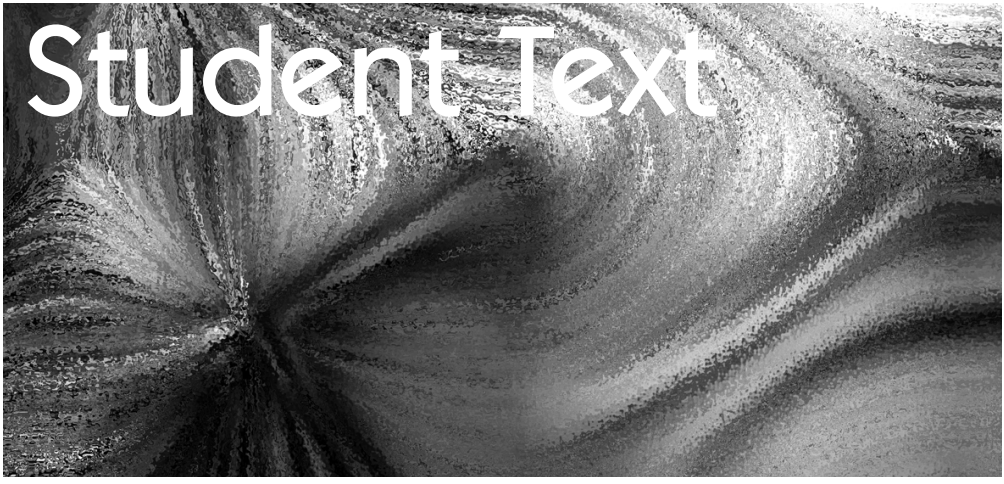
For **Edexcel** Specifications

Advanced Subsidiary

Biology or Biology (Human)



AS Unit 1
Molecules and Cells

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Contents

1.1 Molecules

Content

The structure and properties of some important biological molecules

Water

- Its dipolar nature and formation of hydrogen bonds
- The importance of water as a solvent
- Other roles of water related to its high latent heat of vaporisation, specific heat capacity, density and surface tension.

Carbohydrates

- Hexoses and pentoses are monosaccharides and their role as monomers
- The structure and roles of the monosaccharides alpha and beta glucose, ribose and deoxyribose
- The roles of fructose and galactose
- Disaccharides and polysaccharides composed of monomers joined by glycosidic bonds
- Condensation and hydrolysis reactions involved in the synthesis and degradation of disaccharides and polysaccharides
- The structure and roles of the disaccharides sucrose, maltose and lactose
- The structure and roles of the polysaccharides starch (amylose and amylopectin), cellulose and glycogen

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- The general nature of lipids as fats, oils and waxes
- The general structure of a triglyceride synthesised from glycerol and fatty acids, the formation of ester bonds
- The nature of saturated and unsaturated fatty acids
- The roles of lipids as energy stores, and in protection, waterproofing, insulation and buoyancy
- The structure and properties of phospholipids and their role in the structure and properties of cell membranes

Proteins

- The nature of amino acids as monomers in the formation of polypeptides and proteins
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- Condensation and hydrolysis reactions in the synthesis and degradation of polypeptides and proteins
- The role of ionic, hydrogen and disulphide bonds in the structure of proteins as illustrated by insulin and collagen
- The nature and roles of fibrous and globular proteins as illustrated by collagen and insulin

Nucleic acids

- Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) as polynucleotides composed of mononucleotides
- The basic structure of a mononucleotide
- Thymine, uracil and cytosine as pyrimidines; adenine and guanine as purines
- Condensation reactions in the formation of mononucleotides and polynucleotides (DNA and RNA)
- The structure and roles of messenger and transfer RNA
- The structure of DNA, base pairing; the double helix
- The mechanism of replication of DNA (semi-conservative)
- The nature of the genetic code, the gene as a sequence of bases on the DNA molecule which codes for a sequence of amino acids in a polypeptide chain
- The processes of transcription and translation in the synthesis of proteins; amino acid sequences are specified by DNA
- The function of the ribosomes
- Codons and anticodons in relation to messenger and transfer RNA
- The Human Genome Project
- (Practical work to include qualitative and quantitative biochemical tests for starch, reducing and non-reducing sugars and proteins using iodine solution, Benedict's reagent and biuret reagent, as appropriate)

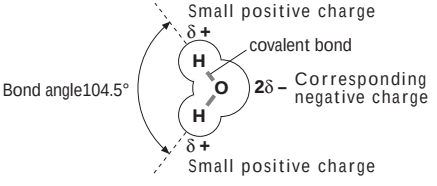
Introduction

It may surprise you to know that the bodies of living organisms are made up of fewer than 20 different chemical elements and just six of these bioelements make up 99% of the total body mass (oxygen 65%, carbon 18%, hydrogen 10%, nitrogen 3%, calcium 2%, phosphorus 1%). Most of the important biological molecules (apart from the most important of all - water) are organic, which means that they are made up from carbon compounds (note that most but not all compounds containing carbon are organic. Exceptions include carbon dioxide and carbonates).Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids, all of which exist as individual sub-units (**monomers**) or repeating chains of sub-units (**polymers**). Polymers are very large molecules, built up by reactions called **condensation reactions** in which a molecule of water is lost as each sub-unit joins the next. They may be broken down by the reverse process, **hydrolysis**, which involves the addition of a molecule of water for each bond broken. The following account describes the structure of carbohydrates, proteins and lipids and the relationship of molecular structure to their function in living organisms.

WATER

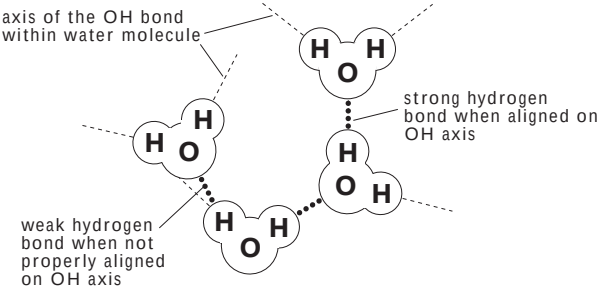
Almost everyone 'knows' the chemical formula for water - H_2O. It is a molecule made up of two hydrogen atoms combined with a single oxygen atom. As molecules go H_2O appears rather simple, yet its molecular structure results in many unique properties upon which life depends.

Each hydrogen atom combines with the oxygen atom by means of a pair of shared electrons (covalent bond). The nucleus of the oxygen atom has a strong positive charge which tends to pull the negatively charged electrons away from the smaller hydrogen atoms. As a result, a negative charge develops near the oxygen atom and positive charges occur near the hydrogen atoms. Molecules which have charged regions are called polar molecules. Water has both positive and negative charges and is therefore described as **dipolar**.



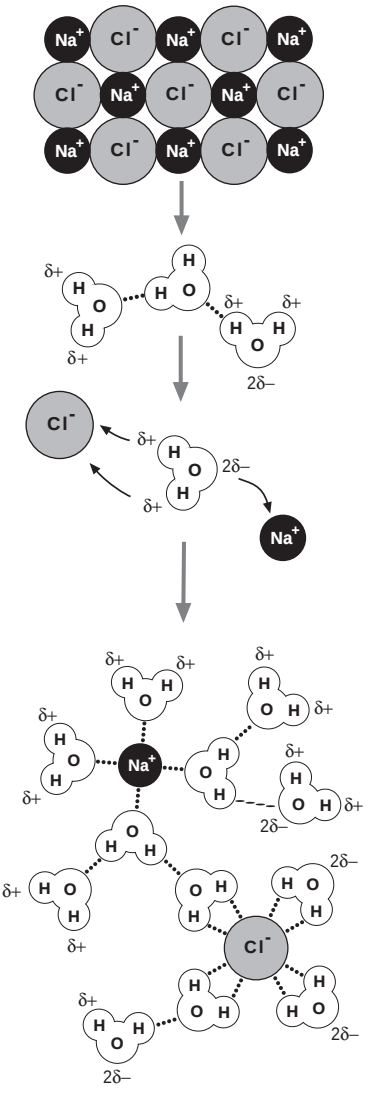
Electron distribution within the water molecule causes the oxygen end of the molecule to have a partial negative charge and the hydrogen sites to have a partial positive charge

The positive and negative charges cause water molecules to attract each other by means of relatively loose linkages called **hydrogen bonds** (H-bonds). This mutual attraction explains why water is a liquid at standard temperature and pressure (STP), whereas other, similar sized, non-polar molecules like methane (CH_4), ammonia (NH_3) and hydrogen sulphide (H_2S) are gases. The H-bonds in water are broken when energy is supplied, e.g. when water is heated. When they break the liquid becomes a gas (vapour). At and below 0°C the H-bonds are at their shortest and strongest and lined up to form a tight regular crystalline structure which is ice. Crucially for life on earth, ice is less dense or 'lighter' than liquid water; in other words ice floats, and aquatic organisms are protected beneath an insulating layer of surface ice. If it did not, and water froze from the bottom up, aquatic organisms would be exposed in progressively shallow surface waters.



Water molecules also attract other polar molecules. When mixed with salt (NaCl), for example, electrostatic links are made between the positive and negative charges of the salt and the water. Water is an excellent solvent allowing many molecules and charged ions to be held in cells and transported around the body. Any molecule with a polar region will dissolve, including sugars and alcohol, and water molecules gather around large protein molecules to form a special kind of solution called a **colloid**, as found in the cytoplasm of cells.

Molecular model of water solubility



As you have seen, the chemical structure of water determines its properties. The table below lists these properties with examples of their biological importance.

Property	Example of biological importance
State	Solid (ice) at 0ºC and vapour above 100ºC (at sea level), providing a wide range of temperature at which it exists as a liquid.
Solvent	'Universal' solvent in which many substances dissolve.
Density	Water supports aquatic organisms. Ice is less dense than water, therefore ice floats and organisms can survive beneath it.
Viscosity	Allows free flow for transport of materials inside and outside of organisms.
Surface Tension	Water has a strong surface film allowing small organisms to be supported on top or underneath it
Capillarity	Water rises in small bore tubes against the pull of gravity because the polar water molecules are attracted to themselves and to a number of surfaces. Capillarity is important in water transport in plants.
Incompressibility	Water provides a hydrostatic skeleton. When enclosed in any structure with a strong surrounding wall, water provides much support and protection to structures within it - e.g. earthworm body cavity, human abdominal cavity
Latent heat of vaporisation	As water molecules evaporate they absorb heat energy from the surface from which they are evaporating, cooling them down in the process, e.g. sweating.
Specific heat	Water has a high specific heat. This means that it gains and loses heat slowly which is important in the temperature control of aquatic environments and internal fluids.
Transparent	Allows the transmission of light e.g. for photosynthesis in aquatic organisms.

CHECKPOINT SUMMARY

- *Water molecules are dipolar with an electronegative pole and an electropositive pole*
- *The dipolar nature of water causes molecules to be attracted to each other by hydrogen bonds resulting in most of the key properties of water in relation to living organisms*
- *Liquid at normal temperature and pressures, solid (ice) at 0ºC, boiling point 100ºC, providing a wide range as a liquid*
- *It has high cohesion resulting in a strong surface film allowing small organisms to be supported on top or underneath it, and the ability for the water column*
- *'Universal' solvent in which many substances dissolve*
- *Water supports aquatic organisms, ice is less dense than water, therefore ice floats and organisms can survive beneath it*
- *Low viscosity allows free flow for transport of materials inside and outside of organisms*
- *Water rises in small bore tubes by capillarity e.g. important in water transport in plants*
- *Being incompressible water provides a hydrostatic skeleton, e.g. earthworm body cavity, human abdominal cavity*
- *Latent heat of evaporation results in a cooling process e.g. sweating*
- *Water has a high specific heat which is important in temperature control of aquatic environments and internal fluids*
- *Transparency allows the transmission of light e.g. for photosynthesis*
- *Inorganic ions dissolve freely in water and have a wide and varied role in living organisms.*

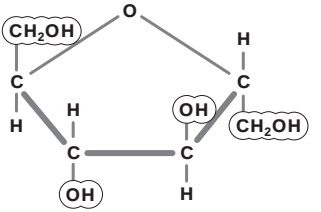
CARBOHYDRATES

Monosaccharides

Carbohydrates are a family of molecules made up, as the name suggests, from carbon and the components of water (hydrogen and oxygen). Carbohydrates are manufactured by plants and are passed on to other organisms via food chains. You should already be familiar with the name and chemical formula of one of the simpler carbohydrate molecules, glucose, which is made from CO_2 and H_2O in the process of photosynthesis.

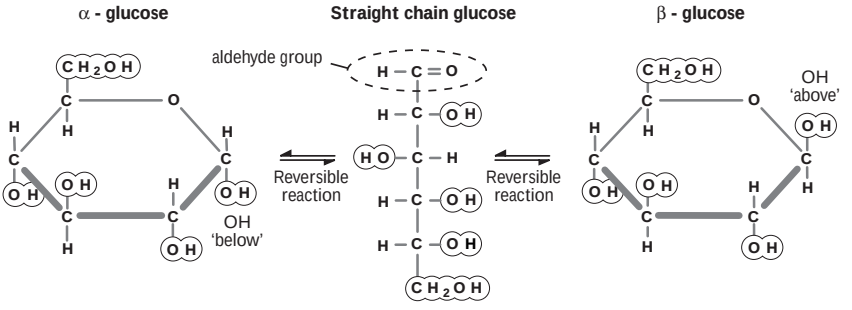
$$6CO_2 + 6H_2O \rightarrow \underset{\text{glucose}}{C_6H_{12}O_6} + 6O_2$$

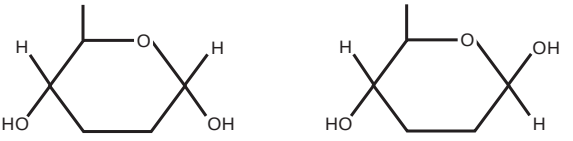
Glucose has six carbon atoms in the form of a framework to which the oxygen and hydrogen atoms are attached. In dry glucose powder the carbon atoms are arranged in a straight chain, but if it is dissolved in water, the chains form themselves into ring structures. Two different ring forms exist called **alpha** (α) **glucose** and **beta** (β) **glucose**. The carbon atoms are numbered 1 to 6 starting in a clockwise direction from the position of the oxygen atom. As discussed later, the different structures of alpha and beta glucose result in the formation of polysaccharides with different properties.



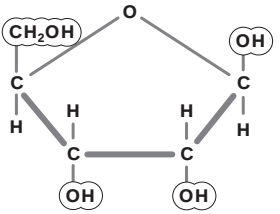
Fructose

Alpha and beta glucose are **isomers**; they have the same chemical formula but a slightly different arrangement of atoms in the molecule. Fructose and galactose are also isomers of glucose. You will notice that fructose has the same number of carbon, hydrogen and oxygen atoms as glucose but it has a keto group (C=O) instead of the aldehyde group (CHO). This gives fructose slightly different chemical properties, for example it is sweeter than glucose.

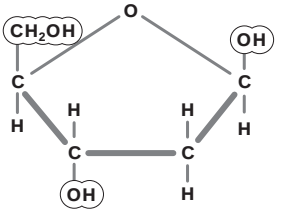




Glucose, fructose and galactose are molecules containing six carbon atoms; they are six carbon sugars called **hexoses**. The simplest carbohydrates are **trioses** (they have three carbon atoms), one example of which, **glyceraldehyde**, you will encounter later in this unit, they do not exist as free sugars but are very important intermediates in biochemical pathways. A very important group of monosaccharides has five carbons. These are the **pentoses,** and they includes **ribose** and **deoxyribose** which play an essential role in the structure of RNA and DNA.



Ribose

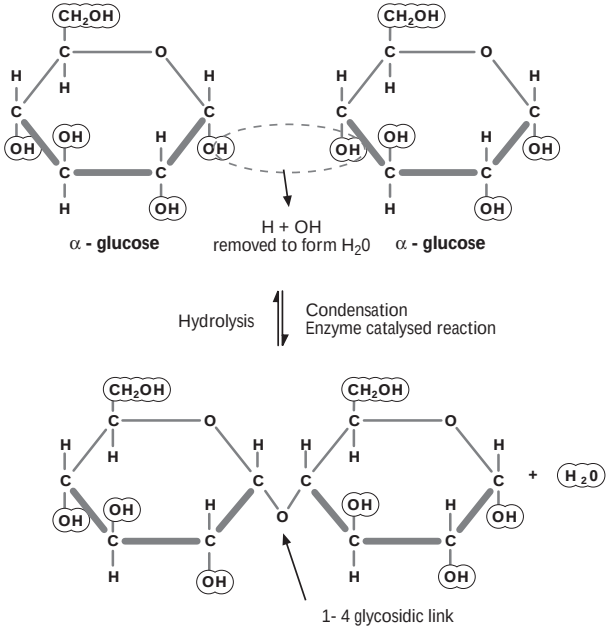


Deoxyribose

These monosaccharides: hexoses and pentoses have a role as monomers in long chain molecules known as polymers. Mono, di and polysaccharides may be interconverted from one form to another by condensation, hydrolysis and isomerisation reactions, each particular reaction being catalysed in living cells by enzymes.

The condensation of monosaccharides to form disaccharides and polysaccharides

Ring structures like these are monomers which are able to link together to form pairs or long chain molecules (polymers). The terms monosaccharide, disaccharide and polysaccharide are used to describe the number of glucose molecules making up a chain (mono = 1, di = 2, and poly = many). If the units of glucose link up to form larger molecules, the first carbon atom of one alpha glucose molecule links to the fourth carbon atom of another with the elimination of a molecule of water. This enzyme controlled reaction results in an oxygen bridge called a **glycosidic bond**. The formation of more than one bond by condensation leads to longer and longer 'multi-molecules' or polymers.



Hydrolysis of disaccharides and polysaccharides

Most of the carbohydrate taken in by animals as food is in the form of polysaccharides or disaccharides. In the process of digestion, enzymes catalyse the hydrolysis of glycosidic bonds to produce monosaccharides which are then absorbed into the blood. A similar process occurs in the germination of seeds. Seeds often store the polysaccharide starch which must be converted to glucose to provide the energy required for growth. The onset of germination is marked by the uptake of water, followed by the activation and release of hydrolysing enzymes.

The hydrolysis of polysaccharides in industry is achieved in two or three stages, each requiring a specific enzyme. This is illustrated by the commercial production of the glucose / fructose sweetener for soft drinks from low cost corn (maize) starch. A number of enzymes are used in this process, e.g.:

- alpha amylase which breaks down starch to shorter, branched amylopectin chains called dextrins;
- glucoamylase which hydrolyses dextrins to glucose.
- glucose isomerase which converts glucose into a glucose/fructose mixture which is sweeter than glucose alone.

Hydrolysing enzymes are used widely in the food industry. Another ingenious example is the use of the enzyme invertase in the manufacture of soft centred chocolates like after eight mints. The minty centres are made from a thick sucrose paste with mint flavouring, and just before the chocolate coat is added, a small quantity of invertase is added which slowly hydrolyses the sucrose to a semi-liquid glucose fructose mix after the chocolate has set hard.

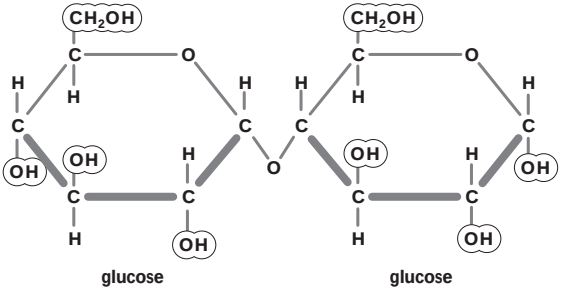
Disaccharides: maltose, sucrose and lactose

Maltose is a disaccharide formed from two molecules of alpha glucose, linked by an alpha 1-4 glycosidic bond, it is the first breakdown product of the action of amylase on starch. It derives its name from germinating barley (malt) used in the brewing industry as a source of sugars for fermentation into alcohol. The germinating barley activates amylase enzymes which hydrolyse its starch stores to maltose.

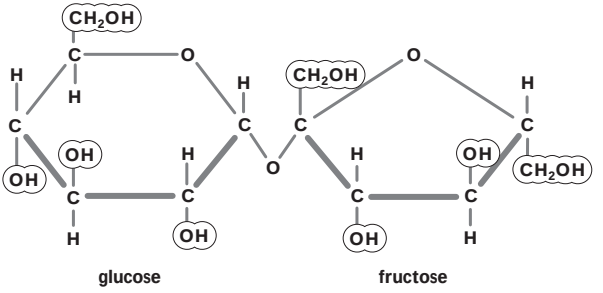
Sucrose is formed by a similar condensation reaction between a molecule of glucose and a molecule of fructose. Sucrose is the best known disaccharide because it is the sugar most produced by plants, and is the commonest sugar used to sweeten foods, being commonly known as 'table sugar'.

Lactose is sometimes referred to a s milk sugar because it is present in the milk of mammals and is, consequently , essential in the diet of infants. Lactose is a disaccharide made up of one molecule of glucose and one of galactose linked by a glycosidic 1-4 bond. Many people are lactose intolerant, not being able to digest (hydrolyse) it to its monosaccharides, so that it passes into the large intestine where it encourages the growth of bacteria which feed on it, resulting in the accumulation of lactic acid in the large intestine, and associated acute symptoms of discomfort.

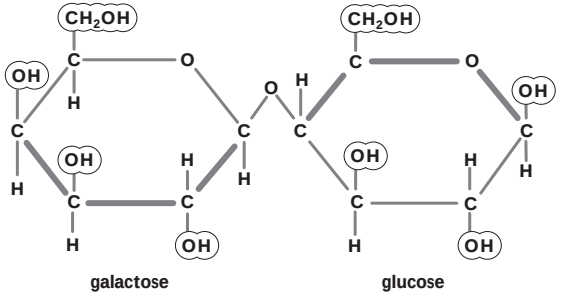
Maltose



Sucrose



Lactose



Structure and roles of the polysaccharides: starch (amylose and amylopectin), glycogen and cellulose

Glucose is the universal fuel for respiration, but it cannot be stored in cells because a high glucose concentration would result in the entry of large amounts of water by osmosis which could cause cells unprotected by a cell wall or surrounding cells, to swell up and burst. For this reason, glucose in excess of energy requirements is converted for storage to the relatively insoluble polysaccharides **starch** in plant cells and **glycogen** in animal cells. These substances are similar in chemical structure, both being formed from chains of alpha glucose molecules.

