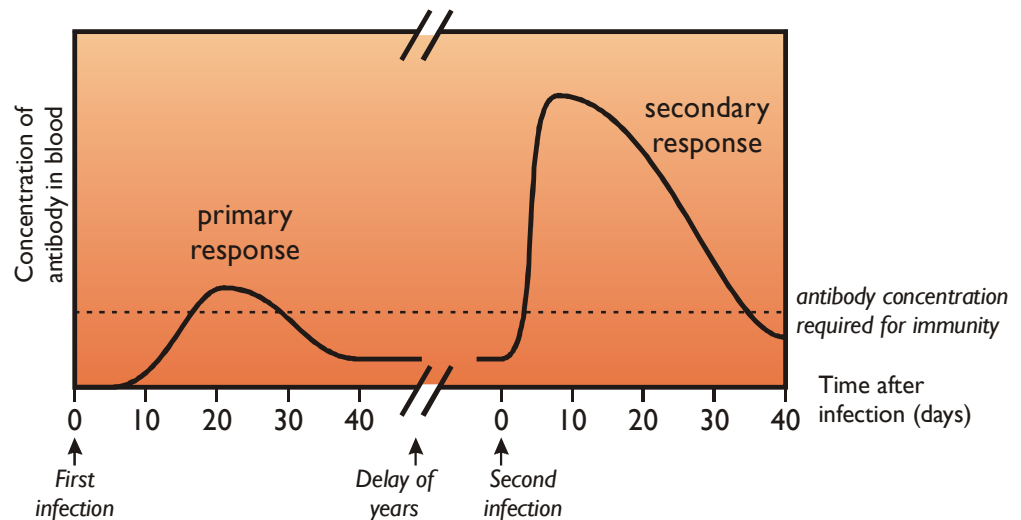
Antigenic Variability

Some pathogens have antigens that remain constant, so we remain immune to them, and can only catch them once (e.g. chicken pox, measles or mumps). Other pathogens develop new strains every few years, with different antigens (e.g. the common cold and the flu). The body does not have memory cells against the new antigens, so infection by a new strain of the microbe causes a new primary response, with all the trappings of the accompanying disease. These pathogens with many different strains show antigenic variability. It is caused by mutations during replication of the pathogen.

Immunisation

We have been able to make use of the immune system's memory to artificially make people immune to certain diseases even without ever having caught them. The trick is to inject with an antigen that will promote the primary immune response, but has been modified so that it is non-virulent (or non-pathogenic), i.e. will not cause the disease. The immune system is thus fooled into making memory cells so that if the person is ever infected with the real virulent pathogen, the more powerful secondary immune response is triggered and the pathogen is killed before it can cause the disease. This technique is called vaccination and is commonly used to provide artificial immunity to a number of potentially-fatal diseases. In the UK children are commonly vaccinated against diphtheria, tetanus, whooping cough, polio, measles, mumps, rubella and TB. If enough people are vaccinated in a population (typically 85-95%), then even the few that are not, or cannot be, vaccinated are protected by herd immunity, since there are not enough hosts for the pathogen to survive and reproduce.

Passive Immunity

Injecting antigens to promote an immune response is called active immunity, but it is also possible to inject antibodies against certain pathogens into the blood. This is called passive immunity and is used when someone has already been infected (or is likely to become infected) with a pathogen. The antibodies in it assist the body's normal immune response and help it deal with serious diseases. Antibodies are either prepared from the blood serum of an infected human (or rarely animal), called an antiserum, or are made by genetic engineering. Passive immunisation is not very common, but can be used for rabies, tetanus, measles and hepatitis B, and is being tried to combat AIDS.

Passive immunity also occurs naturally when a mother passes antibodies to her child. Antibodies can pass across the placenta to the foetus and are also found in colostrum, the milk produced in the first few days after birth. Since the baby's digestive system does not function at this stage, the immunoglobulin proteins can be absorbed intact. This passive immunity helps the new-born baby survive in a world full of pathogens, and is one reason why breast feeding is so important.

The different kinds of active and passive immunity are summarised in the table.

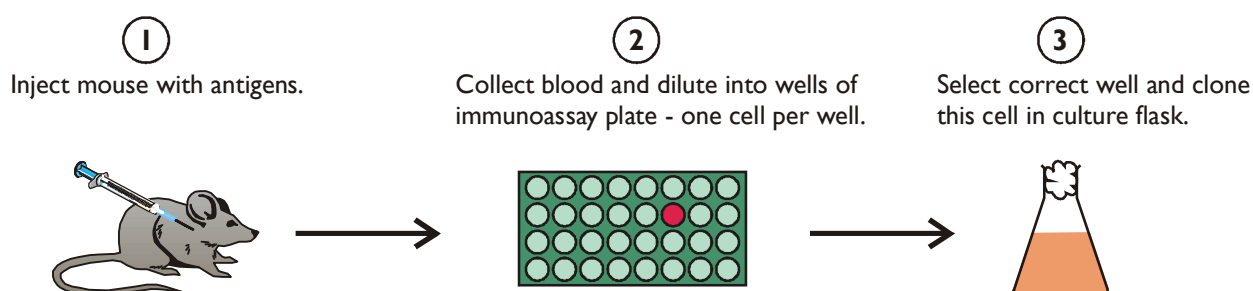|	Active Immunity (antigens received)	Passive Immunity (antibodies received)
Natural	Achieved through the primary immune response following an infection	Achieved through the passing of antibodies from mother to child through the placenta and milk.
Artificial	Achieved through injection of modified antigens (vaccination).	Achieved through injection of antibodies (antiserum).

Monoclonal Antibodies

The unique tertiary structure of each antibody protein allows it to bind specifically and tightly to one particular antigen. Scientists quickly realised that the remarkable specific binding property of antibody proteins *in vivo* would make them very useful tools in medicine and research *in vitro*. [*In vivo* means "in life", i.e. in a living organism; and *in vitro* means "in glass", i.e. in a test tube.] <u>Monoclonal antibodies</u> are antibodies of one particular shape made by a clone of a single B-lymphocyte.

Making Monoclonal Antibodies

Antibody proteins are far too complicated to be synthesised chemically *in vitro*: they have to be made by living cells. In 1975 Kohler and Milstein developed a method to make monoclonal antibody proteins using mice.

①	②	③
Inject mouse with antigens.	Collect blood and dilute into wells of immunoassay plate - one cell per well.	Select correct well and clone this cell in culture flask.

1. Inject a mouse with the antigen protein that you want antibodies for. The mouse will show a primary immune response and make a clone army of B-lymphocytes with antibodies specific for that antigen.

2. After a few days, extract B-lymphocyte cells from the rabbit's blood. The blood contains a mixture of thousands of different B-cells, each making their own specific antibodies, so we need to isolate the B-cell we want. Dilute the blood cells into hundreds of wells in an <u>immunoassay plate</u>, so that there is just one cell per well. The cells multiply in their wells and secrete antibodies – a different antibody in each well.

3. Test each well for production of the antibody required and grow the B-cells from that well in a culture flask, where they multiply by mitosis, making millions of identical cloned cells, each secreting identical antibodies – monoclonal antibodies.

Using Monoclonal Antibodies

Monoclonal antibodies have many uses, but they are all based on the same principle. If monoclonal antibodies are mixed with a sample containing a mixture of proteins, the antibodies will bind specifically and tightly to only one protein in the sample.

mixture of proteins   monoclonal antibodies   one protein identified
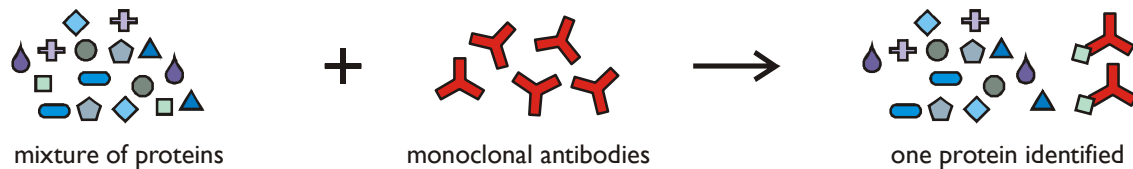
The monoclonal antibodies can have another molecule chemically attached to the constant region, which can make the antibody coloured, or fluorescent, or attach it to a surface. This allows the target protein to be visualised.