Starch is a mixture of **amylose** (unbranched chains of alpha glucose) and **amylopectin** (branched chains of alpha glucose). When combined together, the alpha glucose units have their oxygen bridges on the same side of the chain. Hydrogen bonds form between them and cause the chain to coil into a helix, so starch molecules have a dense, tightly packed structure and are relatively insoluble in water.

To dissolve starch, it is necessary to heat it. This breaks the hydrogen bonds and, as the coils begin to open, water molecules rush to occupy the exposed charged (polar) sites binding all over the surface. This is why starch suddenly swells in hot water and is the secret of starch based sauce thickeners used in cooking.

Glycogen has a similar structure to amylopectin but has a large number of shorter branches. It is more suited to animal metabolism as it is capable of being broken down more rapidly than starch. It is never present in food in significant amounts, due to its relatively low concentration in muscles (meat) and liver, and the fact it is rapidly broken down on the death of the cell.

- CHECKPOINT SUMMARY
- *Simplest carbohydrates are monosaccharides (single sugars) which have varying numbers of carbon atoms e.g. pentoses 5C and hexoses 6C*
- *Monosaccharide have a role as units (monomers) in the formation of long chains of repeating units (polymers)*
- *Simple formula for glucose - $C_6H_{12}O_6$*
- *In straight chains in dry powder form*
- *Forms ring structure in solution*
- *alpha and beta glucose are isomers differing in the 3D arrangement of their atoms*
- *Monosaccharides (glucose, fructose, galactose) combine with the elimination of water, form glycosidic bonds- a condensation reaction*
- *Glucose and fructose combine to form sucrose; glucose and galactose combine to form lactose; and two molecules of glucose combine to form maltose*

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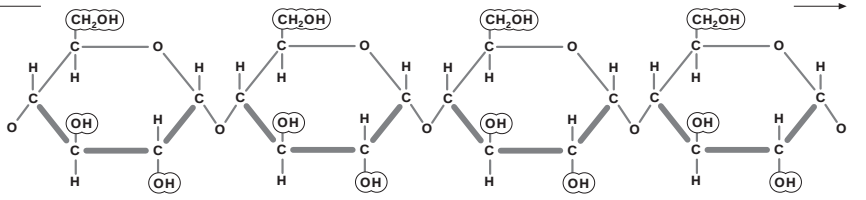
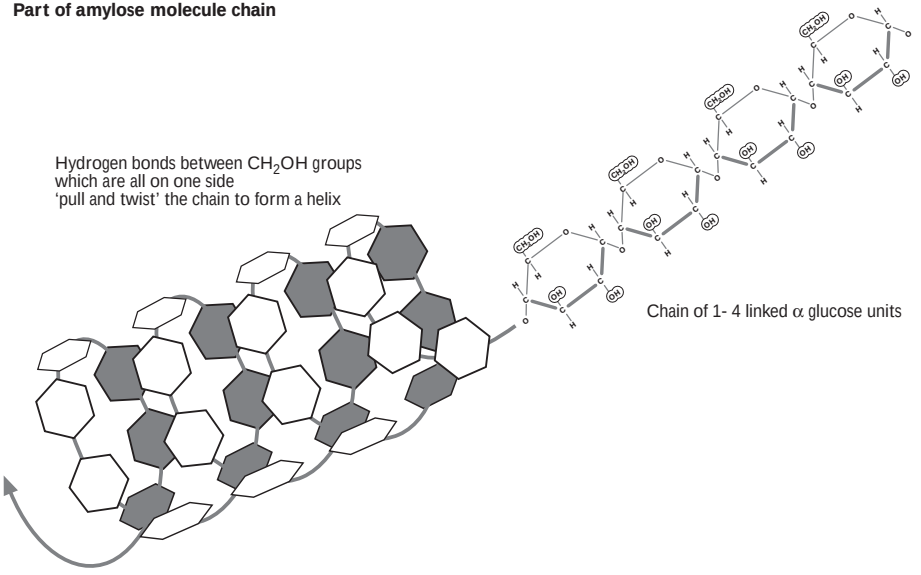


Part of amylose molecule chain



Cellulose is the major component of plant cell walls. The characteristic fibrous structure of cellulose is created by chains of 1-4 linked beta glucose molecules. The beta molecules, when joined together, have their oxygen bridges on alternate sides of the chain, and thus cannot form into a helix, but by forming hydrogen bonds with other chains lying side by side they make flat ribbons which form cellulose fibres.

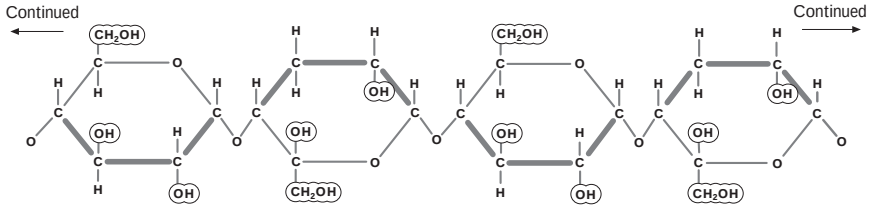
The basic meshwork of cellulose fibres in plant cell walls is strengthened and cemented by other materials such as pectin and lignin. Cellulose is the main component of numerous textiles eg cotton, linen, hemp, paper, timber products, and plastics eg celluloid.

Starch and cellulose have very different properties which arise as a result of one difference between alpha and beta glucose. Starch has no distinct structure (amorphous), no structural strength, is slightly soluble in water, and digestible by vertebrates. Cellulose has a distinct fibrous structure, great structural (tensile) strength, is insoluble in water and indigestible by vertebrates. (Herbivorous vertebrates harbour mutualistic microorganisms in specialised compartments of their gut which secrete cellulase enzyme which digests cellulose.)

CHECKPOINT SUMMARY

- *Glycosidic bonds are broken by the addition of the elements of water across the bond (hydrolysis reaction)*
- *Starch is a mixture of amylose (unbranched chains) and amylopectin (branched chains)*
- *Both being long chains (polymers) of alpha glucose molecules*
- *When combined the alpha glucose molecules have their oxygen bridges on the same side of the chain, hydrogen bonds form between them and cause the chain to coil into a helix*
- *Starch molecules, relatively insoluble in water*
- *Glycogen ('animal starch') is a glucose polymer found in the liver and muscles of animals*
- *Cellulose is a polymer of beta glucose which have their oxygen bridges on alternate sides of the chain and so cannot form a helix, but form hydrogen bonds with other adjacent chains to make flat ribbons which form fibres.*

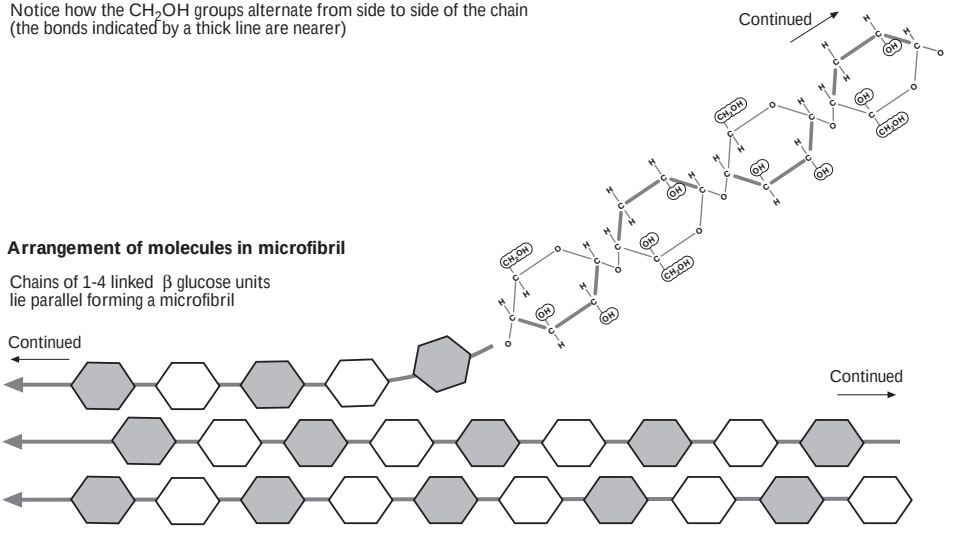
Part of a cellulose molecule chain



Notice how the CH_2OH groups alternate from side to side of the chain (the bonds indicated by a thick line are nearer)

Arrangement of molecules in microfibril

Chains of 1-4 linked β glucose units lie parallel forming a microfibril



Hydrogen bonds form between alternating CH_2OH units bind molecules together side by side into microfibrils.

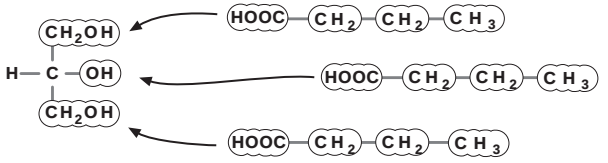
LIPIDS

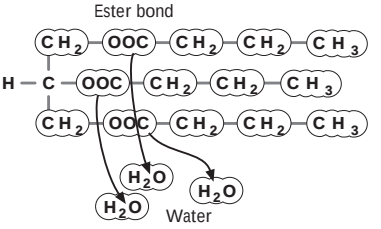
Lipids are a family of molecules which include fats, oils, waxes, phospholipids and steroids. Although they vary in chemical structure they share two important characteristics: they are energy rich substances, and they are insoluble in water.

Structure and function of a triglyceride

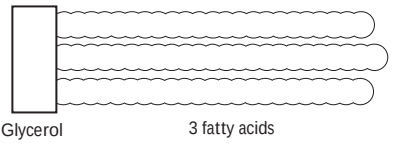
Fats and oils are built on the same basic chemical plan which consists of a molecule of **glycerol** joined to three **fatty acid** chains. They are **triglycerides** ('mono' and 'diglycerides' are structures with one and two fatty acid chains respectively). The fatty acids are linked by **ester bonds** to glycerol in a condensation reaction similar to that seen in the formation of polysaccharides and polypeptides.

Molecular model of development of a triglyceride

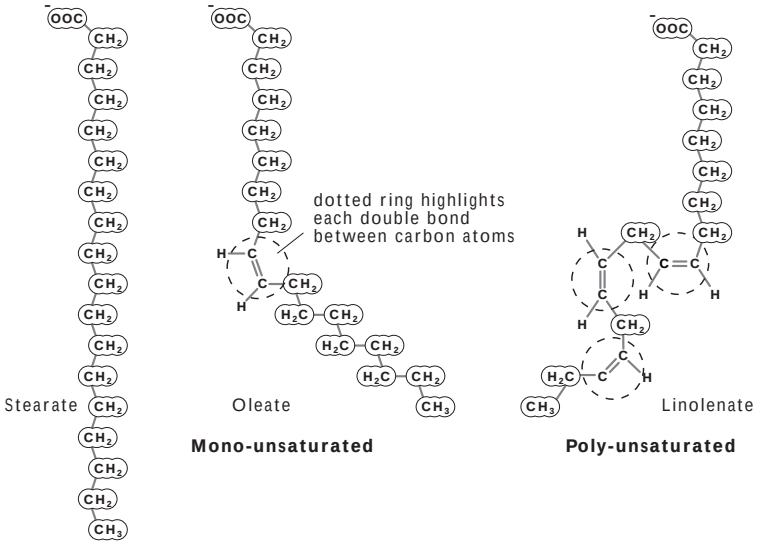




Model of a triglyceride



Fatty acids are hydrocarbon chains with a carboxylic (-COOH) group at one end. It is the -COOH group which forms the ester bond with glycerol. Hydrocarbons are non polar and, like fuel oils which have a similar structure, they contain a lot of energy locked up in their C-C and C-H bonds. Fats are mostly of animal origin and are solid at room temperature (RT); oils occur mostly in plants and are liquid at RT. The difference in melting point is due to the different nature of the hydrocarbon chains. In fats, each of the possible bonding sites between carbon and hydrogen is fully occupied (they are **saturated**), but in oils, one or more double bonds occur between the carbon atoms in the chain (they are **unsaturated**). Wherever a double bond occurs, the fatty acid chain acquires a kink, so the fatty acid chains of oils occupy more space than those of fats, giving them greater mobility and a lower melting point so that they are liquid at RT. The terms 'mono', 'di', and 'polyunsaturated' refer to the number of double bonds in the fatty acids.

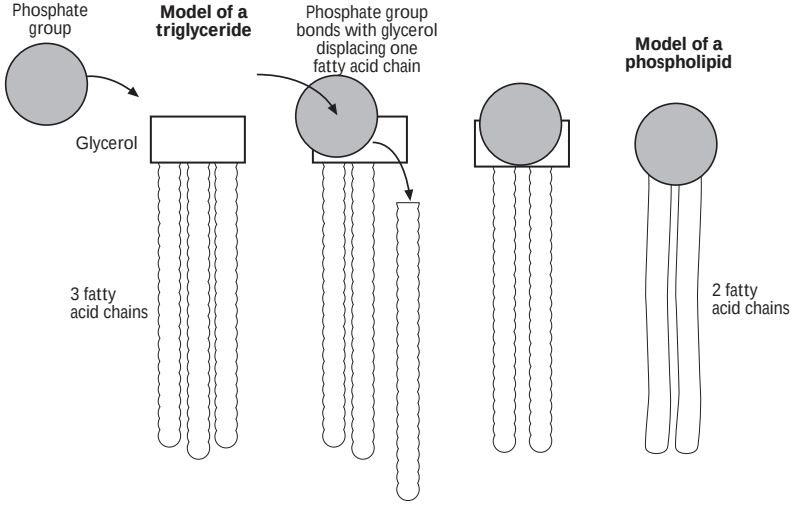


When more food energy is taken in by the body than can be used, it may be converted to fat which is stored in large adipose cells under the skin and around internal organs. **Adipose** tissue not only acts as a long term food store; it also insulates the body against rapid changes in temperature, gives mechanical protection to internal organs and, and aids buoyancy in aquatic animals.

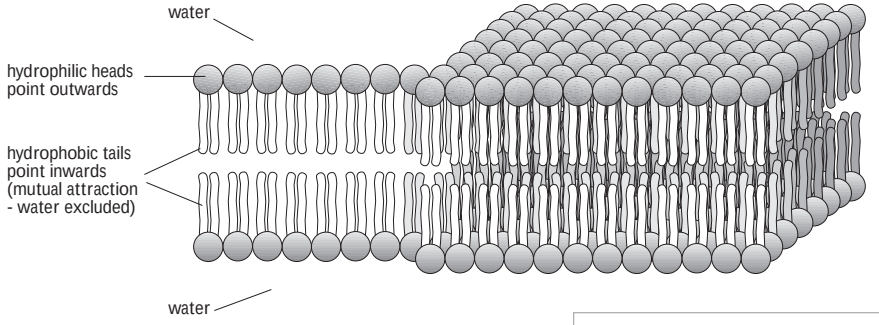
Waxes differ from fats and oils in that they have only one fatty acid chain and it is not joined to glycerol, but to a longer chain alcohol. They are produced in many animals and plants as a waterproof layer on external surfaces acting to reduce water loss (e.g the cuticle of plant leaves, and the surface layer of some insect exoskeletons).

Structure and function of a phospholipid

Phospholipids are essential to cells because they form the structural basis of all membrane systems. They have two fatty acid chains (which may be saturated or unsaturated) joined to a molecule of glycerol, the third position being occupied by a phosphate group (phosphate plus choline or ethanolamine).



The phosphate group is strongly polar and readily dissolves in water, this is the **hydrophilic** (water loving) 'head' region of phospholipids. The non polar fatty acids are repelled by water and project in the opposite direction, and these are the **hydrophobic** (water hating) 'tails'. As a result, in the presence of water, phospholipid molecules form spontaneously into a characteristic **bilayer**. It is this bilayer which forms the molecular framework of all cell membranes.



Cholesterol is another important lipid component of cell membranes although it is very different in chemical structure to the lipids so far described. It has a ring structure and belongs to a group called **steroids** which include the sex hormones testosterone and oestrogen. Cholesterol molecules are strongly hydrophobic and they take refuge between the tails of phospholipids in membranes. Their presence in the bilayer has two effects. It slows down the lateral movement of individual phospholipid molecules, making the bilayer more stable, and it prevents the movement of certain substances through the bilayer, ensuring that membrane transport occurs through the correct (protein) channels.

CHECKPOINT SUMMARY

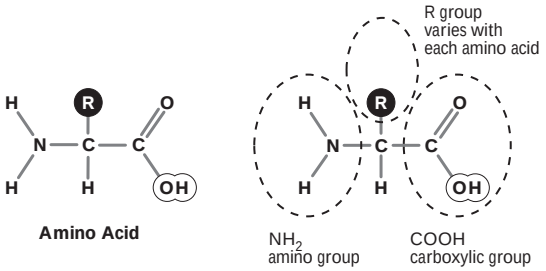
- *Lipids include fats, oils, waxes, phospholipids and steroids.*
- *Triglycerides are composed of three (tri) fatty acids combined by condensation reactions to form ester bonds with glycerol.*
- *Fatty acids have long hydrocarbon chains and a carboxyl group (COOH).*
- *Saturated fatty acids (and hence saturated fats) have no double bonds in the hydrocarbon chain which is 'saturated' with hydrogen.*
- *Unsaturated fatty acids have one double bond.*
- *Polyunsaturated fatty acids have two or more double bonds.*
- *Fats (triglycerides) are energy rich storage products in plants and animals.*
- *Lipids provide waterproofing in plant cuticles and animals e.g. insect cuticles. provide mechanical protection and insulation as sub-cutaneous fat in mammals, and bouyancy in aquatic mammals.*
- *Phospholipids are formed from triglycerides by the replacement of one fatty acid by a phosphate group.*
- *Phospholipids form the structural basis of biological membranes by forming a bilayer.*
- *The bilayer is formed with their hydrophobic poles adjacent and their hydrophilic poles in contact with the aqueous phase.*

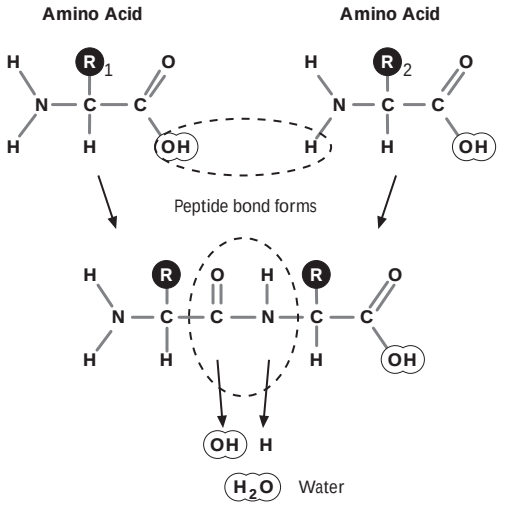
PROTEINS

Proteins are polymers made up of **amino acids,** joined together in long chains, which may be folded and twisted in various ways. There are as many as 100 000 different kinds of protein molecule in human cells. This variety of structure is made possible by the fact that the twenty different types of amino acids commonly found in proteins can be arranged in any order in chains up to 2000 units long.

Amino acids and peptide bonds

The chemical structure of an amino acid consists of a central carbon atom bonded to a hydrogen atom, a carboxyl group (-COOH), an amino group ($-NH_2$), and a side chain which varies with each amino acid, referred to as the 'R' group. **Peptide bonds** form between the amino ($-NH_2$) and carboxyl (-COOH) groups of adjacent amino acids to make chains called peptides (dipeptide = 2 amino acids linked together, polypeptide = many amino acids). As in carbohydrates, these linkages are made by a condensation reaction involving the elimination of a molecule of water.





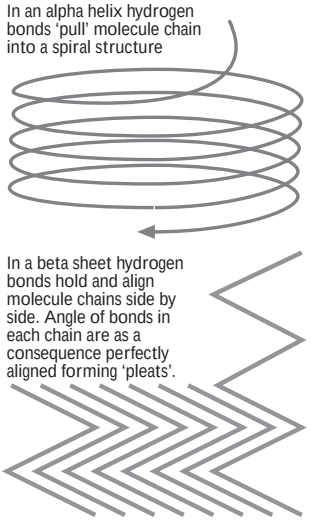
Primary structure

The amino acid sequence is the starting point or primary structure of proteins. The final shape of proteins depends upon additional bonds which form between the -NH, -C=O, and -R groups which 'stick out' of the sides of the polypeptide chain.

Secondary structure

The secondary structure of proteins is determined by hydrogen bonds between adjacent -NH and -C=O groups. Two very different formations are possible, the **alpha helix** and **beta sheet**. Both of these secondary structures may occur in the same protein

Secondary structure of proteins



Tertiary structure

The tertiary (third level) structure of proteins is the complex 3D folding determined by bonds which form between R groups. There are three possible types depending upon the chemical properties of the different R groups. R groups may be acid, basic, polar or non polar.

The relationship of tertiary structure to function

The types of bonding which hold protein molecules in position determine the shape and properties of the protein.

Hydrogen bonds are formed between polar -R groups (in addition to the H-bonds already described)

Ionic bonds are formed between the negatively charged acidic -R groups and positively charged basic -R groups. The forces are like those which exist between the Cl^- and Na^+ ions in salt.