Some uses of monoclonal antibodies include:

• Antibodies can be used as a "magic bullet" to target drugs to one specific cell type in the body. Monoclonal antibodies are made to an antigen only found on the target cell, and the drug is bound to the constant region of the antibody. The antibody/drug complex is then be injected into the patient and the antibody will ensure that the agent is carried only to the target cells and nowhere else.

• Antibodies can be made to target a toxic agent (e.g. a radioactive substance) to cancer cells and nowhere else in the body.

• Antibodies to the protein hormone hCG, produced in pregnancy, are bound to a test strip and used to detect the presence of hCG in urine in a pregnancy test strip.

• Antibodies are used to detect the presence of specific proteins in very low concentrations in the ELISA assay.

• Fluorescent antibodies are used to stain particular cell organelles in microscope slides.

• Antibodies can be used directly in passive immunity to help the body's normal immune response to a serious infection (see p80).

Appendix 1 – Mathematical Requirements

Biology is a quantitative science, and a reasonable mathematical ability is expected in an A-level biology exam. The AQA specification states that you can be tested on any of these mathematical topics:

Calculations
- Use standard form; ratios, fractions and percentages.

- Calculate x^n; $1/x$; $\sqrt{x}$; mean; and standard deviation.

- Calculate percent change and rate of change.

- Calculate circumferences and areas of circles; and surface areas and volumes of cuboids and cylinders when provided with appropriate formulae.

- Use units with prefixes (n, μ, m, k, M, G) and use an appropriate number of significant figures.

- Make estimates of the results of calculations without using a calculator.

- Rearrange equations and substitute numerical values into equations using appropriate units.

Handling data
- Understand the terms mean, median and mode and standard deviation.

- Understand the use of logarithms for quantities that range over several orders of magnitude.

- Construct and interpret frequency tables, bar charts and histograms.

- Use a scatter diagram to identify positive and negative correlation between two variables.

- Plot graphs from data (using appropriate institute of biology conventions) and read data from graphs.

- Understand the principles of sampling as applied to biological data.

Some of these mathematical requirements are explained on the next two pages.

SI Units

Biological measurements are always made using standard "SI" units. There are fundamental and derived units. The main units used in biology are:

Quantity	Unit	Symbol	Notes
length	metre	m	
mass	kilogram	kg	
amount of substance	mole	mol	
time	second	s	Never use sec. Minutes (min), hours (h), days (d) and years (y) are also used when more appropriate.
temperature	degree celcius	°C	The kelvin (K) is the SI unit of temperature, but is rarely appropriate in biology, where °C is much more common. Never use the term centigrade. 0°C = 273.15 K.
force	newton	N	$1\ N = 1\ kgm\ s^{-2}$
pressure	pascal	Pa	$1\ Pa = 1\ Nm^{-2}$
energy	joule	J	$1\ J = 1\ Nm$
volume	litre	L	$1\ L = 10^{-3}\ m^3 = 1\ dm^3$. Volume should strictly be measured in m^3, but the litre is more useful in biology and is widely used. Try not to mix the figure 1 and the letter l (use L). Exams usually use cm^3 (= mL).
concentration		$g\ L^{-1}$	or $mol\ L^{-1}$
speed		$m\ s^{-1}$	
rate of reaction		$mol\ s^{-1}$	or any measure of progress / any unit of time (e.g. $g\ min^{-1}$)

All SI units can take these <u>prefixes</u> in front of them to make them smaller or larger:

10^{-3}	milli	m	10^{3}	kilo	k
10^{-6}	micro	μ	10^{6}	mega	M
10^{-9}	nano	n	10^{9}	giga	G
10^{-12}	pico	p	10^{12}	tera	T

The Thousands Rule. The prefixes increase or decrease by factors of a thousand, so by choosing the right prefix, all values can be in the range 1–999.

e.g   10 mm   instead of 0.01 m
        2.56 MPa   instead of 2 560 000 Pa
        75 μL   instead of 0.075 mL.

Values may also be expressed in standard form (or scientific notation) e.g. 3.2×10^6 cells mL^{-1}. You should be able to convert between these forms e.g. 4×10^{-8} m = 40 nm.