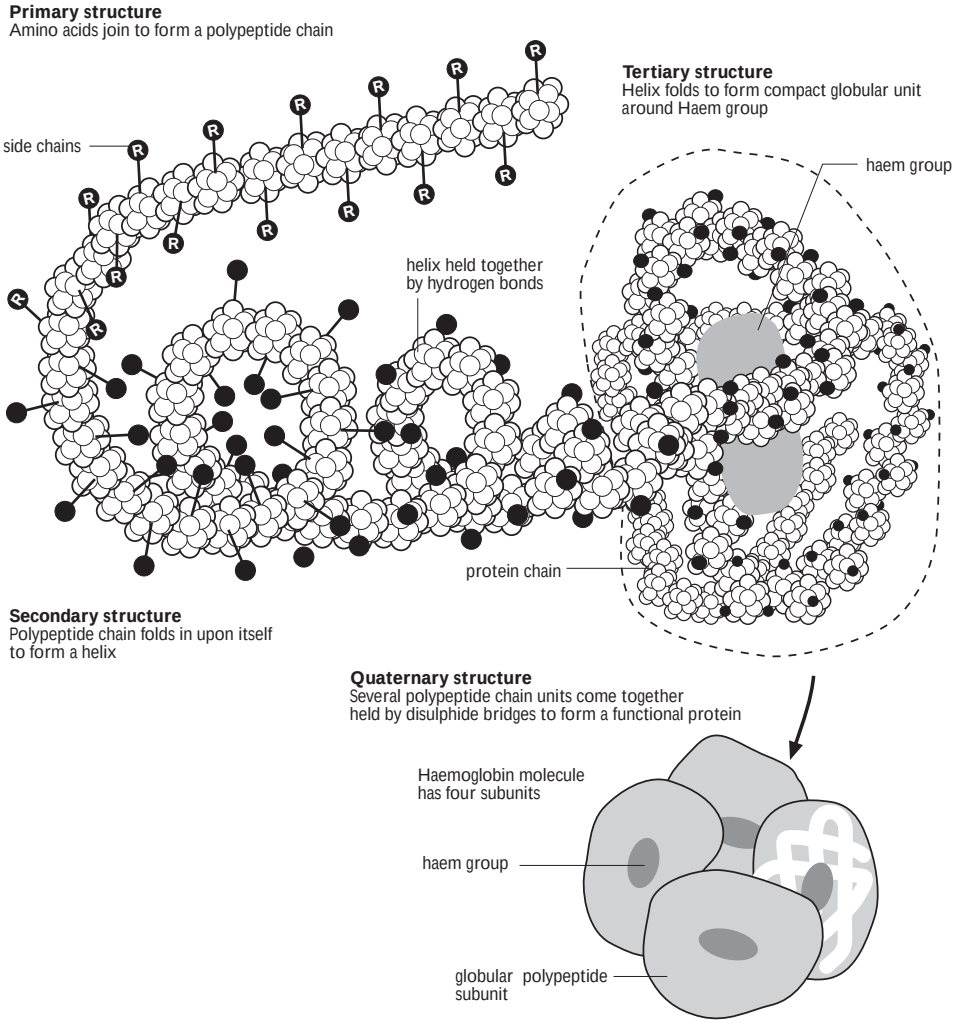
Hydrophobic bonds occur between the -R groups of non polar amino acids e.g. valine (Val) and leucine (Leu).

Disulphide bridges are much stronger covalent bonds formed between the sulphur atoms of the amino acid cysteine (Cys).

Proteins may be modified further by being linked to mineral ions, as in the case of haemoglobin or joined to carbohydrates and lipids to form **glycoproteins** and **lipoproteins**. This 'finishing off' process happens within the endoplasmic reticulum of cells.

Quaternary structure

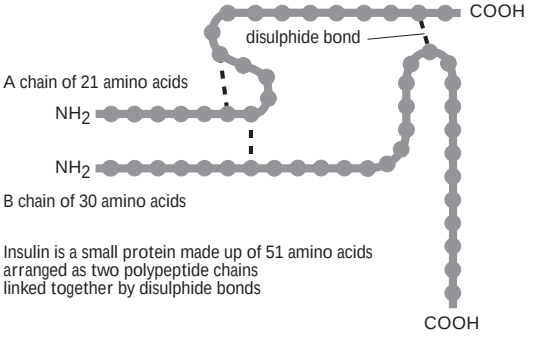
The quaternary (fourth level) structure of proteins arises from the combination of separate polypeptide chains to form a larger complex, e.g. haemoglobin, which is composed of four polypeptide chains with iron containing haem units.



Globular proteins

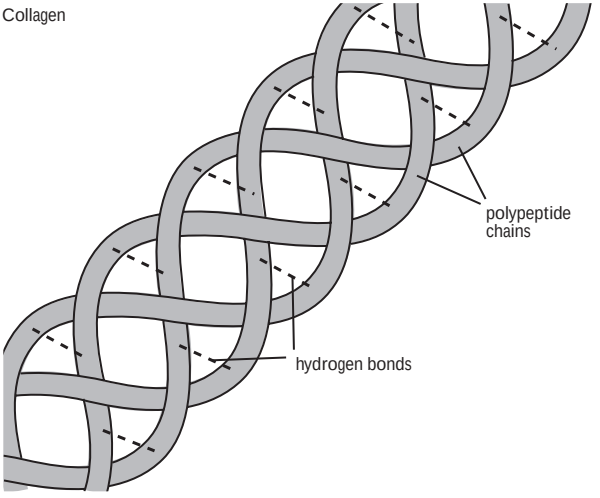
Globular proteins have a pronounced tertiary structure with many folds and twists, making them very susceptible to temperature and pH changes. Non polar groups tend to be repelled by water (hydrophobic) and tuck inside the molecule whilst the polar groups are attracted to water (hydrophilic) and they face outwards forming hydrogen bonds with water molecules. Globular proteins therefore tend to be soluble. Globular proteins are involved in metabolic activities e.g. enzymes, hormones, antibodies, and haemoglobin.

Insulin is made up of two peptide chains, one 21 amino acid units long, the other 30 units long, joined together by disulphide bridges. It is produced in the pancreas cells as a single longer chain (a **precursor** molecule) which is chopped into the two smaller pieces by a hydrolysing enzyme in a process called **activation** before being secreted into the bloodstream.



Fibrous proteins

In fibrous proteins the peptide chains are extended and inter-twined to form long thread like molecules. They do not dissolve in water and are relatively unaffected by changes in pH or temperature (they are not easily denatured). Fibrous proteins are mainly structural in function, giving support to tissues e.g. keratin in hair and skin, and collagen.



◆ CHECKPOINT SUMMARY

- *All amino acids share a common structure consisting of a hydrogen atom, an amino group (NH_2), a carboxyl acid group (COOH), and a 'R' group, all attached to a carbon atom.*
- *The 'R' group can range from a single hydrogen atom to a long complex chain. Thus the simplest amino acid has the formula of CH_2NH_2COOH.*
- *Amino acids combine in a condensation reaction which form a peptide bond, which is broken by hydrolysis (digestion).*
- *There are about 20 different types of amino acids found in proteins of living organisms.*
- *Proteins are polymers of up to 2000 amino acids (monomers) joined by peptide bonds (polypeptides).*
- *The combination of the 20 different types of amino acids gives rise to as many as 100 000 different types of proteins in living organisms.*
- *The sequence of amino acids in the polypeptide chain determines the primary structure of proteins.*
- *Hydrogen bonds between adjacent NH_2 and COOH groups result in the secondary structure of either alpha helix or beta sheet. Both of these structures may occur in the same protein.*
- *Bonds that form between the 'R' groups determine the tertiary structure.*
- *Combination or association of two or more polypeptide chains determines the quaternary structure of a protein.*
- *Haemoglobin has a quaternary structure involving four polypeptide chains and four iron containing haem groups.*
- *The three dimensional structure of protein is vital for the activity of enzymes, all of which are proteins.*
- *Proteins with complex tertiary and quaternary structures are referred to as globular proteins, e.g. enzymes and some hormones e.g. insulin, and are metabolically active.*
- *Fibrous proteins are less complex proteins and largely structural in function e.g. collagen which forms the most common connective tissue in the human body e.g. in skin, bones, tendons, etc.*

Collagen is the most important structural component of skin, bone and tendons. It is the collagen in skin which forms leather after treatment in the tanning process, and it is collagen which gives bones their 'bendability'. Bone without collagen is like concrete without the reinforcing steel rods. The word 'collagen' is Greek for 'glue maker' because it was extracted from bones by boiling them up in water and used as a strong wood glue. Chemically, collagen molecules are made up of three polypeptide chains, each consisting of about 1000 amino acid units which twist around each other like the strands of a rope (a triple helix). The molecules of collagen are insoluble and have a natural tendency to link up with each other forming fibrils several millimetres long.

BIOCHEMICAL TESTS

Test for reducing sugars e.g. glucose

All the common simple carbohydrate sugars, with the notable exception of sucrose (table sugar), have the ability to **reduce** other chemicals. They are thus called reducing sugars and they can be identified by heating with **Benedict's reagent**. Benedict's reagent contains copper sulphate and has a clear light blue colour. This changes to brick red as the copper is reduced to a solid precipitate of copper oxide in the presence of a reducing sugar. The quantity of reducing sugar in a positive test can be estimated from the amount of precipitate formed using a colorimeter or a standard colour comparison sequence.

Test for non reducing sugars

Sucrose give a negative test with the Benedict's test, but when heated to boiling with dilute hydrochloric acid it is hydrolysed into its constituent monosaccharides e.g. glucose and fructose, which when made alkaline and re-tested with Benedict's reagent give a positive result.

If a mixture of both reducing and non-reducing sugars are present, which would be highly likely with plant tissue extracts, the precipitate from the initial positive Benedict's test would need to be removed by filtration, and the resultant clear filtrate re-tested for sucrose as described above.

Test for Starch

A solution of iodine in potassium iodide is added to the sample at room temperature. The presence of starch is indicated by a colour change from light brown to blue-black.

Test for Protein - the Biuret test

2cm^3 of potassium hydroxide is added to 2 cm^3 of the tissue extract in a test tube followed by a few drops of dilute copper sulphate solution. The presence of protein is indicated by a change in colour from light blue to purple.

◆ CHECKPOINT SUMMARY

- ◆ *Generally all these tests are qualitative, i.e the results show whether a substance is present or not.*
- ◆ *They are not generally quantitative, i.e. the results do not give an accurate measure of how much of the substance is present.*
- ◆ *The Benedict's test for reducing sugars can be semi-quantitative in so much as the colour and amount of the precipitate found in a positive test is roughly proportional to the amount of reducing sugar present. Positive results can be compared to results from solutions of known concentration of reducing sugar.*
- ◆ *The same considerations apply to the iodine in potassium iodide test for starch. The colour for a positive test grading from blue-black to pale brown with decreasing amounts of starch present.*
- ◆ *The test for lipids is physical rather than chemical, depending on the observation of the formation of an emulsion.*
- ◆ *The biuret test is not a simple test for proteins but is used as such by Biologists*
- ◆ *These tests are commonly referred to as 'Food' tests as traditionally they are used on samples of food.*

NUCLEIC ACIDS

Structure of DNA and RNA

Nucleic acids are so named because they are acids found in the nucleus. They are polymers made up of repeating sub-units, in this case, **nucleotides** (mononucleotides) linking up to form a type of polymer known as a **polynucleotide**. What makes DNA so important and so unique is that it contains within its structure the genetic code, and it can produce copies of itself (replicate).

The basic structure of a mononucleotide

A mononucleotide consists of three main parts:

- a pentose (5 carbon) sugar;
- a phosphate group;
- a nitrogen-containing organic base.

There are four different types of nucleotide in DNA each with a different organic base. The bases are;

adenine (A); guanine (G), cytosine (C); and thymine (T)

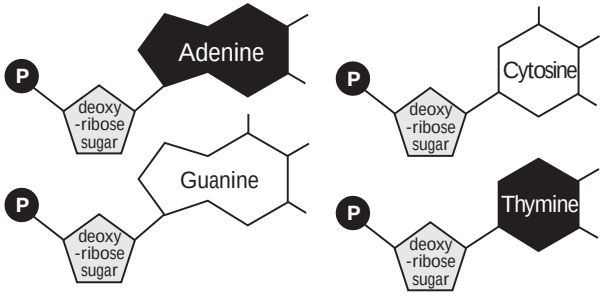
Adenine (A); guanine (G) belong to group of bases called **purines**.

Cytosine and thymine belong to a group of bases called **pyrimidines**

The components of the nucleotides are combined by condensation reactions, with the phosphate group and nitrogenous base being joined to the pentose sugar. Chains of nucleotides in turn link up by condensation reactions to form one strand of the DNA polynucleotide, with the sugar and phosphate molecules forming the 'spine' of the strand, with the bases to one side of the strand.

DNA consists of two such chains or strands linked by **hydrogen bonds** which form between pairs of bases. As the hydrogen bonds form, so the two strands of the DNA molecule twist into the spiral structure known as the double helix. Hydrogen bonds form only between specific pairs, namely A with T and C with G. The strands are therefore complementar**y** to each other. The sequence along one strand determines the sequence of the other (complementary) strand. The understanding of the structure of the DNA double helix was one of the most significant breakthroughs in Biology.

The Nucleotides of DNA



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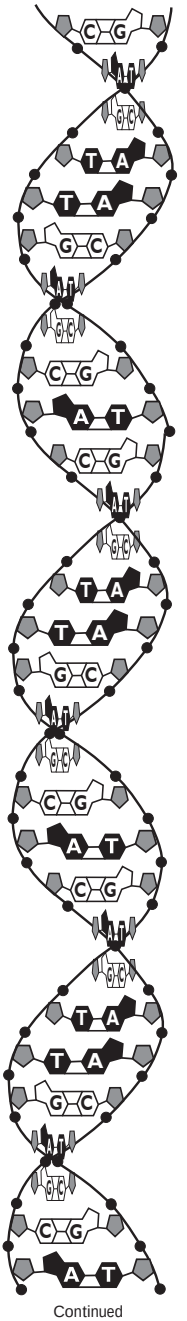

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RNA (Ribonucleic acid), like DNA, is a polymer made up of repeating nucleotides. It differs from DNA in the following three ways.

RNA is single stranded, not double stranded.

The pentose sugar in RNA is ribose, not deoxyribose (molecules of ribose contain an additional oxygen atom).

The pyrimidine base **uracil (U)** is found instead of thymine (T).

There are three different types of RNA, each designed to carry out a specific function in protein synthesis.

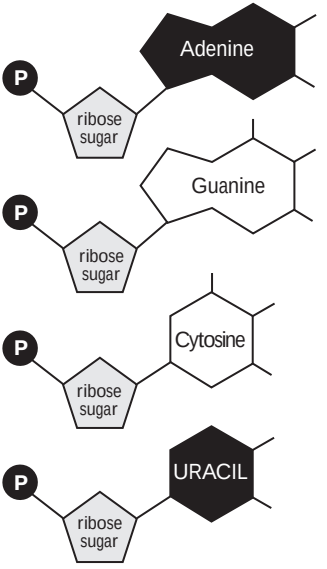
Messenger RNA (mRNA) consists of a single unfolded strand, between 75 and 3000 nucleotides long, produced in the nucleus as an exact copy of a selected section (gene) of the DNA code. The mRNA molecules travel out of the nucleus to the ribosomes where proteins (polypeptides) are synthesised.

Transfer RNA (tRNA) is between 75 and 90 nucleotides long, twisted into a characteristic shape which allows one end to attach to a specific amino acid, and the other to expose a triplet of bases called the **anti-codon** which attaches to mRNA at the site of protein (polypeptide) synthesis. tRNA molecules bring amino acids to their correct position as the protein (polypeptide) is being assembled. Each type of amino acid is carried by a different type of tRNA molecule with a specific anti-codon.

Ribosomal RNA (rRNA) consists of more than 1000 nucleotides and is found in ribosomes which are composed of an approximately equal mixture of proteins and rRNA. Ribosomal RNA is manufactured within the nucleolus of the nucleus.

The full functions of these RNAs is described in the section on the synthesis of proteins (polypeptides).

The Nucleotides of RNA

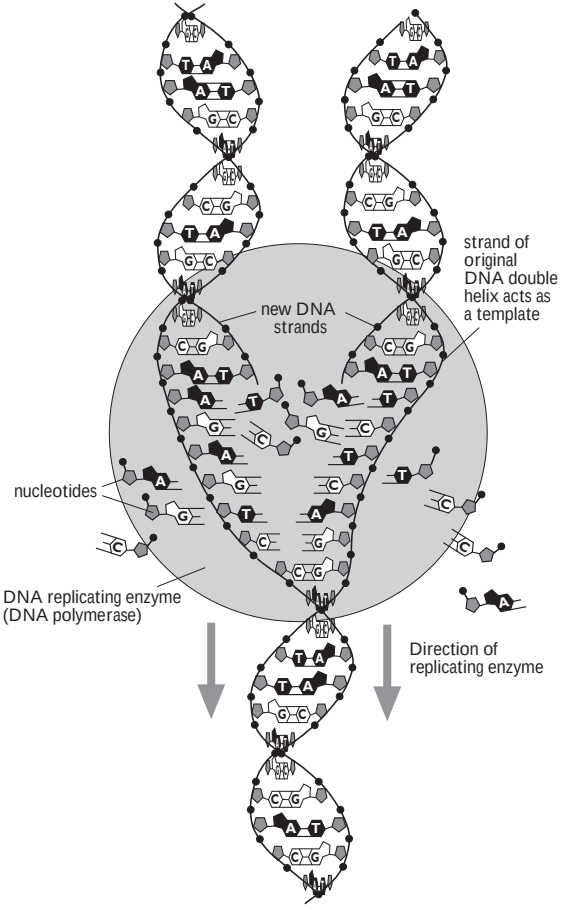


Replication of DNA

In the process of DNA replication, one double stranded DNA helix gives rise to two new DNA double stranded helices (plural of helix). To achieve this the two strands of a DNA helix unwind and separate, and each acts as a pattern (template) for the formation of another new strand. Each of the new daughter DNA molecules has one of the original strands and a new strand. For this reason, the process of replication is said to be semi-conservative, half of the original molecule is conserved in each of the two new DNA molecules. The process requires a supply of the four different types of nucleotides, energy in the form of ATP, and an enzyme called DNA polymerase. DNA polymerase is required to catalyse the condensation reactions by which free nucleotides combine to form a copy of the template strand.

Experimental evidence

for the semi-conservative mechanism of DNA replication includes experiments in which the mass of the DNA content of *Escherichia coli* (a bacterium), grown on different nutrient media was determined and compared. One batch of microorganisms was cultured on a medium containing a 'heavy' isotope of nitrogen (^{15}N), the DNA was extracted and compared with that of organisms cultured on a medium containing normal 'light' nitrogen (^{14}N). The *E.coli* cells grown on 'heavy' nitrogen were then transferred to a new medium containing only light nitrogen and allowed to divide once to produce a new generation. The DNA of these daughter cells was found to be halfway between the 'heavy' and 'light' values, indicating that half the DNA was old and half was new (semi-conservative replication).



The genetic code

The DNA base sequence forms a four letter language which, as you have seen, can be copied every time a cell divides. This DNA base sequence determines the sequence of amino acids in a polypeptide chain (the primary structure of proteins). A sequence of nucleotides and their bases which codes for one polypeptide is known as a gene. Each gene codes for a different polypeptide chain. The primary structure of a polypeptide is the basis of the particular structural shape and characteristic of the final protein, e.g. enzymes, structural materials and membrane proteins. The base code determines the structure, functions and individuality of every organism.

Remember that up to twenty different types of amino acids may occur in a single protein. The DNA code has a four letter base language (A, G, C, T). A triplet of bases (sequence of three in a row) codes for each amino acid, for example 'CGC' codes for the amino acid alanine (Ala) whilst 'GCT' codes for arginine (Arg), so the code 'CGC-GCT-CGC' would be translated as 'Ala-Arg-Ala' in a polypeptide. Note that the code is non-overlapping, i.e. it must be read '**C**GC-**G**CT-**C**GC', not '**C**GC-GC**G**-C**G**C-**G**CT-CT**C**-' etc.

Sixty four (4^3)different triplet codes (codons) are possible with this four letter base language, more than sufficient to code for the twenty different types of amino acids. In fact most amino acids are coded for by more than one codon. As a result of this 'spare capacity' in the code, the code is referred to as a 'redundant' code. The process by which this code is used in protein synthesis, is described below.

Protein Synthesis

Protein synthesis involves all three of the RNA types described above working in combination, and is achieved in two stages. As the DNA cannot leave the nucleus, the code has to be read and copied (transcribed) into mRNA, in a process called **transcription**. The mRNA moves out of the nucleus and associates with the ribosomes, where the code (now embedded in the mRNA) is used in the synthesis of a polypeptide in the process of **translation**.

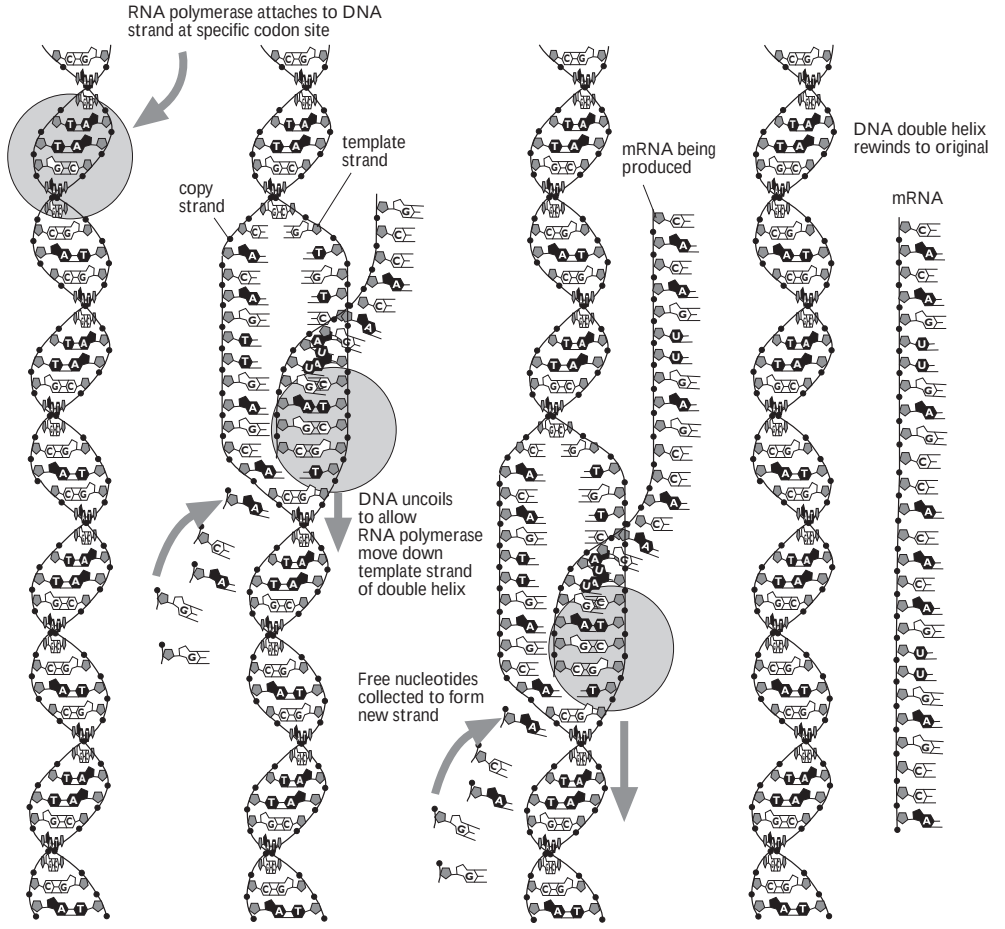
Transcription begins as an enzyme, **RNA polymerase**, attaches to the 'Start' codon of a gene. The DNA base sequence of this 'Start' codon is always 'TAC'. As RNA polymerase attaches to the DNA, the hydrogen bonds binding the two strands of the DNA double helix break apart to expose the sequence of triplet base codons on both strands. The enzyme then travels rapidly down the length of the gene like a zip fastener unzipping the DNA. As it does so, a molecule of mRNA is formed as a copy of one of the DNA strands. Only one of the two DNA strands is copied into mRNA, called the **template strand**. The mRNA is therefore an exact replica (save that 'U' is substituted for 'T') of the other strand, consequently referred to as the **copy strand**. When the enzyme reaches the 'Stop' codon of the gene, transcription ends, the enzyme is free to start the process again, and mRNA is released out through a pore in the nuclear membrane to the ribosomes where translation occurs.

A complicating feature of the process of transcription in eukaryotic cells (cells with a nucleus) is that the length of DNA (the gene) to be copied into mRNA, has sections of 'junk code' which do not code for any of the required amino acids in a polypeptide chain. The 'junk' sequences are called **introns** and the parts to be transcribed and translated are called **exons**. The whole length is transcribed into mRNA, but before it is released from the nucleus, the introns are cut out with the assistance of enzymes, leaving a single, 'unpolluted', strand.

CHECKPOINT SUMMARY

- *DNA and RNA are long chain polynucleotide molecules with repeating sub-units of mononucleotides combined as a result of condensation reactions.*
- *Mononucleotides consist of a 5 carbon sugar, a phosphate, and an organic base.*
- *In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine.*
- *In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine.*
- *RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine.*
- *There are three types of RNA - messenger, transfer and ribosomal.*
- *The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine.*
- *DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.*
- *The synthesis of new strands of DNA is catalysed by enzymes, including DNA polymerase.*
- *A gene is a length of DNA which carries the genetic information for the synthesis of a single polypeptide chain. The gene determines the primary structure of the polypeptide, that is the sequence of amino acids.*

Transcription

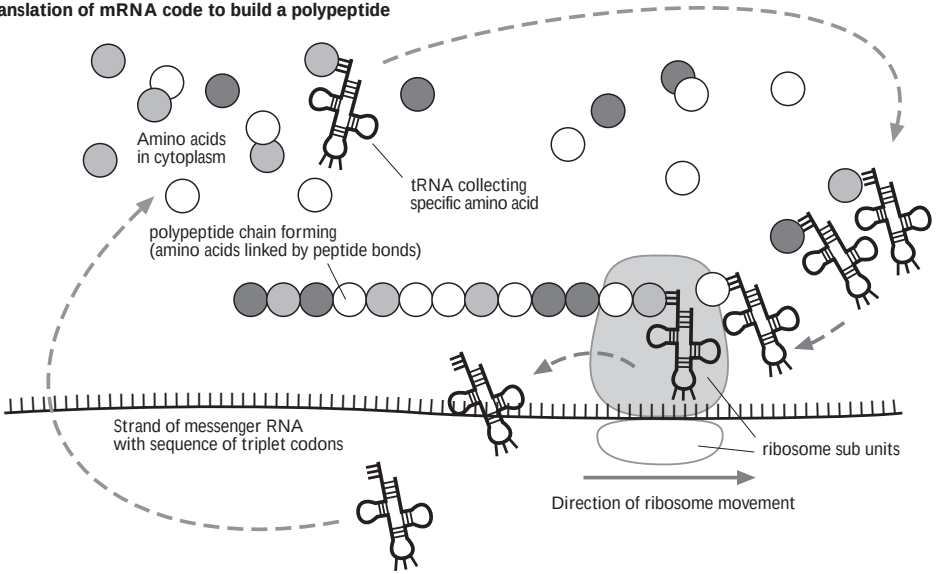


Translation occurs at ribosomes which are made up of two sub-units, one larger than the other, which come together only for the purpose of translating a length of mRNA. The mRNA binds to the small sub-unit at its 'Start' codon (AUG). It is then joined by the large sub-unit along with a molecule of tRNA which has the anti-codon 'UAC' and which carries the amino acid methionine. As the three different RNA types come together, the interface between the small and large sub-unit of the ribosome forms two binding sites allowing two triplet codons be lined up side by side. The process of translation can then proceed.

A second tRNA now occupies a position on the mRNA alongside the first and, as it does so, a peptide bond is formed between the two amino acids. The ribosome moves along the length of the mRNA, and once a tRNA molecule has delivered the appropriate amino acid it is released to repeat the process. New tRNA molecules with their amino acids come to occupy the ribosomal coding sites, and the polypeptide chain grows ever longer. When the ribosome reaches the 'Stop' codon (UAG) on the mRNA, it splits into its separate sub-units releasing the mRNA and the now complete polypeptide. A number of ribosomes move along the mRNA at the same time so that many polypeptide molecules are formed simultaneously. It is calculated that, in the average mammalian cell, more than a million new peptide bonds are being formed every second.

Generally mRNA molecules only have a short existence, sometimes just a few minutes, so that if continuous supplies of a particular protein is required the above processes must be repeated.

Translation of mRNA code to build a polypeptide



DNA control of enzyme synthesis

All enzymes are proteins, and are synthesised in the processes of transcription and translation in the same way as all proteins. The amount produced is regulated by any factors which influence these processes. These include the switching on and off of genes, and the length of life of the mRNA. Some enzymes are only produced (induced) in the presence of the substrate, and for this to be possible there must be some feedback signal from the cytoplasm of the cell which detects the presence of the substrate and results in the appropriate gene being switched on, for example lactase enzyme is produced in response to the presence of lactose.

The Human Genome Project

The 'genome' is the complete set of genetic information contained within a single cell. It is copied nearly exactly in every cell of the body. As you have seen, the DNA lengths which make up the individual genes contain 'junk' sequences called introns which are not translated into protein. In addition there are many repeating sequences of DNA lying between the genes which have no known role in protein synthesis. In fact 95% of the human genome is 'junk' DNA.

The Human Genome Project, which began in the mid 1980s, aimed to map and sequence all the nucleotides including those of the 'junk' sections. '**Mapping**' entails discovering the exact location of each gene on the different chromosomes: '**sequencing**' means determining the position of every letter of the base code on every length of DNA. The Human Genome Project has received worldwide cooperation and the first complete drafts emerged in early 2000. In all, there are about one billion codons in the human genome (3 billion nucleotides) and each chromosome contains about 4000 genes on its DNA.

You may be wondering why the scientific world should consider it so important to know these precise details. Different answers might be given by people working in different fields. For the academic geneticist, the genome is an autobiography of the human species. It contains genes acquired at every stage of the evolution of our species from the earliest prokaryotic life forms, and reveals patterns of inheritance which explain relationships so far undiscovered from the fossil record and other sources. For medicine it opens up a completely new horizon: the possibility of diagnosing the susceptibility of particular individuals to particular diseases, as the following example illustrates.

Huntington's chorea is a disease which results in degeneration of mental processes (dementia) and loss of motor control. The onset of this condition is characterised by uncontrolled, random flicking movements. It is inherited in a gene which is located on chromosome number 4. The gene normally contains the codon CAG repeated over and over, normally ten to fifteen times, but many more in people susceptible to the disease. The exact age at which Huntington's disease develops seems to depend upon the number of times this codon is repeated. 39 repetitions, for example gives a 90% chance of dementia by the age of 75, and 50 repetitions predicts the onset of dementia at the age of 27.

You might argue that knowing what the future holds in store is less than small comfort if there is no chance of prevention or cure but it

CHECKPOINT SUMMARY

- *The sequence of nucleotide bases on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living organisms.*
- *Three bases (a triplet codon) code for one amino acid.*
- *There are 64 (4^3) possible combinations of any three from four bases, but only 20 amino acids to be coded for.*
- *Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'.*
- *A gene is a sequence of nucleotide bases of a DNA molecule which codes for a polypeptide.*
- *A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons.*
- *Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon.*
- *Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood.*
- *Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells.*
- *The genetic code (a gene) for a polypeptide is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes.*
- *The ribosomes 'read' the genetic message on the mRNA, and tRNA delivers amino acids in the sequence determined by the code.*
- *There are 20 different types of tRNA, each specific to a particular amino acid.*
- *Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid.*
- *The amino acids combine by means of peptide bonds to give a polypeptide chain.*
- *The polypeptides subsequently form proteins.*
- *As enzymes are proteins their synthesis is controlled by DNA.*

does give options which did not exist before the Human Genome Project. Knowing that you are in a high risk category for a particular disease might affect your decision about taking up smoking, boxing or rugby football, for example. More importantly, doctors will soon be able to treat the individual patient and not the population, for example by prescribing a drug according to the individual's genotype and not simply referring to the national statistics on adverse reactions and side effects.

Such knowledge could be used to discriminate against people with certain sequences, in education, employment, health insurance, marriage, and many other situations; leading to an 'underclass' condemned on the grounds of their base sequences. Considering the readiness with which many people are willing to 'label' others, the base sequence could become the ultimate 'label'.

Spiritually, morally and ethically the debate includes the consideration of emergent properties, that is how much more we are than simply the sum of our parts. Mapping and sequencing are analytical approaches, and do not address the problem of the human condition in its entirety in the context of the ever changing environment.

1.2 Enzymes

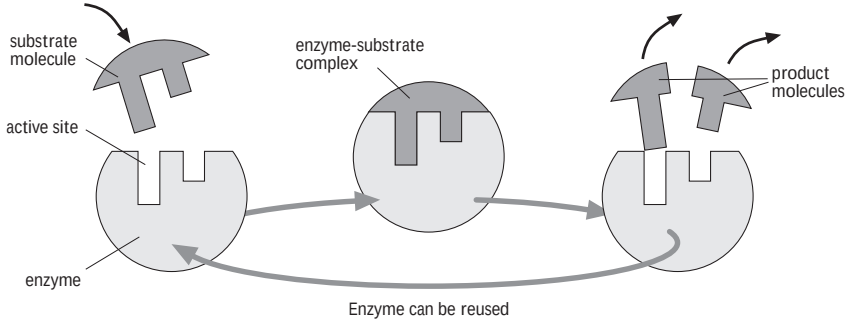
Content

- The structure of enzymes as globular proteins, and the concept of the active site and specificity
- Enzymes as catalysts which reduce activation energy
- The effects of temperature, pH, substrate and enzyme concentrations on enzyme activity
- Active site-directed and non-active site-directed inhibition of enzyme action
- The commercial uses of enzymes as illustrated by pectinases in food modification and proteases in biological detergents
- The advantages of the immobilisation of commercial enzymes, as illustrated by lactase
- (Practical work to include experiments to investigate the effects of temperature, pH and enzyme concentration on enzyme activity using suitable enzymes, illustrations of enzyme immobilisation using lactase, the use of pectinase in the production of fruit juice)

Introduction

Most students of science can name an enzyme. Usually it is a digestive enzyme like amylase or pepsin, but enzymes are not just concerned with breakdown reactions like digestion, they can also help build things up. In fact, every chemical reaction in every living cell is made possible by an enzyme. Several thousand enzymes have been isolated and studied.

Enzymes are protein molecules. Compared to many other molecules they are relatively large (macromolecules) of the globular type with a complex tertiary structure. What gives each enzyme its particular qualities and mode of action is the way in which its peptide chains are folded and twisted into specific shapes. As you will see in the following account, enzyme action depends upon shape recognition between enzymes and other reacting molecules called **substrates**.

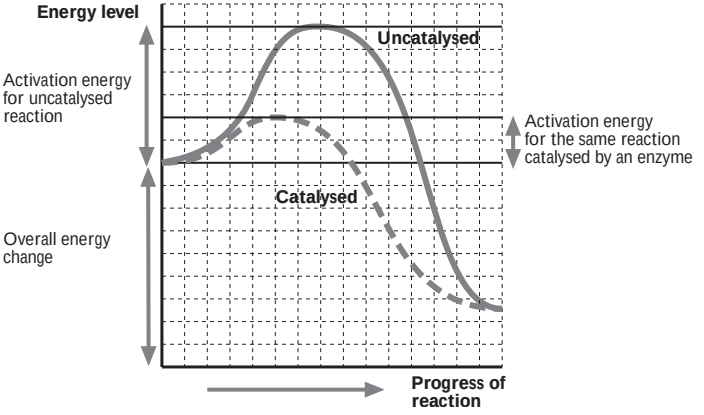


Enzymes as catalysts

Enzymes are **biological catalysts**. A catalyst is a substance which speeds up a reaction without being changed itself in the process. As they are not used up or changed, enzymes can be used repeatedly; therefore they are effective in relatively small amounts.

Usually enzyme molecules are much larger than the reacting molecules (substrates). In an enzyme-catalysed reaction, the substrate(s) become attached to a particular region on the enzyme called the **active site** which has a specific shape and structure matching that of the substrate(s), forming the **enzyme - substrate complex**. This association is temporary and lasts only long enough to allow the substrate molecules to interact to form the products. Once the products are formed, they are released from the active site and the enzyme is free to repeat the process.

The formation of the enzyme-substrate complex lowers the **activation energy** which is the energy needed to start a reaction. In the laboratory, it is common for reacting substances (substrates) to be heated in order to make the reaction occur more quickly. The heat energy causes the molecules to move about more rapidly (i.e. it gives them more kinetic energy) so that they collide more frequently, forming the product. In other words heat is used to overcome the activation energy. Living cells operate at a constant, relatively low temperature so therefore they rely on enzyme catalysts to overcome the activation energy.

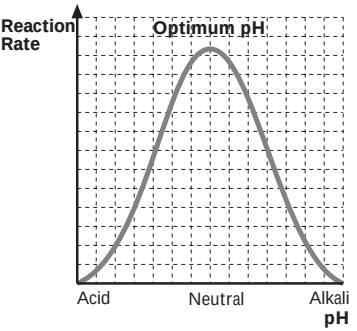


The active site matches the substrate, so different shaped substrate molecules require different shaped active sites. Most biological reactions therefore involve different **specific** enzymes. This is possible because proteins which make up enzymes can be made in an infinite variety of shapes as a result of the nature and sequence of the amino acids and their R groups.

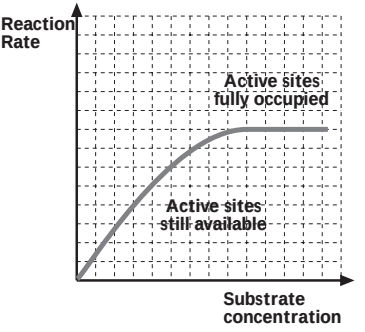
This model of enzyme action is referred to as the **lock and key model**, but it is known that the shape of the active site may change as the substrate makes contact with it, adjusting to make a close fit, rather like a soft glove around a hand, a mode of action called **induced fit**.

The effects of temperature, pH, substrate and enzyme concentration on enzyme activity

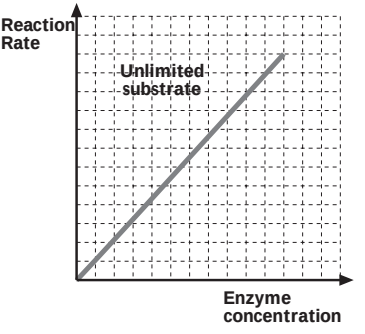
pH is a measure of H^+ ion concentration, which is a measure of acidity and alkalinity. A pH of 7 is neutral, less than 7 is acid, and more than 7 is alkaline. Small changes in pH can affect the association process between enzyme and substrate which happens at the active site. The attraction between substrate and enzyme is often the result of small electric charges at the active site, and these are disrupted by changes in H^+ ion concentration. Extremes of pH, i.e. very acid or alkaline conditions, cause permanent denaturation. Enzymes have an optimum pH for efficient functioning. Usually this is around pH7, the normal pH of cells. Notable exceptions are the enzyme pepsin, which is found in the stomach and works best in highly acidic conditions in the range pH 1-2, and the enzyme trypsin which is found in the duodenum and has an optimum pH of 9.



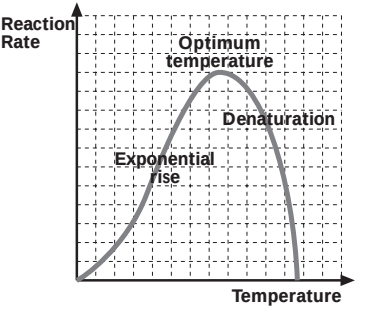
Temperature affects the rate of all chemical reactions. As the temperature increases, so does the kinetic energy of the reacting molecules. More enzyme/substrate complexes are formed as the reactants collide more frequently, and the reaction rate rises exponentially, doubling for every 10ºC rise in temperature. However, enzymes, like all proteins, suffer irreversible alterations in molecular shape above certain temperatures as a result of the breaking of chemical bonds within the molecule, so that in enzyme-catalysed reactions, there is an **optimum** temperature above which the reaction rate drops sharply. For an enzyme, any change in the shape of the active site means a loss of function, the enzyme is said to be **denatured**. For most enzymes, the optimum temperature is around 40ºC, but there are exceptions. For example, some bacteria living in hot springs have enzymes which function at temperatures above 85ºC, whilst the ice fish of the Antarctic possess enzymes which operate at -2ºC.



Enzyme Concentration exerts a direct effect on the rate of the reaction as long as there is a plentiful supply of substrate. Any increase in the number of enzyme molecules will result in a proportional increase in the number of enzyme-substrate complexes and therefore an increase in the rate of reaction.



Substrate concentration has a similar effect. At low substrate concentrations, not all the active sites of the enzyme molecules will be occupied, so the rate of the reaction depends upon the concentration of substrate alone. As the concentration of substrate increases, so all of the available active sites will become filled. The upper limit is determined by the amount of enzyme molecules available.



Enzyme Inhibitors

Enzyme inhibitors are substances which interfere with the active site of the enzyme.

Active site-directed inhibitors block the actual active site directly.

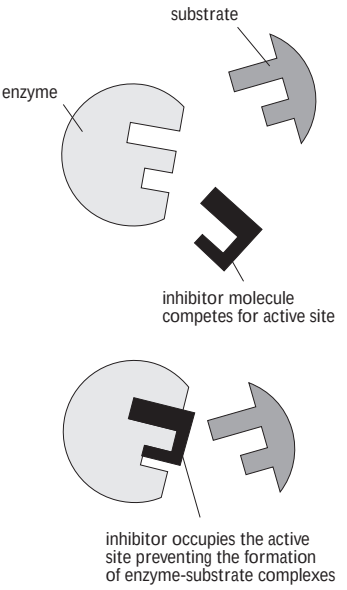
Non-active site-directed inhibitors attach a part of the enzyme away from the active site, but alter the enzyme's molecular shape so that the enzyme-substrate complex cannot be formed.

Competitive inhibitors are active site-directed substances which have a molecular structure resembling that of the substrate. They attach themselves to the active sites forming inhibitor/enzyme complexes and cause the reaction to slow down or stop altogether. The effect of competitive inhibitors depends upon the relative concentrations of the substrate and inhibitor, and also the relative stability of the enzyme/substrate and inhibitor/enzyme complexes. This type of inhibition is seen in the action of a group of antibiotics called sulphonamides, which compete for the active site of a key bacterial enzyme involved in synthesising an essential growth factor, not found in the cells of the host.

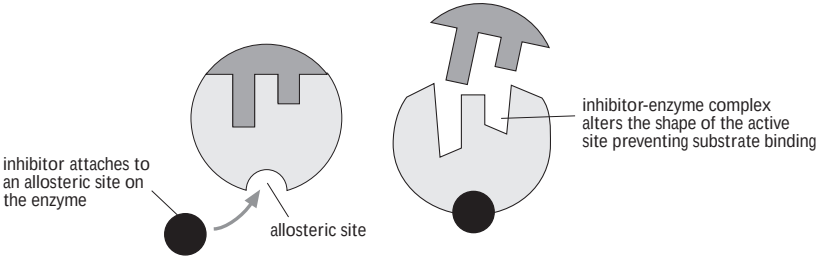
Non-competitive inhibitors are non-active site-directed and are substances which bind onto the enzyme at a place other than the active site called an **allosteric site,** causing the enzyme to change shape sufficiently to stop enzyme-substrate complexes being formed. Non-competitive inhibitors are not similar to the substrate molecules and are not affected by the substrate concentration. The ions of some heavy metals such as arsenic and cyanide may bind onto allosteric sites and act as poisons, stopping important enzyme controlled reactions. (Some enzyme molecules possess an allosteric site which must be occupied by an **activator** substance to give the enzyme its correct working shape. Without the activator, the enzyme will not work.)