- Never use centi (c, 10^{-2}) or deci (d, 10^{-1}), e.g. cm, dm. They don't follow the thousands rule and so cause confusion.

- Names of units are always spelt with a small letter, even if they're named after scientists (e.g. joule).

- Symbols do not need a full stop (like an abbreviation) or an s (like a plural) e.g. 3 min not 3 mins.

- There should be a space between the value and its symbol (e.g. 6 g not 6g)

- There is no space between a prefix and a symbol (e.g. 7 mN not 7 m N)

- Use a space to indicate thousands, not a comma (e.g. 72 000 not 72,000)

- Use the index $^{-1}$ for division in units, not a slash (e.g. ms^{-1} not m/s)

- If you don't know already, find out how to use subscripts and superscripts in your word processor.

The Greek Alphabet

Greek letters are often used in biology (and other sciences), so for reference, here is the 24-letter Greek alphabet. To get these on a PC, use the Roman equivalent letter, and set the font to "Symbol". Mu (μ) is also available in any font using ALT-0181.

Name	uppercase letter	lowercase letter	Roman equivalent	Name	uppercase letter	lowercase letter	Roman equivalent
Alpha	A	α	a	Nu	N	ν	n
Beta	B	β	b	Xi	Ξ	ξ	x
Gamma	Γ	γ	g	Omicron	O	o	o
Delta	Δ	δ	d	Pi	Π	π	p
Epsilon	E	ε	e	Rho	P	ρ	r
Zeta	Z	ζ	z	Sigma	Σ	σ	s
Eta	H	η	h	Tau	T	τ	t
Theta	Θ	θ	q	Upsilon	Y	υ	u
Iota	I	ι	i	Phi	Φ	φ	f
Kappa	K	κ	k	Chi	X	χ	c
Lambda	Λ	λ	l	Psi	Ψ	ψ	y
Mu	M	μ	m	Omega	Ω	ω	w

Area and Volume

You should know, and be able to use, common formulae such as:

circumference of circle = $2\pi r$ area of circle = πr^2

surface area of cube = $6s^2$ volume of cube = s^3

You may also be given, and have to use, other formulae such as:

volume of cylinder = $\pi r^2 h$ surface area of cylinder = $2\pi rh$ (+ $2\pi r^2$ for the ends)

volume of sphere = $4\pi r^3/3$ surface area of sphere = $4\pi r^2$

Percentage Change

A common exam question is to calculate percentage change. The formula is:

$$\% \text{ change} = \frac{(\text{final value - starting value})}{\text{starting value}} \times 100$$

Percent changes can be positive (increases) or negative (decrease). It is also possible to have changes greater than 100%.

For example:
- if the skin temperature of an athlete changes from 37.0 °C to 37.5 °C, there is a change of   +1.35 %
- if the area of a decomposing leaf changes from 150 mm² to 80 mm², there is a change of   -46.7 %
- if the leaf area of a growing tree changes from 0.6 m² to 3.7 m², there is a change of   +517 %

Appendix 2 – The Unit 1 Exam

The three AS biology units are assessed as shown in this table:

Unit	Assessment	Details	Raw marks	UMS marks
Unit 1	1h 15min exam	5-7 short answer questions plus 2 longer questions: 1 comprehension and 1 continuous prose.	60	100
Unit 2	1h 45min exam	9 short answer questions plus 2 longer questions: 1 data handling and 1 HSW.	85	140
Unit 3	AS EMPA	2-3 practical sessions with short written task sheets plus a 1h 15min exam.	50	60

Biology is not just about learning facts (though there is a lot to learn): it's largely about understanding principles and being able to apply these principles to unfamiliar situations (which is what happens in real life). It's also important to understand How Science Works, and the role of evaluation and critical thinking. So the A2 biology exams test all these aspects. Of the 60 raw marks in the unit 1 exam, about 25 will be for biological knowledge; 25 will be for applying that knowledge to unfamiliar situations and analysing data; and 10 will be for How Science Works, including planning, analysing and evaluating experiments. So expect lots of questions about data analysis. These are designed to test your knowledge of unit 1 biology in unfamiliar contexts.

Exam Technique

- 40% of all exam marks lost are lost due to poor exam technique.