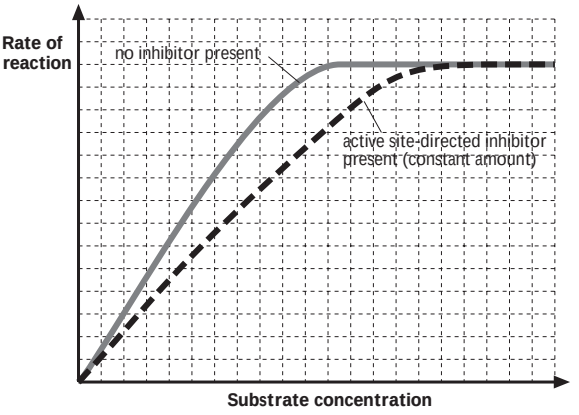
Inhibitors and activators are important in regulating the activity of enzymes in the complex metabolic pathways of the body.

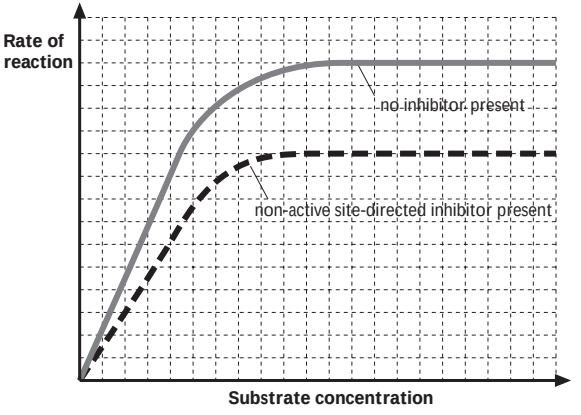
Active site-directed



Non-active site-directed







CHECKPOINT SUMMARY

- *All enzymes are protein in nature, and fall into the group of 'globular' proteins with a complex 3D structure*
- *Enzymes are catalysts which speed up the rate of reaction of much larger amounts of reactants without themselves being used up in the process*
- *Unique amongst catalysts they are denatured by extreme conditions of pH and temperature etc.*
- *Their activity depends upon their complex 3D shape which creates an active site which combines with the substrate to form an enzyme/substrate complex which lowers the activation energy of reactants*
- *Their complex 3D shape also accounts for enzyme specificity as the active site has a shape complementary to the shape of the substrate*
- *Disruption of the 3D shape accounts for denaturation*
- *pH has an effect on the 3D shape of enzymes, especially the active site which has an electric charge*
- *Any effect on the active site alters the reactivity of the enzyme. There is an optimum pH, either side of which reactivity drops*
- *Extremes of pH permanently disrupt the active site and thus denature the enzyme*
- *Similar effects are seen with temperature, except low temperatures do not denature enzymes. The rate of enzyme catalysed reactions, like all chemical reactions, are reduced with low temperatures*
- *Optimum conditions of pH and temperature vary with the particular enzyme, which reflects the conditions in which the cell exists*
- *The relative amounts of enzyme and substrate affect the rate of reaction as it affects the formation of the enzyme substrate complex. Only one can be rate regulating or limiting at any one time*
- *Active site-directed inhibition interferes with the active site so that combination with the substrate is prevented, e.g. competitive inhibition when a substance with a shape similar to the substrate occupies the active site*
- *Non-active site-directed inhibition involves combination of a substance (often an end product) with an allosteric site, which alters the overall shape of the enzyme, and thus the active site.*

The commercial use of enzymes

Although enzymes have only been investigated scientifically for just over a century, they have been used for by humans for thousands of years: they are, for example, essential in brewing (the name enzyme meaning "in yeast"), the production of cheeses, and the practice of hanging meat before eating it is based on the tenderising action of protease enzymes. While we are still far from identifying and understanding the range of enzymes and enzyme functions in living cells, knowledge of some enzyme catalysed reactions has led to the development of enzyme-based technology. A range of enzymes now have important commercial applications in industries including the food and drink industry, the paper industry, and the pharmaceuticals industry. For a range of financial and biological reasons most of the enzymes used in these industries are obtained from fungi and bacteria.

Since it has become possible to extract pure enzymes from cultures of bacteria and fungi on a commercial scale, they have been put to use in many different ways.

Biological Washing Powder is one which contains digestive enzymes in addition to the normal detergent. Protein digesting enzymes **(proteases)** help break down protein stains like egg and blood. Fat digesting enzymes **(lipases)** deal with grease, whilst starch digesting enzymes **(amylases)** work on starch based food products such as gravy. The broken down products of these 'biological stains' are then easily washed out.

Washing powder manufacturers invest heavily in the development of new, genetically designed, enzymes which can operate at the temperatures and pH levels of the average washing machine. Although some early forms used enzymes that could only resist temperatures of 40ºC, newer products can be used in washes at 60ºC or above with improved results. Fabric Softener may contain the cellulose digesting enzyme **cellulase.** It is present in concentrations just sufficient to erode the tiny cellulose fibres on the surface of cotton garments, making them feel softer and smoother.

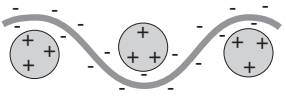
The extraction of fruit juice involves the release of the juice of a fruit from within the sap vacuoles of its cells. In order to extract it more efficiently, two types of enzyme are added before the fruit is crushed. One is cellulase, which weakens the cellulose cell walls. The other is **pectinase,** an enzyme which attacks the glue-like substance **pectin** which forms the middle lamella, sticking cells together. Mixtures of the enzymes are available commercially from a fungal source (*Aspergillus niger*). In the preparation of citrus fruit juices (orange, lemon, lime and grapefruit), the fruit skins are subjected to abrasion first so that the enzymes can penetrate the fruit tissues, then after soaking in the enzyme mixture (usually at around 35ºC) for a few hours, the peel and pith can be easily separated from the juice containing tissues. The resulting yield of juice is greater and purer than from the traditional practice of crushing the fruit whole, then separating the solid matter.

Immobilisation of commercial enzymes

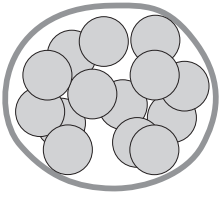
Since enzymes, like all catalysts, do not get used up in the processes they catalyse, they can be reused again and again. This is particularly desirable since enzymes are expensive to produce. One of the difficulties of reusing enzyme is, however, the difficulty of recovering the enzyme from the substrate where it is in a very low concentration. This can be a time consuming and expensive exercise. If the enzyme is not recovered then not only is the process wasteful, but may also be harmful as the final product will be contaminated with enzyme.

For these reasons the development of immobilised enzymes has improved enzyme technology enormously. Immobilised enzymes are enzymes that have been attached to another (insoluble) material in some way and hence prevented from moving freely within the substrate mixture. They may be attached to a fixed membrane or gel made of a range of substances including silica, starch, or cellulose. In other cases the enzyme may move within the substrate but only once encapsulated within beads of another material. In the former systems enzymes never become mixed with the product whereas encapsulated enzymes are mixed in with the final product but are easily extracted by filtering or other methods.

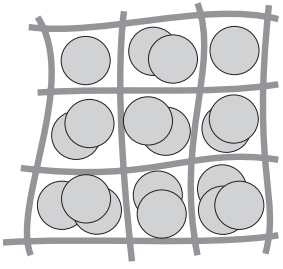
Absorbtion



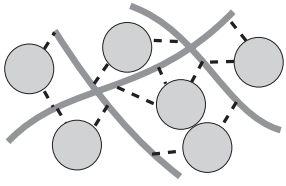
Encapsulation



Entrapment



Covalent bonding



There are many advantages to immobilising enzymes in addition to cost and ease of extracting a pure product.

- Immobilised enzymes may be more easily added to and removed from reactors to allow the rate of reaction to be more carefully controlled. This means that enzymes can be used to partially but not completely digest a substrate.
- Immobilised enzymes can lead to much greater production efficiency. They can be used in a continuous enzyme reactor where substrates are constantly added and products constantly produced without the need to add or extract enzyme.
- Immobilised enzymes tend to be more stable (better able to resist alteration to shape and activity.) In particular they are less likely to be inactivated or denatured by changes in pH, presence of other chemicals, or high temperatures (they are more thermostable).
- Immobilised enzymes can also be used for longer before their activity decreases and are less likely to lose activity during periods of storage.

A disadvantage of immobilising enzymes is that the rate of reaction may be slower because the enzyme has reduced kinetic energy and is often partitioned from the substrate by a membrane through which the substrate must diffuse.

Use of immobilised lactase to produce lactose-reduced milk

Lactose (milk sugar) is a disaccharide made up of two hexose sugars (glucose and galactose). It is essential to the diet of infants, but adults may develop 'intolerance' to lactose which means that they are unable to digest it and it remains unaltered in the intestine. This, in itself, is not a problem but undigested lactose may be acted on by bacteria to produce lactic acid (lactate) which can cause abdominal pain and diarrhoea.

Lactose containing milk can be converted to lactose-reduced milk by trickling it over a column of alginate beads containing immobilised lactase enzyme. By testing the milk outflow at the bottom of the column for the presence and relative quantity of monosaccharide products (glucose and galactose), the flow rate can be adjusted to its optimum efficiency.

CHECKPOINT SUMMARY

- *Enzymes have many commercial uses as a result of their catalytic properties and specificity, e.g. pectinases in food modification and proteases in biological detergents*
- *The extraction of fruit juices aided by addition of enzymes; cellulase which weakens cellulose cell walls, and pectinase which attacks glue-like substance pectin which forms middle lamella, sticking cells together*
- *The resulting yield of juice is greater and purer than from the traditional practice of crushing the fruit whole, then separating the solid matter*
- *A biological washing powder is one which contains digestive enzymes in addition to the normal detergent*
- *Protein digesting enzymes (proteases) help break down protein stains like egg and blood. Fat digesting enzymes (lipases) deal with grease, whilst starch digesting enzymes (amylases) work on starch based food products such as gravy. The broken down products of these 'biological stains' are then easily washed out*
- *Newer products with thermostable enzymes can be used in washes at 60°C or above with improved results*
- *Immobilisation of commercial enzymes enables them to be reused again and again, and prevents them being lost to contaminate the end product*
- *Immobilised enzymes are enzymes that have been attached to another (insoluble) material in some way and hence prevented from moving freely within the substrate mixture*
- *They may be attached to a fixed membrane or gel made of a range of substances including silica, starch, cellulose. In other cases the enzyme is encapsulated within beads of another material and mixed within the substrate, and are recovered by filtering or other methods*
- *Immobilised enzymes can lead to much greater production efficiency with continuous flow of reactants past the immobilised enzyme*
- *Immobilised enzymes tend to be more stable and are less likely to be inactivated or denatured by high temperatures, changes in pH, or presence of other chemicals*
- *Immobilised enzymes can also be used for longer before their activity decreases*
- *A disadvantage of immobilising enzymes is that the rate of reaction may be slower because the enzyme has reduced kinetic energy and is often partitioned from the substrate by a membrane through which the substrate must diffuse*
- *An example is immobilised lactase used to produce lactose reduced milk.*

1.3 Cellular Organisation

Content:

Prokaryotic cells

- The structure of a bacterial cell and its inclusions as illustrated by *Escherichia coli*.
- The roles of the cell wall, cell surface (plasma) membrane and its invaginations, flagella, bacterial chromosomes, plasmids, glycogen granules and lipid droplets.

Eukaryotic cells

- The organisation of eukaryotic cells as illustrated by a leaf palisade cell and a liver cell.
- Light and electron microscopy compared (magnification and resolution)
- The structure and roles of the nucleus, nucleolus, rough and smooth endoplasmic reticulum, Golgi apparatus, lysosomes, chloroplasts, mitochondria, ribosomes, centrioles and microtubules, the cellulose cell wall.
- The structure, properties and roles of the cell surface (plasma) membrane.

Transport across membranes

- How molecules and ions move into and out of cells.
- The principles involved in passive transport by diffusion and facilitated diffusion.
- The principles of osmosis in terms of the diffusion of water molecules from a higher to a lower water potential through a partially permeable membrane.
- The factors which affect water potential
- The principles involved in active transport; endocytosis and exocytosis.

Aggregations of cells

- Tissues as aggregations of cells of common origin, structure and function, as illustrated by the tissues of a mesophytic leaf.

Introduction

All living organisms are composed of cells. It is calculated that an average human adult is made up of around 10^{14} cells, each coordinated with the others to form functional tissues, organs and systems. At the other extreme, some organisms are composed of a single cell; Amoeba, bacteria and yeast are examples of single celled organisms.

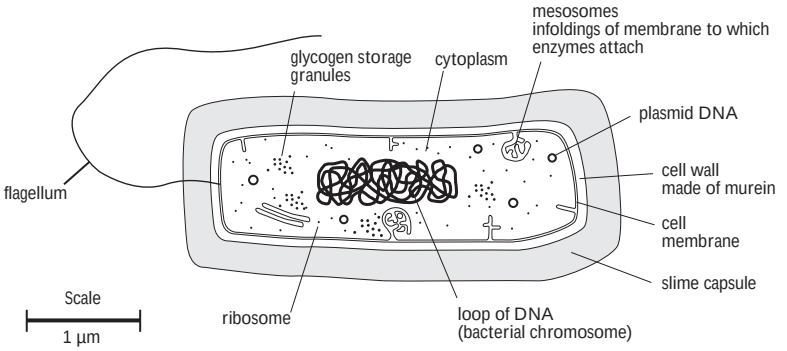
Cells are grouped into two major types, **prokaryotic** and **eukaryotic**, the most important distinction between them being the presence or absence of a nucleus ('*karyon*' in Greek means 'nucleus', '*eu*' means true, and '*pro*' means 'before'). Eukaryotic cells are so named because they have a true nucleus and prokaryotic cells are built on an earlier design without a true nucleus.

PROKARYOTIC CELLS

Prokaryotes are all single celled organisms and they are classified in a separate kingdom called **Kingdom Prokaryotae**, and include the bacteria. Prokaryotic cells are much smaller than eukaryotic cells, ranging from 1-10 μm in size (eukaryotic cells range from 10-100 μm). Although bacteria are all constructed according to the same basic pattern, they are a very diverse group with individual types capable of inhabiting parts of the earth as distinct from each other as hot sulphurous springs to permafrost, some are photosynthetic, others (decomposers) live off the dead remains of other organisms, assisting in the breakdown, decay and recycling of essential nutrients. A few have gained notoriety as disease agents (pathogens) due to their parasitic life style e.g. *Mycobacterium tuberculosis* (TB), *Salmonella enteriditis* (food poisoning).

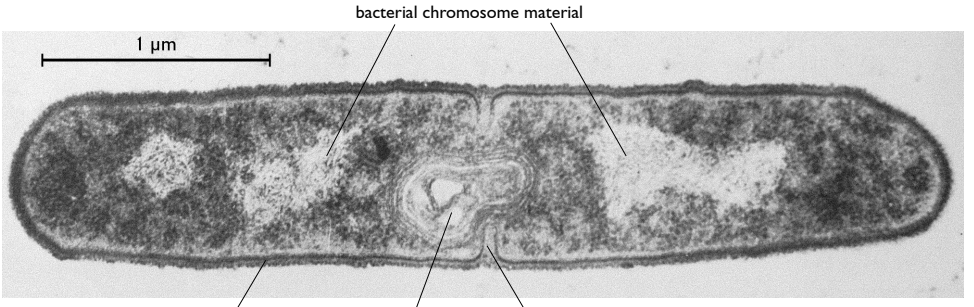
Not all bacteria are able to move, but some possess **flagella**, capable of propelling the organism through water. The cell wall is not made of cellulose, but is formed from a mixture of polysaccharides and amino acids called **murein**. Many bacteria which cause disease (pathogenic bacteria) have a **slime capsule** as their outermost layer which helps in protection against the body defences of the infected organism.

Bacteria generally divide and reproduce themselves much quicker than eukaryotes. Each bacterium divides into two (binary fission) to give two new daughter cells. Growth of bacterial populations can show a doubling every 20 minutes or so in ideal conditions.



Escherichia coli (*E. coli*) is a bacterium common in the gut of humans and other animals, it can occasionally however cause disease. *E. coli* has flagellae all over its surface. Like other Prokaryotic cells, it does not possess **organelles** (organelles are membrane bound structures found in Eukaryotic cells, e.g. nucleus, mitochondria, chloroplasts.) Its outer membrane folds inwards, however, to form special areas (**mesosomes**) for the attachment of enzymes. The DNA is not enclosed by a nuclear membrane but exists as a single main coil, **E**

EM of a section through a dividing rod-shaped bacterium



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with smaller loops of DNA (**plasmids**) containing just a few genes, scattered in the cytoplasm. Food energy reserves are stored in glycogen granules and fat droplets.

Due to their small size, the detail described above can only be seen under the electron microscope. Under the typical laboratory light microscope bacteria will be seen as tiny darkly staining specks, even at the highest magnification.

EUKARYOTIC CELLS

Eukaryotic cells (all cells other than the prokaryotic cells) do possess a membrane bound nucleus, within which is to be found DNA and histone proteins which form into chromosomes just before nuclear and cell division. Eukaryotic cells also possess membrane bound organelles; e.g. mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, and if photosynthetic - chloroplastids. The occurrence and distribution of these organelles depends upon the type of eukaryotic cell that is under consideration. Like prokaryotic cells they all possess ribosomes. The description of a 'typical' eukaryotic cell is not possible, due to the wide range of structure and function. However, the two groups (plant and animal cells) can be illustrated by reference to plant leaf palisade cells and animal (mammalian) liver cells.

◆ CHECKPOINT SUMMARY

- ◆ *Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles*
- ◆ *Escherichia coli (E. coli) is a bacterium found in the large intestine of animals, normally it is harmless but can cause disease under certain circumstances; new mutations can be fatal.*
- ◆ *The DNA is located in a central strand (bacterial chromosome), and as circular plasmids in the rest of the cytoplasm*
- ◆ *The plasmids can be exchange between bacteria, a fact exploited by genetic engineering*
- ◆ *The cell wall is not cellulose as in plant cells, but is formed from a mixture of polysaccharides and amino acids called murein*
- ◆ *The cell surface (plasma) membrane is partially permeable and controls the transport of substances into and out of the cell*
- ◆ *The cell wall provides protection and support, preventing bursting (lysis) of the cell in more dilute solutions*
- ◆ *Many disease causing (pathogenic) bacteria have a slime capsule covering the cell wall which helps in protection against the body defences of the infected organism*
- ◆ *Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing the principle of increased surface area of membranes with true mitochondria*
- ◆ *Ribosomes are present in both prokaryotes and eukaryotes*
- ◆ *Glycogen granules and lipid droplets act as energy stores*
- ◆ *Flagella provide bacteria with mobility.*

Plant and animal cells as seen under the light microscope

Plant cells are generally easier to see under the microscope, as the cell membrane of each cell is surrounded by a relatively thick **cellulose cell wall** which gives each cell a more visible boundary.

Leaf **palisade cells** are located just beneath the upper epidermis of the leaf and are specialised for the purpose of photosynthesis. Under the light microscope several typical plant cell structures can be seen in suitably stained specimens. They have a cellulose cell wall surrounding their cell surface membrane (plasmalemma), a large central vacuole surrounded by the tonoplast membrane, numerous chloroplasts which are aligned along the elongated vertical walls in such a way as to absorb the maximum amount of light. If living cells are observed it is possible to see the chloroplasts move within the cytoplasm to positions favouring optimum light utilisation.

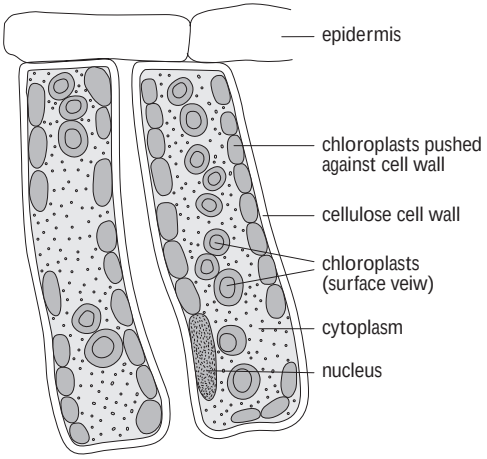
Liver cells shows typical animal cell features. There are three obvious differences from plant cells which can be seen with a light microscope: the liver cell does not have a cellulose cell wall; the liver cell has many tiny scattered 'vacuoles' (more properly termed vesicles); they lack chloroplasts. Very little detail of the structure of liver cells can be seen under the typical laboratory light microscope, in fact only the darkly staining nucleus and paler staining cytoplasm and cell surface membrane.

To observe more of the structure of these cells the greater resolving power (resolution) and magnification of the electron microscope is required.

◆ CHECKPOINT SUMMARY

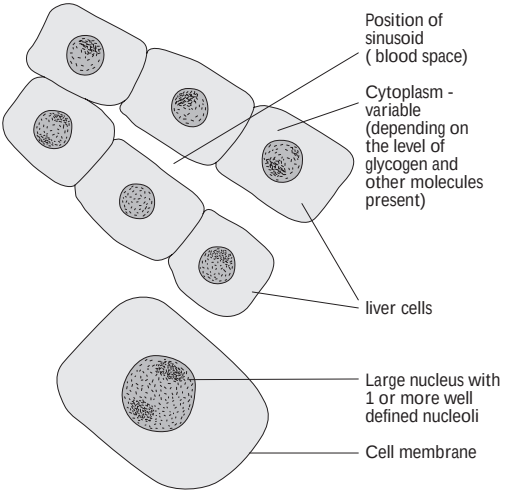
- *Eukaryotes do have a true membrane bound nucleus containing chromosomes of DNA and histone proteins, and membrane bound organelles as described previously*
- *Eukaryotic cells typically form tissues in multicellular organisms*
- *Cell differentiation and specialisation prevent the easy definition of typical animal and plant cells*
- *'Typical' cells would be those considered to show features common to the vast majority of cells in plants or animals*
- *A mesophyll cell from a leaf could be considered as a 'typical' plant cell*
- *A liver cell from a mammal could be considered as a 'typical' animal cell*
- *Under the best light microscope both the typical animal cell and the typical plant cell can be seen to possess a cell surface membrane, cytoplasm, nucleus with nucleolus, mitochondria, endoplasmic reticulum, and Golgi body*
- *In addition the typical plant cell can be seen to possess a cell wall around the cell surface membrane, a large central vacuole surrounded by the tonoplast membrane, and chloroplastids.*

Palisade mesophyll Cells



N.B. central vacuole not visible

Human liver cells (enhanced)



Light microscopes and electron microscopes

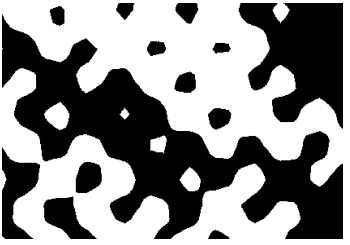
Magnification

For the visual study of cell structure it is necessary to enlarge (magnify) them by the use of microscopes. Light microscopes that you are most likely to come across in general Biology laboratories usually magnify up to 400 times (special techniques could take this up to 1000 times). However, to continue magnifying objects past a certain point reveals no further detail. In order to see more detail it is necessary to increase the resolving power (resolution).

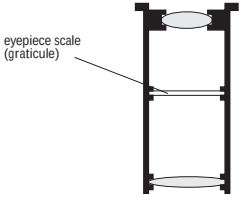
Resolution

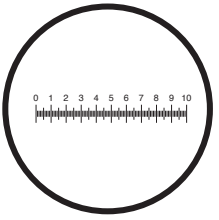
Resolution or resolving power can be defined as the ability of a system (e.g. microscope, the eye) to distinguish (resolve) detail in an object. The amount of detail of a specimen that can be seen is determined by the resolving power of the microscope system being used. In the case of looking at material under the light microscope, the resolving power depends upon the light being able to distinguish this detail, which is determined by the wavelength of light. To resolve the detail, light must be reflected back from the structures. Any structures less than 0.2 micrometres (µm), that is 200 nanometres (nm) in diameter cannot be distinguished by light, nor can two structures closer than 0.2µm be seen as separate.

(1 µm = one thousandth of a millimetre, 1nm = one millionth of a millimetre)

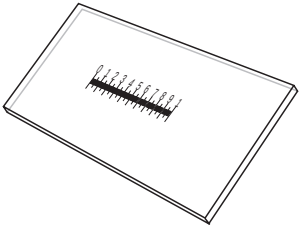


Measurement under a light microscopeusing an eyepiece graticule and slide (stage) micrometer

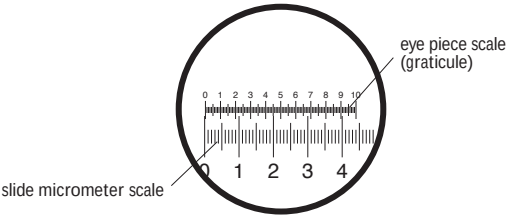




Eyepiece scale as seen when held up to the light away from the microscope.



Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)



Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope.

To improve the resolution, it is necessary to use a device which uses a beam of electrons, instead of light, called the **electron microscope**. Electron beams have a much shorter wavelength than light and give a hundred times better resolution. The magnification of an electron microscope picture (electron micrograph) can also be increased significantly to reveal this extra detail. The most powerful electron microscope can magnify up to x 500 000.

There are two types of electron microscope. In the **transmission electron microscope (TEM)**, an extremely thin section of the specimen is held on a copper or nickel grid and an electron beam passes right through it. Inside the electron microscope tube, electrons are accelerated by high voltage and focussed into a fine beam by a strong electromagnet (condenser lens). The beam passes through the specimen and is focussed further by additional electromagnets to give an image on a fluorescent screen, similar to the screen of a television set.

Electrons are disrupted by particles in the specimen to produce lighter and darker areas on the screen according to the relative density of those parts. If a photographic film plate is substituted for the fluorescent screen, the resulting picture is called an **electron micrograph**. A vacuum is maintained inside the tube of the electron microscope so that the electron beam is not scattered by molecules of the air e.g. oxygen and water. For this reason, it is impossible to view living specimens. Interpreting electron microscope images is difficult as they show the structure of dead cells which have been subjected to extreme mechanical and chemical trauma in the preparation process and are held in a vacuum.

Specimens are prepared by immersion in a series of increasingly pure solutions of alcohol which serve to dehydrate the cell components, then they are typically embedded in a block of resin which is cut into very thin sections by a machine resembling a high tech. bacon slicer called a microtome with a diamond or glass cutting surface. As the difference in density is not pronounced between one structure and another, it is common to 'stain' the specimen with the salts of heavy metals such as lead and uranium which increase the scattering of the electrons where the 'stains' are taken up and improve the contrast.

In the **scanning electron microscope (SEM)**, the beam of electrons passes back and forth across the surface of the prepared specimen producing a three dimensional effect. This technique is used to show surface features.

Remember that as light is not involved there is no colour in electronmicrographs, although they are often coloured 'artificially'.

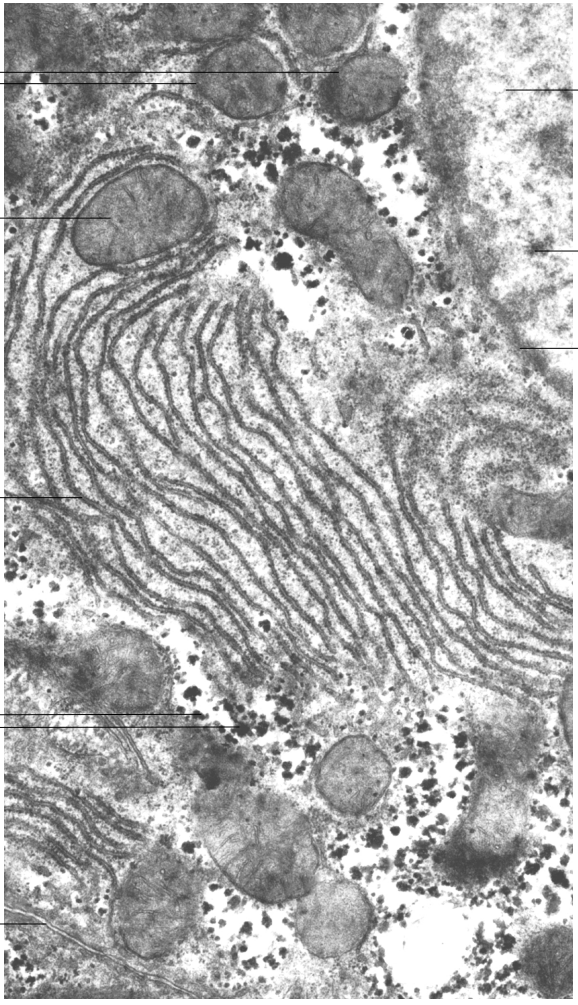
◆ CHECKPOINT SUMMARY

- ◆ *If the specimen can be observed with the naked eye without the need for magnification the calculation of the linear magnification of any drawing of it is straightforward - being the number of times any linear dimension of the drawing is greater than that of the specimen*
- ◆ *Magnifications of images of slides of biological material are given in terms of the magnification of the microscope image, that is by multiplying the power of the two lenses.e.g. 10 x 10 = 100*
- ◆ *However reproductions of the image as seen down a microscope introduce another layer of magnification which is often overlooked*
- ◆ *To calculate the linear magnification of drawings of microscope images of specimens it is necessary to know how many times the drawing is larger than the actual specimen*
- ◆ *The actual size of the specimen is measured with an eye piece graticule and stage micrometer*
- ◆ *Resolution is the ability to see detail*
- ◆ *The higher the resolution the more of the detail present can be seen*
- ◆ *Increasing magnification only reveals extra detail if the detail can be resolved*
- ◆ *Resolving power of light microscope limited by the wavelength of light*
- ◆ *Put simply some detail is so small that it cannot be 'hit' by light, and therefore cannot be seen*
- ◆ *Endless magnification of the image produced by a light microscope yields no extra information*
- ◆ *Wavelengths of electron beams are much shorter than that of light and give the electron microscope much greater resolving power than the light microscope*
- ◆ *Therefore extra magnification up to x250 000 yields an equivalent amount of detail.*

The principal features and organelles of a eukaryotic cell as seen by the electron microscope

The electron microscope reveals a wealth of detail of cell structure.

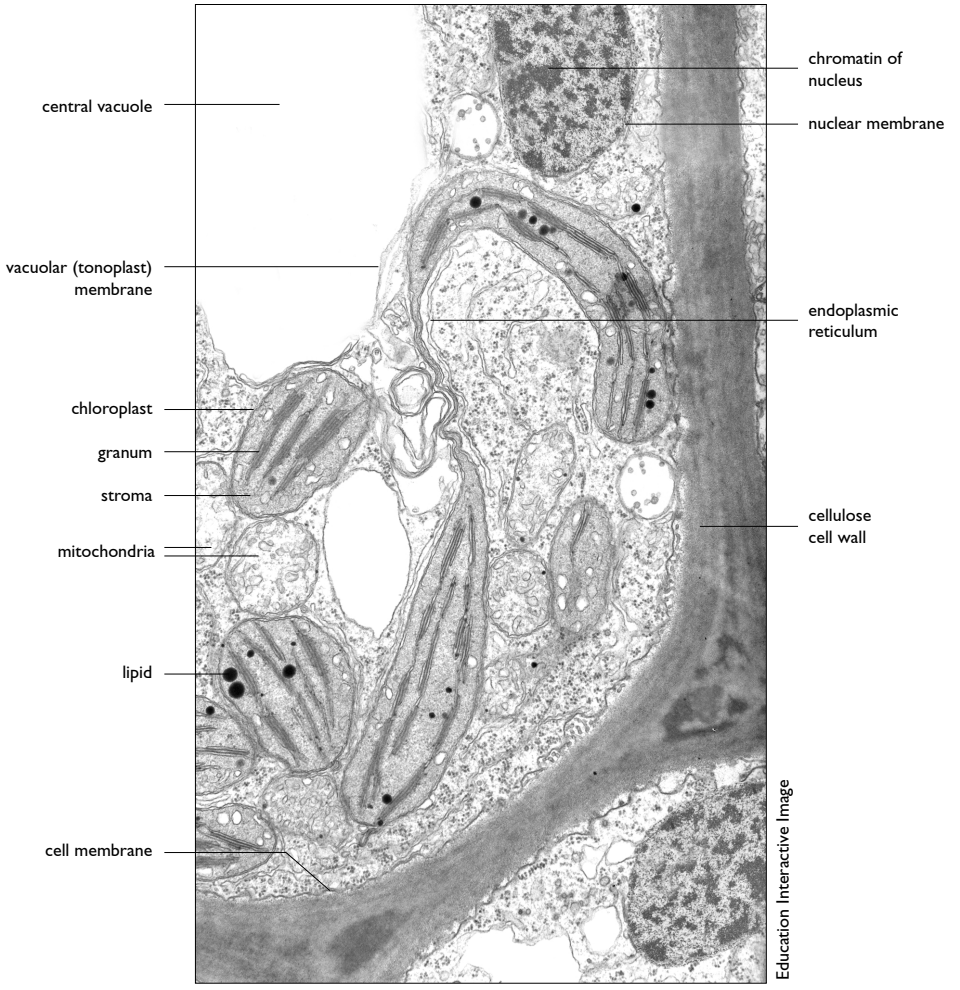
EM part of an animal cell (liver)



Nucleus

Even with the optical microscope, the nucleus is clearly visible, having an average diameter in animal cells (liver) of about 5 μm, and in plant cells (mesophyll) of about 15 μm. The nucleus stands out because of the material **chromatin**, a mixture of DNA and protein, which takes up the stains used in slide preparations. Chromatin is the material of which the chromosomes are made. The nucleus is surrounded by a double membrane, the **nuclear envelope** which

EM part of plant leaf cell



has the same structure as, and is continuous with, the other membrane systems of the cell. Three or four thousand large pores (up to 100nm diameter) perforate the nuclear envelope but the passage of materials in and out is controlled by plugs of protein and RNA which appear to fill the pores. Genes in the nuclear chromosomes direct all the cell's activities and determine its life span and reproductive cycle. The nucleus is rightly called the command centre, but it does not contain all of the genetic material of the cell. Mitochondria and chloroplasts also contain DNA and have genes of their own.

Nucleolus

This is an even more darkly stained region in the nucleus, which consists of a mass of RNA, used in the manufacture of **ribosomes**. Ribosomes are relatively small structures, about 20 nm in diameter, which occur in large numbers, often several thousand per cell either bound to internal cell membranes or free in the cytoplasm. They are built in two sections called sub-units, made of approximately equal quantities of RNA and protein. They act as the sites of assembly for new proteins, manufactured both for export and for internal use.

Rough and smooth endoplasmic reticulum

In addition to the outer surface (plasma) membrane and those which surround the organelles, cells (especially secretory cells), have extensive internal membrane systems, known as the endoplasmic reticulum (**ER**). The ER is a maze of membrane bound tubules and layers of flattened sacs enclosing a fluid filled interior, which acts as an intracellular (inside the cell) transport and storage system. It is continuous with the nuclear membrane and similar in structure.

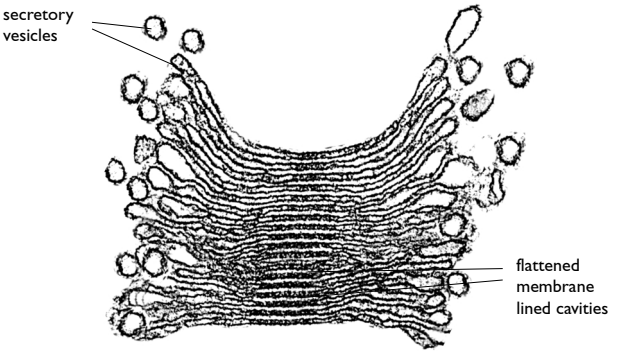
The rough ER has ribosomes associated with it on the cytoplasmic side of the membranes which are the sites of protein synthesis.

The smooth ER tends to be more tubular, and appears to have a complex set of functions, including fat metabolism.

The rough ER and smooth ER are not related in structure or function.

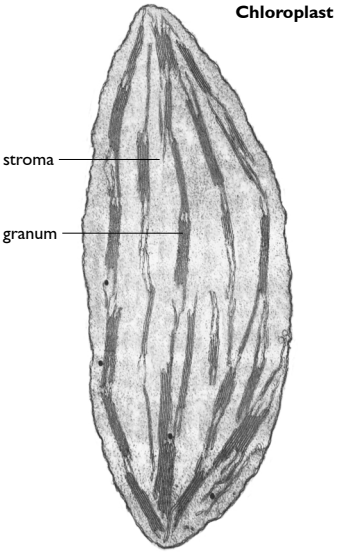
Golgi apparatus

This is more clearly seen in animal cells and is a region of stacked smooth ER specialised for the purpose of collecting, processing and redirecting proteins and fats made in the rough ER. Materials are transported between the ER, the Golgi apparatus, and the plasma membrane, sealed in membrane sacs called vesicles which bud off from one membrane, and then fuse with another. Substances are taken in (**endocytosis)** and expelled (**exocytosis**) by the plasma membrane in this way. For example, digestive enzymes produced by cells of the pancreas are secreted by exocytosis into the pancreatic duct.



Lysosomes

Some of the vesicles produced by the Golgi apparatus are specialised for particular functions. Animal cells, have lysosomes which contain a cocktail of digesting enzymes. They break down and dispose of unwanted materials or damaged structures in the cell, expelling waste debris by exocytosis, and recycling reusable molecules within the cell. They also meet and fuse with vesicles carrying imported food substances, or immobilised bacteria, from the cell surface, and digest the contents. It is clear from this that the membranes surrounding lysosomes must act as impermeable barriers to the passage of these digestive enzymes. If the contents of a lysosome spilled out into the cytoplasm, for example, the digestive enzymes would digest the cell itself. This process (**autodigestion**) occurs after the death of a cell and plays an important part in its removal.

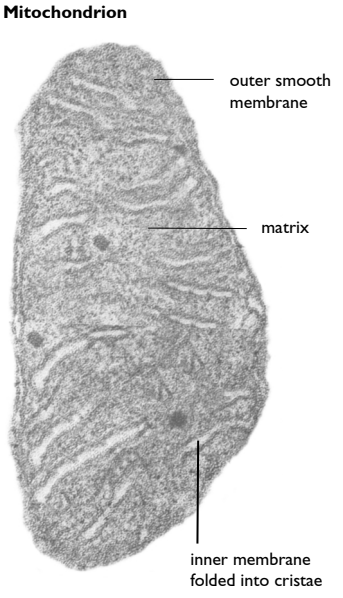


Chloroplasts

contain the green pigment chlorophyll which absorbs light for photosynthesis. In photosynthesis light energy is used in the synthesis of organic compounds (e.g. glucose) from carbon dioxide and water. Chloroplasts, like mitochondria, have a double membrane, but there is also a third membrane system inside the chloroplast called the **thylakoid** membrane system. It is on this that the chlorophyll molecules and enzymes of the light dependent stages are located. The thylakoid membrane system is arranged in the form of stacked discs (**grana**) joined by connecting membranes (**lamellae**). The internal space between the membranes is filled with a fluid (**stroma**) containing enzymes for making organic compounds, e.g. glucose and starch.

Mitochondria

Mitochondria (singular = mitochondrion) carry out aerobic respiration, which transfers energy from energy rich substances such as glucose, into the energy rich substance ATP. High energy consuming tissues like those in the muscles or brain may contain up to a thousand mitochondria in a single cell. Mitochondria are rod-like structures which can vary greatly in length and shape, but are never greater than 1μm in diameter. There is an outer and an inner membrane. The area of the inner membrane is increased by infoldings of the membrane (**cristae)**, and it is encrusted on its inner surface with spherical bodies which contain the enzymes for making ATP. The more active the cell, the more cristae and the more enzymes there are, the more the mitochondria increase in size, and increase in number by dividing (they contain their own mitochondrial DNA). The fluid matrix within the inner membrane contains the enzymes responsible for the final stages of the aerobic oxidation of glucose to carbon dioxide and water.



Cytoskeleton An interconnected system of fibrous proteins gives cells their shape and a basis for movement. A number of thread-like protein structures make up the cytoskeleton, notably actin, which forms thin contractile **microfilaments**; and tubulin, a protein which arranges itself into more rigid tubes resembling miniature scaffolding poles called **microtubules**. Microtubules form the supporting skeleton for the tiny cell 'hairs' called **cilia**. Microtubules are also formed and organised within areas called **centrosomes** (microtubule organising centres, or **MTOC**s) which lie very close to the nucleus. During cell division, the centrosome divides to form two new centres of microtubule formation at opposite sides of the cell. A net of microtubules (the spindle) forms between these two new centrosomes on which chromosomes are held and pulled apart.

In animal cells (but not all plant cells) the centrosome contains two structures resembling short cilia, which can be seen in electron micrographs lying at right angles to each other. These are called **centrioles**. Centrioles also form the basal bodies at the base of cilia.

Cellulose cell wall The plant cell wall is made of a fibrous cellulose framework mixed with other materials, notably **pectin**. Pectin is a glue like material (it is used in jam making to set the jelly) and it is concentrated on the surface, sticking adjacent cells together. This sticky region can be seen as a line between the walls of adjacent cells and is called the middle lamella. When the plant cell is fully expanded as a result of the entry of water by osmosis, the cell wall resists further expansion, and the cell is said to be turgid. Turgid cells make a considerable contribution to the support of non-woody structures, e.g. leaves.The cell wall is fully permeable to water and dissolved solutes, but influences the exchange of these across the selectively permeable cell membrane by exerting various physical and chemical forces. The wall is perforated by tiny holes, through which strands of cytoplasm (**plasmodesmata**) pass giving direct communication between neighbouring cells.

In 'woody' parts the cell wall is impregnated with **lignin**. Lignin is a hard, impermeable substance, and lignification typically results in the death of the cell. Such dead lignified tissues are specialised for water transport (eg xylem) and support (eg xylem and sclerenchyma fibres).