- Read the question! You will only get marks for doing exactly what it asks, e.g. if a question says "explain how A causes B" then start at A and finish at B.

- Do what the question says. If it says "use the diagram to..." or "use the graph to...", then you must do so.

- If a question says "give two reasons…" then give exactly two. You will lose marks if you give three.

- Read the whole question before answering any of it. This helps understanding.

- Use technical terms in every answer. In general a technical term used correctly is worth one mark. "Meiosis causes alleles to be recombined" is more likely to earn a mark than "meiosis mixes genes".

- Look at the marks. Don't write too much for a 1-mark answer, and do write 3 good things for a 3-mark answer.

Exam Strategy

- 40% of the marks are aimed at E-grade candidates, so should be fairly easy to get. You could try finding and doing these questions first.

- In longer answers (5 or more marks) try writing your answer in bullet points, where each statement is worth one mark. That will force you to be logical and put a technical term into every sentence, and it will help the examiner to find your points.

Content and Synopsis

- In general questions will only test the content of the specification for that unit. However:

- Some basic ideas (like cells, diffusion, osmosis, proteins, enzymes, etc.) could crop up in any unit.

- Knowledge will often be tested in unfamiliar contexts, so you may need to work out what part of the specification is actually being tested. Don't panic – questions on hippopotamuses aren't really about hippopotamuses.

- The essay in Unit 5 is the only real synoptic question, when you will be expected to introduce ideas from many parts of the course (more details below).

- How Science works will be tested in all units.

Describing and Explaining data

- Underline the words "describe" and "explain" on your paper, to remind you to do the right one.

- If you are asked to describe some results (from a table or a graph) look for different phases e.g. "as X increases Y increases up to 30 days then levels off". Always quote a value from the graph – usually the X-value where the graph chances shape.

- If you have to describe a graph with fluctuating or "noisy" data (a jagged line), try drawing a smooth line of best fit through the data first, and then describe that.

- If you are asked to describe some results from a table it might be a good idea to sketch a quick graph on the exam paper so you can see the pattern more clearly.

- Do not explain the results if you are not asked to.

- If you are asked to explain some results it's often a good idea to describe them briefly first (even if you're not asked to), so you know what you're explaining.

Maths and Statistics

- There will be maths questions! You must be confident with units and prefixes (especially m, μ and n).

- You need to know the formulae for magnification; percent change and gradient of a graph.

How Science Works

- There will be How Science works questions in all exams, so check you know all the terms.

- If you are asked to design an investigation, the marks will be for fair testing (though don't use this term): how to change the independent variables; how to quantify the dependent variable; naming some control variables; doing repeats.

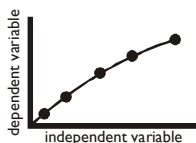You need to understand all the How Science Works words on the next page.

Types of Variable

Dependent Variable
The variable you *measure*, to see how it is affected by the independent variable.

Independent Variable
The variable you *choose to change*, to see how it affects the dependent variable. You may also measure it when you change it.

Confounding Variables
Any variables that could also affect the dependent variable. Confounding variables should be controlled in a fair test.

Control variables
Confounding variables that are kept constant (controlled) during the experiment. If you can't control a variable (such as weather in a field investigation), you should at least monitor it

Experimental Design

Controlled Experiment (FairTest)
When all relevant variables are controlled, so that observed changes in the dependent variable must be due to changes in the independent variable.

Control Experiment (Control)
An additional experiment designed to eliminate alternative explanations for the main experiment, and so show that observed changes in the dependent variable must be due only to changes in the independent variable.

Control Group
A group or sample treated in the same way as the experimental group, except for the factor being investigated e.g. a placebo group in a drugs trial. By comparing the results for two groups it can be shown that observed changes in the dependent variable must be due only to changes in the independent variable.

Placebo
A dummy pill, injection or treatment that has no physiological effect (e.g. a sugar pill or saline injection). Used in a clinical trial to allow for the **placebo effect** - the observation that symptoms can improve when patients believe they are being helped.