◆ CHECKPOINT SUMMARY

- ◆ *The nucleus contains the genetic material (DNA) and can be considered as the control centre of the cell*
- ◆ *Within the nucleus is the nucleolus which is the centre of mRNA and ribosome synthesis*
- ◆ *Rough or granular endoplasmic reticulum is a system of flat sac-like cisternae and interconnecting tubes, which acts as an intracellular transport and storage system*
- ◆ *Ribosomes attached to the RER are centres of protein synthesis*
- ◆ *The rough and smooth endoplasmic reticula are not related in structure or function, and are not joined. Typically there is less smooth endoplasmic reticulum in plant cells*
- ◆ *The smooth endoplasmic reticulum has a wide variety of functions including fat metabolism*
- ◆ *The Golgi apparatus processes proteins from the RER e.g. forming glycoproteins, which are subsequently released from the cell by exocytosis*
- ◆ *Lysosomes cannot be identified solely on appearance, the activity of digestive enzymes must be demonstrated by other means*
- ◆ *Mitochondria are rod-shaped organelles but appear oval in cross section. Chloroplasts are oval in structure and appear oval in cross section*
- ◆ *Chloroplasts have a double membrane, and an internal thylakoid membrane system which provides a large surface area for the attachment of the pigments and enzymes of the light-dependent stage of photosynthesis. Enzymes controlling the non-light dependent stage are found in the matrix*
- ◆ *Centrioles are involved in the movement of chromosomes in nuclear division, but controversy surrounds the identification of centrioles in plant cells*
- ◆ *Microtubules of protein form a cytoskeleton*
- ◆ *The cellulose cell wall of plant cells is fully permeable to the passage of substances, and protects and supports the cell contents, particularly via its effects on turgidity*
- ◆ *The cell surface membrane has a fluid mosaic structure based on a phospholipid bilayer stabilised with cholesterol, with surface and internal proteins*
- ◆ *It is partially permeable to the passage of solutes, controlling the transport of substances into and out of the cell. The surface proteins and glycoproteins act as recognition sites for antibodies and hormones.*

The structure, properties and roles of the cell surface (plasma) membrane

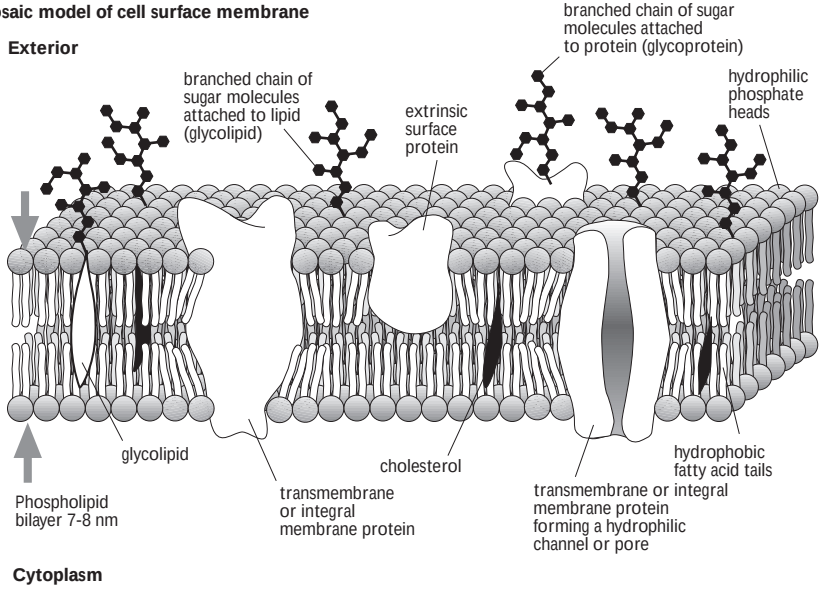
The plasma (cell surface) membrane controls the entry and exit of all materials into and out of all cells. The electron microscope does not reveal any details of its structure, which is modelled on evidence of biochemical investigations. It is fatty in nature and contains proteins and other substances. It is partially permeable, being more permeable to water, than to dissolved solutes which pass through at different rates depending on a complex of factors (see below).

In cells which are specialised for the exchange of materials across the plasma membrane e.g. cells lining the small intestine and parts of the kidney tubules, the surface area may be dramatically increased by extensions known as microvilli.

The cell surface membrane and the membranes which enclose the cell organelles and make up the extensive system of interrelated channels and vesicles within the cell share the same structure. It is a structure which is so flexible that it can break and reform easily yet it is capable of performing complex functions, acting as a barrier, container, transport regulator and as a site of recognition, distinguishing between a wide variety of substances.

The fluid-mosaic model of membrane structure is based on a bilayer of phospholipid molecules, strengthened by cholesterol. Phospholipids adopt this bilayer structure automatically in fluid surroundings because the fatty acid 'tails' of the molecules are water repelling (hydrophobic) whilst the phosphate/alcohol 'heads' are water attracting (hydrophilic).

Fluid mosaic model of cell surface membrane



Proteins of various sizes and shapes are located on and in the bilayer. Some are bonded to the hydrophilic head region and do not penetrate the interior of the membrane (**extrinsic proteins)**, others, have one end buried within the fatty layer and one end sticking outside of the cell membrane (**intrinsic proteins)**. A third kind, (**transmembrane proteins**), pass all the way through the membrane to create special channels. The carbohydrate component of cell membranes consists of short polysaccharide chains joined to membrane proteins or fats forming **glycoproteins** and **glycolipids** respectively, which project outside the cell membrane.

Individual molecules of phospholipid and protein in the membrane have a degree of **mobility** within the membrane. This fluidity, coupled with the bubbled appearance of the proteins on the surface of membranes gave rise to the the term '**fluid mosaic model**' when the structure was first proposed. Cholesterol stabilises membranes by limiting the movement of phospholipid molecules within the membrane.

Cells have the ability to sense and respond to the presence of a great variety of molecules. This ability is due to proteins in the surface membrane which act as **receptors**. Hormones such as insulin and adrenalin, and many drugs and toxins lock onto recognition sites on receptor proteins triggering changes in the cell's behaviour.

Glycoproteins and glycolipids have a very important role in personalising a cell's identity. They are orientated in the membrane bilayer with the chain of sugar molecules to the outside. The possibility for variation in the structure and different combinations of these chains gives an endless range of different surface patterns, and forms the basis of a recognition system. Each individual has his or her own cell surface 'print'. Self recognises self, and immune systems are mobilised when 'non-self' substances are encountered. The glycoproteins and glycolipids of the cell membrane are responsible for an individual's tissue type, a well known example of which is the blood groups.

Transport across cell membranes

The plasma membrane acts as a partially permeable barrier to the passage of substances into and out of the cell, for example water and fat soluble substances pass through more easily than sucrose. The different rate of passage of electrically charged ions generates membrane potentials, which in turn influence the further uptake of charged ions, and are also involved in cell sensitivity, particularly in nerve cells.

The transport of respiratory gases, nutrients and metabolic waste products occurs through the membrane, either directly through the bilayer, or via channels or 'pores' created by transmembrane proteins. In some cases the substances move down a concentration gradient (i.e. from high concentration to low concentration) which is known as **passive transport**. In others, substances are moved against the concentration gradient (i.e. from low concentration to high concentration) in a process requiring energy in the form of ATP, which is known as **active transport.**

Diffusion and the factors which determine its rate

Molecules and ions may move passively by diffusion, which is defined as the overall (net) movement of substances from regions of their higher concentration (higher chemical potential) to regions of their lower concentration (lower chemical potential) until equilibrium is reached. The movement of the particles of substances in diffusion is random in all directions, as a result of their inherent kinetic energy. If there are more in one area than another, the net effect is for the particles to distribute themselves equally throughout that particular space, i.e. the concentration gradient evens out, until at equilibrium there are an equal number of particles moving in all directions.

The speed of these diffusing particles is only increased by an increase in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the **concentration gradient**), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed in the following equation known as **Fick's law**.

$$\text{Rate of diffusion} = \frac{\text{difference in concentration} \times \text{surface area}}{\text{thickness of the exchange surface}}$$

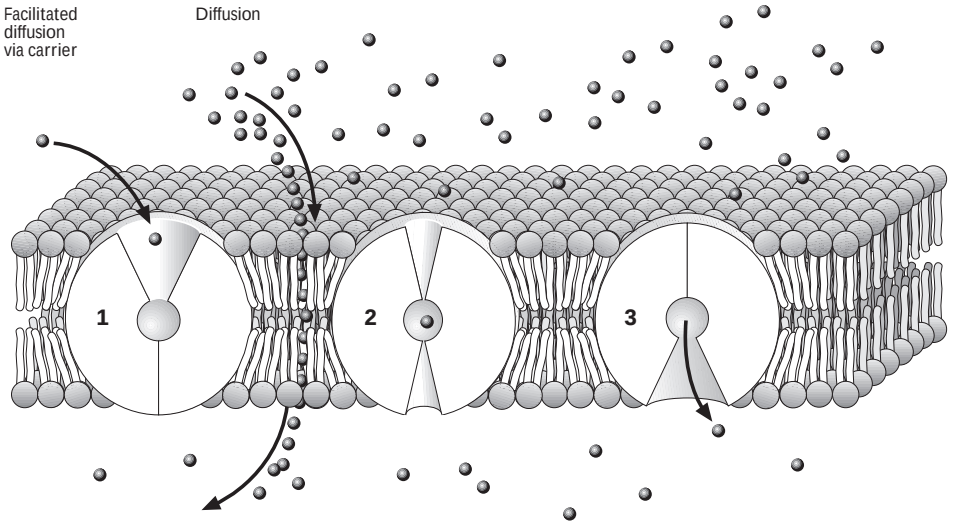
This gives a rough guide to the rate at which a substance such as oxygen, for example, diffuses from the air in an air sac (alveolus) in the lung into the blood in a lung capillary. In principle, the greater the surface area, the greater the concentration difference, and the thinner the separating layers, the greater the rate of diffusion.

The most important factor affecting diffusion across cell membranes, however, is the chemical nature of the substance being transported. Diffusion across the bilayer depends principally upon the solubility of the substance in fat. The phospholipid bilayer is strongly hydrophobic. Polar (charged) molecules such as glucose, some amino acids, and inorganic ions pass through very slowly or not at all, whilst non-polar molecules such as calciferol (vitamin D) move across with ease. It is interesting to note that the first anaesthetics (chloroform and ether) were fat solvents that disrupted membrane structure and function especially in nerve cells.

Facilitated diffusion occurs when non-fat soluble polar molecules e.g. glucose, some amino acids, and inorganic ions, diffuse through hydrophilic channels formed by transmembrane proteins. Polar molecules also become temporarily attached to special binding sites on transmembrane proteins called **carriers**. These are rather like enzymes in that they are shape specific and accelerate a process which is already 'downhill' in terms of energy. All that is needed is a concentration gradient. The attachment of a molecule to the binding site alters the overall shape of the carrier in such a way as to deposit the molecule on the other side of the membrane along their normal concentration gradients.

As has been seen, the transport of inorganic ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and chloride (Cl^-) across cell membranes depends upon transmembrane proteins. Some transmembrane proteins form selective **ion channels** through which the facilitated diffusion of ions occurs down their concentration gradients. These ion channels cannot be coupled to an energy source, and cannot be involved in active transport across the membrane.

Diagram to show diffusion and facilitated diffusion



Osmosis

Cell membranes are **partially permeable**, allowing the passage of substances at different rates. Generally cell membranes are more permeable to water than to many solutes. Water moves according to the same principles of diffusion, but it is not usual to refer to high and low 'concentrations' of water - the term 'concentration' being traditionally associated with any solutes dissolved **in** water in a solution. Therefore the preferred term is the chemical potential of water or **water potential**.

Water always moves (diffuses) from a region of higher water potential to one of lower water potential. You could think of water potential as the **tendency for water molecules to move** i.e. diffuse. The higher the water potential the more easily the water molecules move, and the lower the water potential the less easily the water molecules move.

At standard pressure and temperature, pure water has the highest water potential. If substances are dissolved in solution then the water potential is decreased because the solutes associate with water to a greater or lesser extent and reduce the ease with which the water molecules can move. Therefore any solution has a lower water potential than pure water, and a more concentrated solution has a lower water potential than a less concentrated solution.

The water potential of a fluid can be described as the tendency of water to move into it from pure water. If the fluid **is** pure water, there is no movement, so the **water potential of pure water is zero**. The water potential of any solution is less than pure water and is therefore negative. The more negative the water potential the greater the tendency of water to move into that system.

Where a solution is separated by a partially permeable membrane from pure water or a less concentrated solution, water will diffuse into the more concentrated solution until equilibrium is reached. The special case of the diffusion of water across a partially permeable membrane is known as **osmosis.**

◆ CHECKPOINT SUMMARY

- ◆ *The cell surface membrane acts as a partially permeable membrane regulating the transport of substances across the membrane*
- ◆ *Passive diffusion occurs across membranes down diffusion gradients from high concentration to low concentration. Fat soluble substances diffuse faster than non-fat soluble substances which must pass through the aqueous pores*
- ◆ *The speed of these diffusing particles is only increased by an increase in temperature, which increases their kinetic energy*
- ◆ *In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the concentration gradient), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface*
- ◆ *Facilitated diffusion is diffusion via special carriers which ease the passage of substances through the membrane*
- ◆ *Water always moves across the cell surface membrane passively by osmosis*
- ◆ *Osmosis is the special case of the diffusion of water from regions of higher water potential to regions of lower water potential across a partially permeable membrane which is more permeable to water than to dissolved solutes. (If the membrane was fully permeable the solutes would diffuse down their diffusion gradient and the water would not move*
- ◆ *At room temperature and pressure pure water has the highest water potential which is given the value of zero. Solutions have a water potential of less than zero (negative)*
- ◆ *Increasing pressure increases the water potential and it can become positive, in other words water can be 'squeezed' out of solutions through partially permeable membranes into pure water*
- ◆ *Active transport involves energy in the form of ATP from respiration to pump ions across the cell surface membrane via carriers against their diffusion gradient*
- ◆ *Mass or bulk transport of fluids into and out of the cell is achieved by vacuoles fusing with, or being pinched off from, the cell surface membrane respectively.*

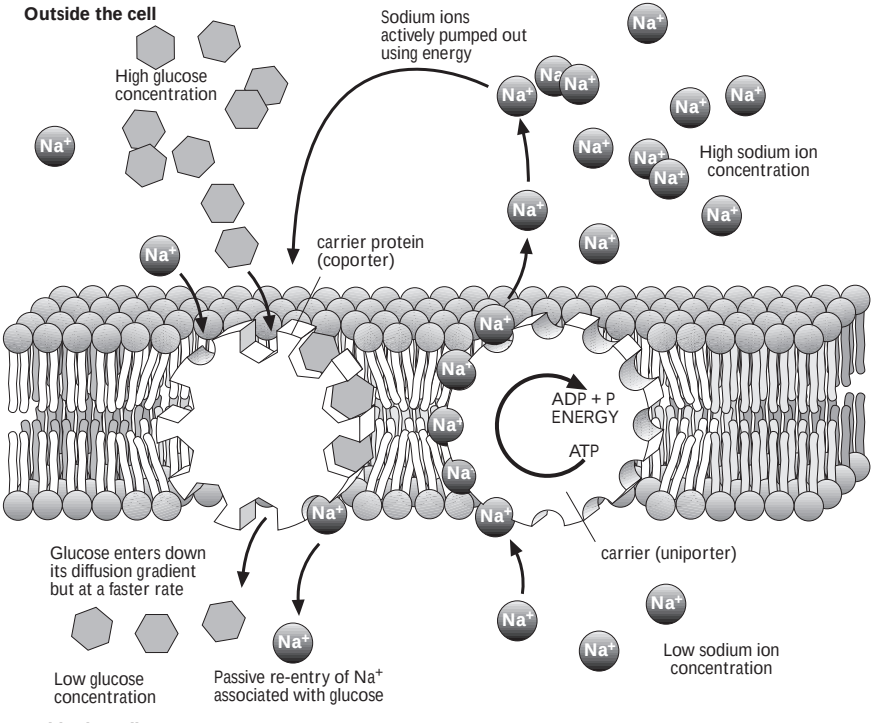
Active transport

Some transmembrane proteins act as active **ion pumps**, which use energy from ATP from respiration, to move ions against their concentration gradients. The ion channels can open and close like 'gates', and the activity of the ion 'pumps' can be varied. In these ways, the membrane can be made selectively permeable to certain ions at certain times.

As a result of the distribution of ions on either side of the membrane, **transmembrane potentials** are created, in which there is an overall difference in electric charge between the inside and the outside of the membrane. In normal conditions, the inside of the cell membrane is negatively charged relative to the outside, and this will assist the uptake of positively charged ions (cations) and oppose the uptake of negatively charged ions (anions). Thus the movement of ions across membranes is influenced by both electrical and chemical gradients i.e. **electro-chemical gradients** (these have particular importance for the function of nerve and muscle cells).

Some of these transmembrane protein carriers carry two substances at once in a **coupled mechanism**. A common example is the sodium/potassium pump (Na^+/K^+ pump), in which the active pumping of sodium out of a cell is coupled with the pumping of potassium in, both against their diffusion gradients. For every two

Diagram to show active transport (ion pumps)



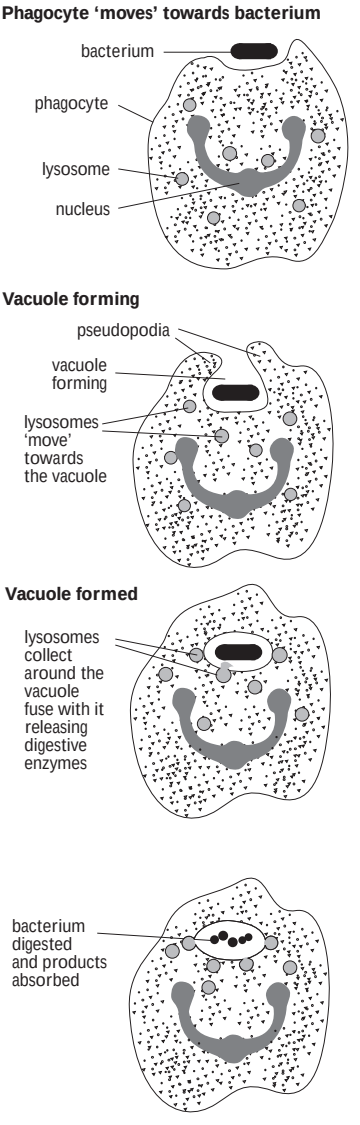
sodium ions pumped out, three potassium ions are pumped in, resulting in high potassium and low sodium levels within the cell. Another example is where the sodium pump is linked to the uptake of glucose. The sodium ions are pumped actively out of the cell and diffuse back into the cell via a carrier associated with glucose. In this case the glucose is moving passively down its concentration gradient, but at a faster rate as a result of its association with the re-entry of sodium which is kept at a high rate by its being continually and actively pumped out of the cell.

Endocytosis and exocytosis

The bulk transport of liquids and solids is achieved by membranes pinching off small bubble-like vesicles which can fuse with other membranes to transfer their contents. Uptake of substances into the cell in this way is known as **endocytosis**, and elimination of substances as **exocytosis**.

The term **phagocytosis** is used to describe the kind of endocytosis in which relatively large solid particles are 'ingested' by a cell, as in the case of white cells which engulf bacteria, or *Amoeba* taking in food. In this process the outer membrane folds inwards to completely surround the ingested particle. It is then transported into the interior of the cell within a sealed vesicle which functions as a temporary digestive chamber and, in *Amoeba*, is termed a food vacuole. Lysosomes containing digestive enzymes fuse with and release their contents into the food vacuole and the soluble products of digestion are transported out for assimilation by the cell. Undigested matter may be expelled from the cell by the reverse process (exocytosis)

Fluid secretions produced by cells are transported to the plasma membrane in small vesicles which fuse with it and release their contents by exocytosis. This process is sometimes referred to as **pinocytosis**.



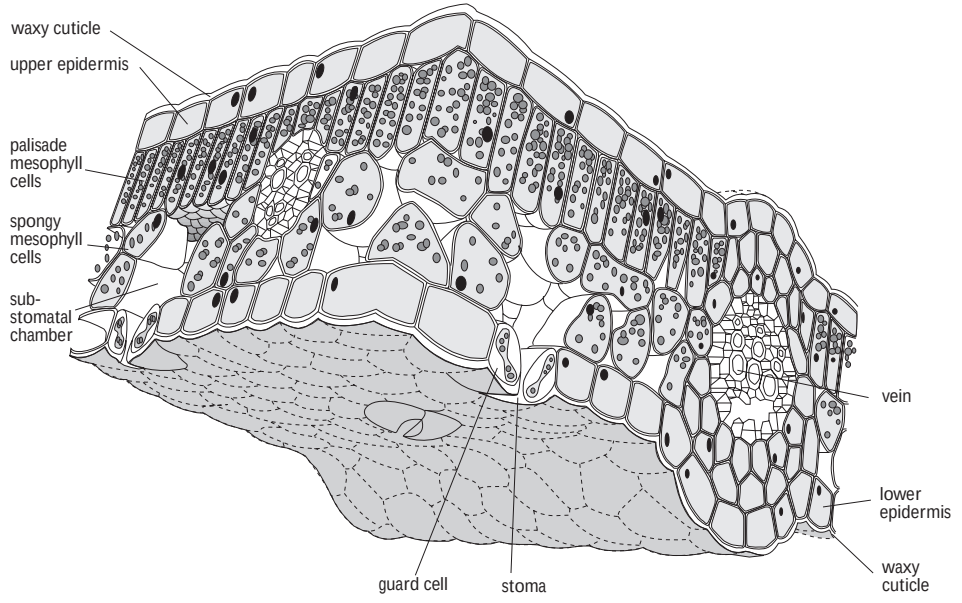
Aggregations of cells

The leaf palisade and liver cells illustrated at the beginning of this unit were chosen to represent typical plant and animal cell features. In life they are grouped with other similar cells performing the same functions into cell aggregations called **tissues**.

The leaf and the liver are **organs**, made up of different tissues organised to carry out a particular function or related functions

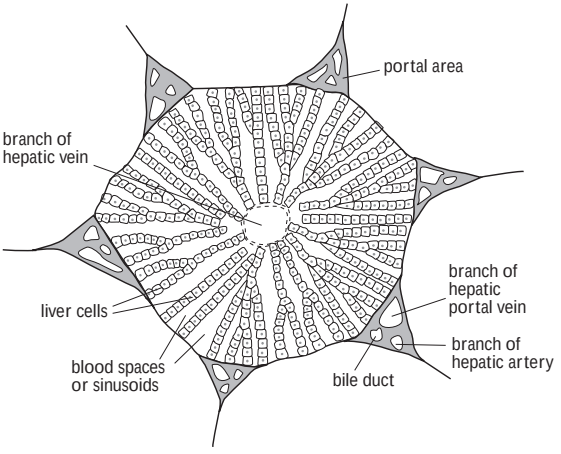
The leaf is an organ specialised for photosynthesis. It consists of various tissues: epidermal tissue of transparent cells with no chloroplasts which secrete a waxy layer (cuticle) and form a protective layer on the leaf surface; palisade tissue forms the photosynthetic layer; spongy mesophyll consists of large, loosely packed, thin walled cells with few chloroplasts, specialised to form a gas exchange surface; and the leaf veins are made up of vascular tissues, xylem and phloem, for the transport of water and organic solutes.