RCT
The best experimental design for a drug trial. RCT stands for Randomised Controlled Trial, or in more detail, a Randomised, Placebo-Controlled, Double-blind Trial. This design ensures that the trial is **valid** free from **bias**.
- **Randomised** means the study and control groups are allocated randomly
- **Placebo-controlled** means the study group (taking the drug to be tested) is compared to a placebo group (who are given a placebo).
- **Double-blind** means that neither the subjects nor the investigators know who is in the study or placebo groups. This avoids bias.

Protocol
A method or technique that has been shown to produce valid and reliable results.

Hypothesis
A suggested explanation of observations or results that can be tested. Also known as a scientific hypothesis. A good hypothesis can be used to make **predictions.**

Quality of Data

True Value
The real value of a measurement, if it could be measured with no errors at all.

Precise Data
1. Measurements that give similar values when repeated. The replicates therefore have a small **range**.
2. Data measured on sensitive equipment with a suitably fine scale, e.g. 20 mm is more precise than 2 cm.

Reliable Data
Findings that can be repeated. This includes by the original investigator; by other scientists; by other techniques; or those that agree with secondary sources.

Accurate Data
Measurement that are close to the **true value.**

Valid Data
The best quality data, i.e. data that is **precise** and **reliable** and obtained from an **unbiased**, **controlled** experiment that addresses the stated aim. Valid data is assumed to be accurate.

Evidence
Any data or observations that are used to support a particular hypothesis.

Anecdote
An observation or story from real life. Anecdotes are not evidence and cannot be used to support a hypothesis, but they can be useful to suggest a new testable hypothesis.

Types of Data

Data
(measurements, singular datum)

Quantitative or Numeric Data
(numbers)

Qualitative or Categoric Data
(words)

Continuous Data
can have any value
e.g. 7.34, -294.6, 2×10^5

Discrete Data
only whole numbers
e.g. no. of atoms

Ordered Data
can be ranked
e.g. small, medium, large

Nominal Data
can't be ranked
e.g. male, female

Errors

Random Errors
Inaccuracies due to mistakes, poor technique, or random variation. Random errors are very common, but can be improved by taking many replicates. Data with a small random error is said to be **precise**.

Systematic Errors
Inaccurate measurements in one direction only, due to poor **calibration** or poor technique. Systematic errors can **not** be improved by taking more replicates. Data with a small systematic error is said to be **reliable.**

Zero Error
A particular kind of systematic error, where the instrument does not return to zero.

Bias
When the observer chooses some results and ignores others, to support a particular view. Also called **cherry picking**.

Anomaly or Outlier
A measurement that falls far outside the expected range and is therefore probably due to experimental error. Anomalies should be rejected, since they skew the mean, but it is very difficult to distinguish between anomalies and normal biological variation.

Calibration
Ensuring that a measuring instrument gives correct readings by fixing known points then constructing a scale between them.

Simple Analysis

Replicates
Repeats of a measurement.

Raw Data
The original measurements or recordings before any manipulation or processing.

Mean or **Average**
The mid-point of the replicates.
= sum of replicates / N

Range
The highest and lowest replicates, or the interval between them.

Standard Deviation (SD)
A measure of the dispersal of the replicates about the mean. In a normal distribution 68% of the replicates will be within 1 standard deviation of the mean, and 95% will be within 2 standard deviations of the mean.

Standard Error of the mean (SEM)*
A measure of the uncertainty, or error, of a calculated mean. The smaller the standard error, the more reliable the mean.

95% Confidence Interval (CI)*
Another measure of the error of the mean. We can be 95% confident that the true mean lies in the range (mean ± CI). The top and bottom of this range are called the **confidence limits**.

Statistical Analysis

Correlation (or Association)
When one variable changes with another variable, so there is a relation between them. The strength of a correlation can be measured using a correlation coefficient. A correlation need not be a **causal relation**.

Causal Relation
When changes in one variable cause the changes in another variable. Can only be shown by a controlled experiment.

Statistical Test*
Something that tests whether observed differences or correlations are significant, or just due to chance.

Null Hypothesis*
The statement that is tested by a statistical test. The null hypothesis is fixed for each test, but always says that there is no difference or no association. The null hypothesis has nothing to do with a scientific hypothesis.

P-value*
The result of a stats test, expressed as a probability. It is the probability that the results are due to chance. If $P < 0.05$ then we reject the null hypothesis, otherwise we accept it.