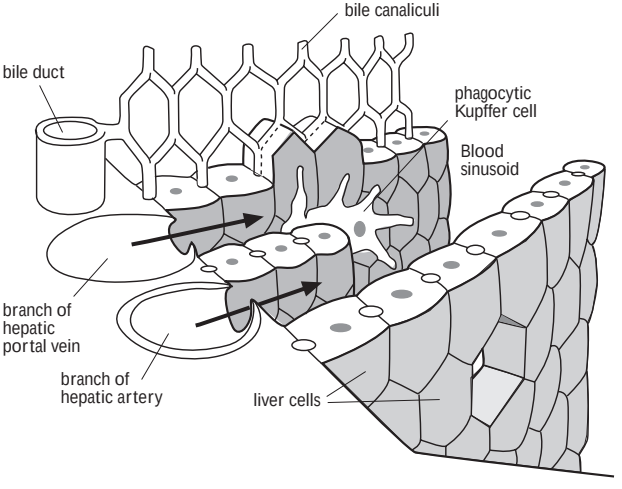
Section of leaf showing internal structure



The liver is an organ of metabolic control, monitoring and controlling large numbers of chemical reactions to help maintain constant optimum internal conditions (homeostasis), e.g. blood glucose levels. It consists of various tissues: sheets of liver cells, blood vessels, bile canaliculi (small ducts) and connective tissue.

Liver lobule





The so called 'classic' liver lobule is only seen in the pig liver, in other animals the structure of the liver is far from clear. The blood flows from the portal area, through the blood sinusoids, into the central vein. As it passes over the large surface area of the liver cells the various adjustments are made.

1.4
The Cell Cycle

Content

Chromosome structure

- Chromosome structure of DNA and histones in the nucleus of eukaryotic cells
- The replication of DNA, the role of the enzymes involved (see 1.1)
- A leaf palisade cell and a liver cell as cells with a diploid chromosome number which have been produced by nuclear division followed by differentiation

Mitosis

- Mitosis: the behaviour of chromosomes during the stages of the mitotic cell cycle, the events of prophase, metaphase, anaphase and telophase
- The significance of mitosis in growth and replacement; the significance of daughter nuclei with chromosomes identical in number and type
- The nature of natural and artificial cloning in plants and animals

THE CELL CYCLE

Introduction

The events that occur in the life of a cell are known as the cell cycle. Some cells are capable of repeated divisions (apical meristems at the tips of roots and shoots in plants, and the germinal epithelium in the epidermis of the mammalian skin), and they have a repeated cycle of events resulting in the production of daughter cells. Most cells, however, become differentiated into specialised cells which lose the ability to divide (terminal differentiation).

Interphase refers to the greater part of the life of a cell when there is no visible activity. If the cell is going to divide the chromosomes appear and nuclear division occurs by a process known as **mitosis**. When the nucleus has divided, the rest of the cell (cytoplasm and plasma membrane) divides in a process known as **cytokinesis**. Together, interphase, mitosis and cytokinesis make up the **cell cycle**. The term interphase should not be interpreted to mean that the cell is resting. Interphase can itself be divided into three distinct periods called G1, S and G2. ('G' refers to the 'gaps' between DNA replication and mitosis.)

G1 is by far the longest period of the cell cycle. During G1, the cell machinery carries out protein synthesis, and growth occurs. All the cells of the body have identical sets of genes in their nuclei but the cells develop in different ways to perform different functions. This development process is called cell differentiation in which different genes are switched on and off so that only one small part of the genetic code is active in each cell.

After a period of growth and differentiation, the length of which depends upon a variety of internal and external factors, protein synthesis is suspended, and the DNA set replicates itself. This is the **S** phase. DNA replication is followed by another 'gap' period, **G2**, before mitosis starts, in which the cytoskeleton of the cell breaks down and the microtubule components begin to reassemble into spindle parts. Without a cytoskeleton, the cell assumes a more spherical shape (see prophase).

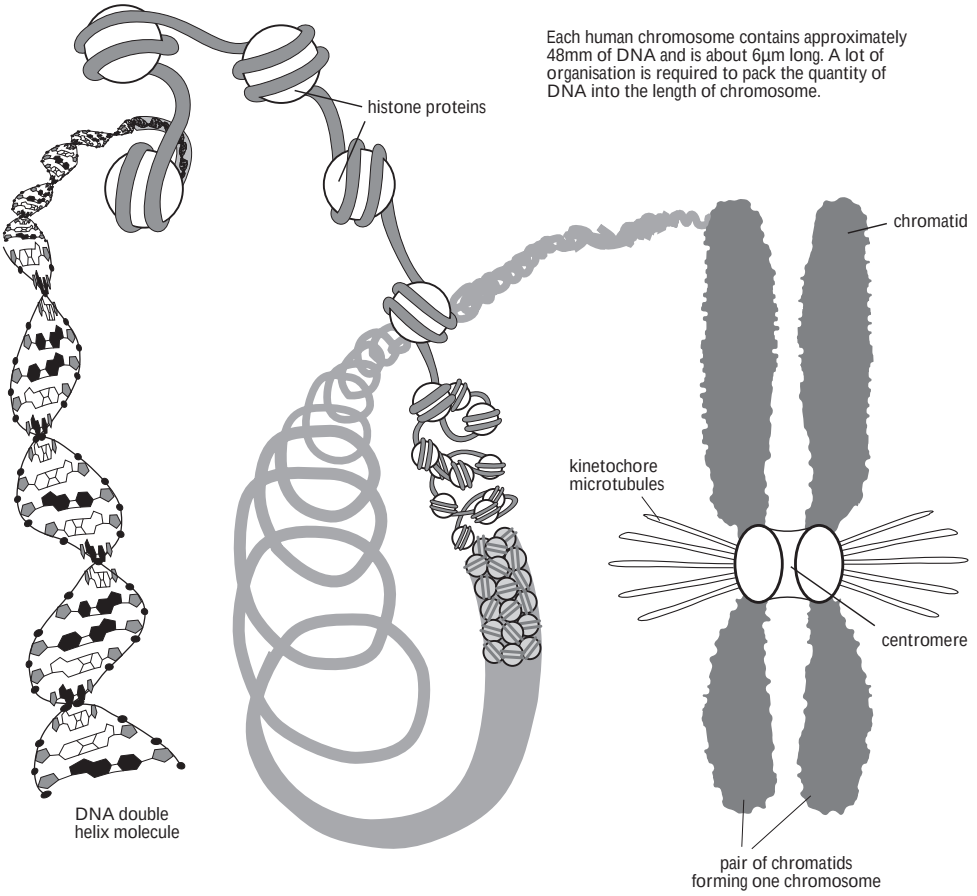
The timing of events within the cell cycle is extremely variable. In ideal conditions, where food and oxygen are in plentiful supply and temperature and pH values are optimal, bacterial cells can complete the cycle to produce a new generation every 20 minutes. Other cells, for example muscle cells, never complete the cycle and remain stuck in G2 until they die, a condition called terminal differentiation.

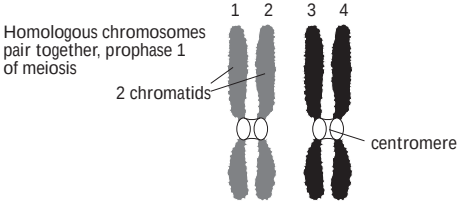
Chromosomes structure

DNA is the carrier of the genetic code by which hereditary information is passed from generation to generation via the nuclei of the gametes and from cell to cell via the nuclei of the cells produced by division of the fertilized egg. In a non-dividing cell, the nucleus contains scattered '**chromatin**' material which is a mixture of DNA and an associated protein called **histone**. When the DNA is in this dispersed form seen in the non-dividing nucleus, it is genetically active, sending out information to the cytoplasm to control the complex of cell processes. When the cell is about to divide the chromatin concentrates or condenses into an inactive state to form the **chromosomes**. In this state the DNA is more resistant to damage during the processes of nuclear and cell division. Moreover, since it is in discrete bodies like the chromosomes, the division of the genetic material between the nuclei can be carefully controlled, reducing the danger of any being lost. It is only in this condensed form that chromosomes are ever visible in the cell and this only happens during cell division, so the familiar ribbon like image of a chromosome is not representative of its active state.

Chromosomes consist of a centromere and two 'arms', the relative lengths of which depend on the position of the centromere. As a result of the replication of DNA before their appearance, chromosomes appear as double structures known as 'sister chromatids'. A chromatid is in effect a new chromosome, but while they remain attached by a single centromere, they are referred to as sister chromatids.

The nucleus of each cell of a **diploid** organism contains two sets of chromosomes; one maternal from the female gamete and one paternal from the male gamete. As a result, each chromosome has a 'partner' in the other set which carries genes for the same characteristics. Two such chromosomes are said to form a **homologous** pair. The nucleus of the palisade cell and that of the liver cell, described in 1.3 are diploid. The cells were produced by divisions of a parent cell by the process of mitosis, described below, in which the DNA is copied exactly (replicated). DNA replication is described in Unit 1.1 and you will find it helpful, before starting this module to read through the section on DNA.





When cells are first formed by the process of division they all look much alike. At a very early stage, however, they start to behave differently as different genes are 'switched' on and off with the result that groups of cells (tissues) become specialised for a particular function. The process of cell specialisation is called **differentiation**. The leaf palisade cell and liver cell described above are produced by nuclear division (mitosis) and cell division followed by differentiation.

Mitosis

Mitosis is the process by which the genetic material of a dividing cell, contained within the DNA of chromosomes, is copied into two new genetically identical 'daughter cells'. Mitosis occurs in the cell division seen in growth, repair, the replacement of cells which have a limited life span like our red blood cells and skin cells, and in asexual reproduction in many plants and lower animals.

The main stages of mitosis

Chromosomes only become visible when stained and viewed under the light microscope when the nucleus of a cell is just about to divide. This is because only at this time is the DNA/histone mixture wound up (condensed) as tightly as it can be. By this stage the DNA has also copied itself (replicated) so each chromosome appears double, and joined at one point (the centromere). Whilst they remain joined in this way the two daughter chromosomes are referred to as sister chromatids.

Nuclear division (mitosis) occurs in four main stages, namely prophase, metaphase, anaphase and telophase.

Prophase is the first phase of mitosis, and as it begins, the DNA has already replicated, all protein synthesis has stopped, and each chromosome is condensed into the characteristic double chromatid form. At the same time the cell rounds up, the nuclear membrane

breaks down into small fragments, and a **s**pindle of microtubules forms between two microtubule organising centres (MTOCs) at opposite poles of the cell.

Metaphase is defined as when the chromosomes become attached to the spindle by structures called kinetochores. Kinetochores consist of microtubules and 'motor' proteins which utilise ATP energy to pull on the spindle. The effect of both sets of kinetochores pulling in opposite directions is that the chromosomes line up along the middle (equator) of the spindle.

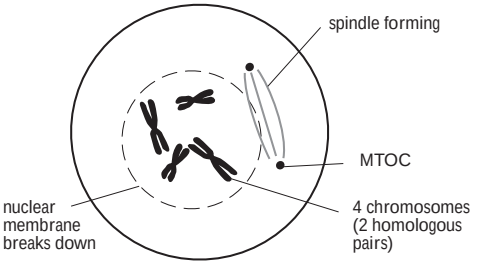
Anaphase is defined when quite suddenly, and all at once, the chromatids of each pair separate, with each being pulled under tension in opposite directions towards the poles of the cell.

Telophase is the stage when the two new, identical sets of chromosomes reach the opposite poles and a new nuclear envelope forms to surround each set.

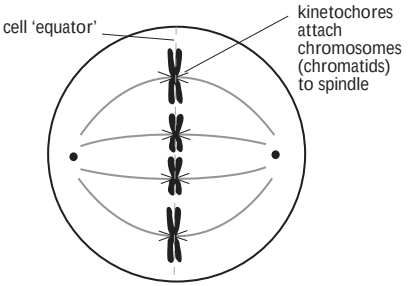
Nuclear division by mitosis is followed by division of the cytoplasm into two equal parts. This process is called **cytokinesis** and it occurs differently in animals and plants. In animal cells the spindle disintegrates during telophase and the microtubule components reassemble into a new cytoskeleton. The equator of the cell is constricted by a ring of contractile proteins (actin) in the process of cleavage, to create two roughly equal cells.

In plant cells the spindle lingers a little longer and seems to act as a guide to bring Golgi apparatus and associated secretory vesicles to the equator. The vesicles deposit their contents, which are the building constituents of a new cell wall, on the equator to form a plate of deposited material (the cell plate). Some of the vesicles remain intact and make connecting channels through the new cell wall (plasmodesmata).

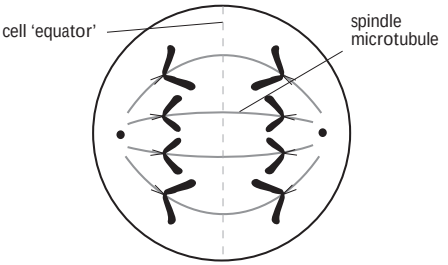
1 Prophase
Cell is rounded
MTOC's migrate to opposite poles
nuclear membrane disintegrates
spindle formed by MTOCs



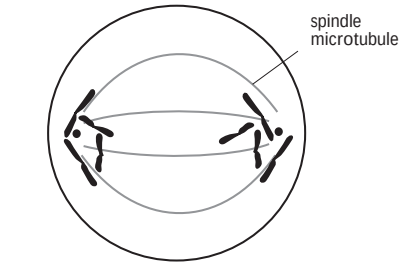
2 Metaphase
Chromosomes held on 'equator' of cell under tension. MTOCs at opposite poles



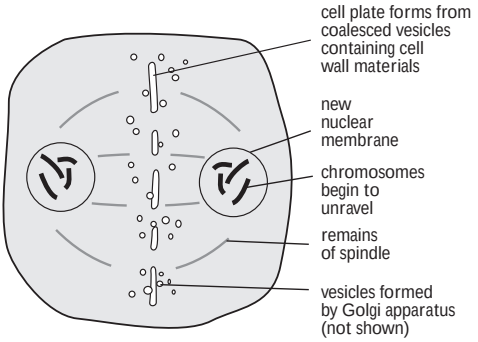
3 Anaphase
Chromatids separate and move towards opposite poles



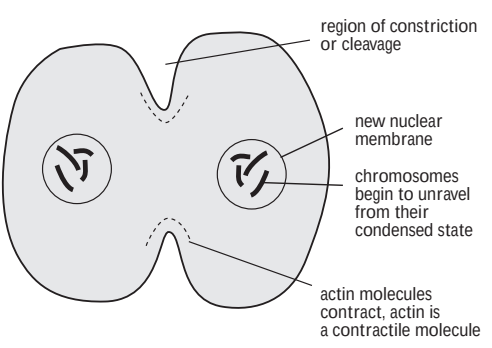
4 Telophase
Chromosomes form beginnings of new nuclei at opposite poles, nuclear envelope reforms



Cytokinesis in plant cells



Cytokinesis in animal cells



The significance of mitosis

The development of a multicellular organism from a fertilised egg by mitosis, means that all the cells of the body are genetically identical to the fertilised egg. The development of an organism from the fertilised egg is the story of the controlled expression of this genetic information, with only a small fraction of the genes being expressed in cells as they differentiate to form tissues specialised for specific (and limited) functions. In mature cells most of the DNA is inactive all of the time.

Asexual reproduction does not involve the fertilisation of gametes, and as a result of mitosis produces offspring which are genetically identical to each other (clones). Many multicellular organisms, especially plants can reproduce their entire bodies in this way, either from specialised structures eg. strawberry runners, or single celled spores.

The production of genetically identical cells in the growth and development of multicellular organisms allows certain cells to retain the ability to develop into any other if needed as a result of some damage, for example stem cells in mammals can divide to form any of the different types of blood cell. In the asexual reproduction of organisms it allows the rapid exploitation of optimal conditions, where any variation would be disadvantageous.

Natural and artificial cloning in plants and animals

Clones are genetically identical organisms. Cloning may occur naturally, for example, identical twins in humans, binary fission in microorganisms and vegetative reproduction in plants, but it can also be achieved artificially. The advantages to agriculture of cloning techniques are obvious; to be able to select the very best crop plant or farm animal and multiply it indefinitely has been the dream and ambition of farmers throughout the ages. Plant growers, since the earliest times,have used the natural properties of plants to multiply by vegetative (asexual) means; indeed some common crop plants, for example the banana, can only be grown by cloning. The cloning of animals is a relatively recent phenomenon, made possible by new techniques for manipulating embryonic cells, and whilst it opens up some exciting new biotechnological horizons, for many it also raises new ethical and legal questions.

- CHECKPOINT SUMMARY
- *Chromosomes consist of DNA and histones (protein only found in the nucleus) in the nucleus of eukaryotic cells*
- *Chromosomes only form just prior to nuclear division, either mitosis or meiosis*
- *Before their formation the DNA has replicated in the S phase of interphase, replication is semi-conservative and controlled by enzymes e.g. DNA polymerase*
- *Leaf palisade cells and liver cells have a diploid chromosome number and have been produced by nuclear division followed by differentiation. Differentiation limits further division*
- *Chromosomes consist of a kinetochore (centromere) which is the centre of chromosome movement, and two arms of variable length depending on the position of the centromere*
- *Telomeres are found at the end of the arms of chromosomes*
- *When stained and viewed under UV light a characteristic pattern of light and dark banding can be seen to be unique to each chromosome of a haploid set*
- *In prophase of mitosis the chromosomes form from the chromatin of the nucleus and appear as double structures made of two chromatids*
- *In metaphase they align on the equator of the nuclear spindle apparatus (NSA) formed from the nuclear membrane*
- *In anaphase the kinetochore divides and the chromatids separate to the poles of the NSA under the control of the centrioles*
- *In telophase each of the two sets of separated chromatids (now considered as new chromosomes) become surrounded by nuclear membranes and disperse as chromatin in the new daughter nuclei*
- *A new cell forms around each of the two daughter nuclei.*

Producing crops by vegetative propagation

Most plants can reproduce asexually, as anyone who has planted a sprouting potato, or taken a geranium cutting will know. This kind of reproduction, which does not involve gametes and the production of seeds, and results in genetically identical copies, or clones, of the parent plant is called **vegetative reproduction**. Over the centuries, crop growers have exploited the natural asexual reproductive properties of plants to multiply selected varieties. Many important food crops such as potatoes, sugar cane and bananas have been developed almost entirely by vegetative propagation.

There are obvious advantages in selecting a single superior individual from a plant population and reproducing it asexually. Since members of a clone are genetically uniform under optimum conditions, they grow to the same height, flower at the same time, and produce fruit of a similar size, features which make them highly suitable for mechanised farming. Vegetative propagation may be used to multiply infertile plants which have arisen from chance mutations but have commercial value, e.g. seedless fruits.

There are, of course, inherent risks in growing large numbers of plants with identical genotypes. The uniformity which makes them so attractive to farmers applies also to their susceptibility to pests, diseases and other environmental hazards. An entire crop may be wiped out by a single type of pathogen. Another drawback is the cost of vegetative propagation. By comparison with seed planting, it is more expensive per plant because it is more labour intensive, and it requires a large capital investment for research and technical facilities. Some plants, notably apple, pear, peach and cherry trees, have proved impossible to multiply by cuttings, but may be propagated vegetatively by the technique of grafting.

Clones may be propagated on for many generations. The Cabernet Sauvignon grape, for example, has been cloned for at least 2000 years, but repeated vegetative propagation can bring about serious disease problems. Without the natural break provided by seed formation and the opportunity for a new virus-free seedling each generation, cloned plants may retain their disease organisms from year to year, and these pathogens may evolve into more virulent strains. For this reason, it is necessary to plant new source vineyards regularly (from seed stock or micropropagated lines), to patent new plants, and to keep accurate records determining the pedigree of the source clone.

'Seed' potatoes are produced by vegetative reproduction from virus free plants grown in areas of windy and wet conditions (Scotland and Ireland) where the aphid (greenfly) carriers (vectors) of many virus diseases cannot easily fly to infect crops. These 'seed' potatoes are bought in, when virus diseases begin to accumulate to levels which decrease crop yield in growing regions. It is not efficient to keep growing potato plants from potatoes kept from the preceding year's crop. New varieties of potatoes are grown from true seeds.

It is now possible to clone plants from cell cultures through the techniques of **micropropagation**. Typically from apical meristems (dividing cells of shoot tips) which are virus free. In some cases, whole new plants can be propagated from single cells. By these techniques new, genetically engineered varieties may be cultured and multiplied rapidly. Plants which are transformed in this way to contain DNA from other organisms are called **transgenic plants**.

◆ CHECKPOINT SUMMARY

- *Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei*
- *The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur)*
- *As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical*
- *The production of genetically identical cells with the full set of genetic information allows the replacement of any cells in repair processes*
- *Mitotic cell division is seen in growth, repair and asexual reproduction*
- *The production of new individuals involves the transfer of genetic information from parent to offspring*
- *Mitosis results in cells identical to the fertilised egg reaching the reproductive organs*
- *Mitosis results in all offspring of asexual reproduction being genetically, lacking genetic variation*
- *In the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous.*

The cloning of animals

Identical animal clones can be produced by simulating the natural process by which identical twins are formed, in other words, by splitting apart the cells of developing embryos at a very early stage after fertilisation. Cows or sheep selected for a particular, desired characteristic may be inseminated artificially with semen from a selected bull and the embryonic ball of cells (**blastula**) is collected from the cow before it naturally implants. Under a microscope the blastula can be divided into two or more 'twins' which are cultured on for a few days, then implanted into a surrogate mother treated with hormones so that her oestrus cycle coincides exactly with the real mother. Alternatively, the blastula can be frozen in liquid nitrogen and stored for later use. This technique is called **embryo transfer**.

A rather more sophisticated extension of these cloning techniques with farm animals is the **nuclear transfer** procedure. Unfertilised eggs are harvested from a donor animal and their nuclei are removed by suction with a micropipette. The 'enucleated' egg cells may then be fused with single cells taken from a selected blastula. Fusion is normally achieved by applying a small electric current to the culture medium. The egg, now equipped with a super selected genotype (set of genes), is grown in culture for a few days, then implanted into a surrogate mother, or frozen. The advantage of this method is that the same selected blastula may yield 30 or 40 cells for nuclear transfer, each of which can be implanted into surrogate mothers and grow into an adult. Furthermore, the original donor may be treated with fertility hormones to repeat the process every three months.

The technique of nuclear fusion described above has been used to insert the genotype of an adult body cell into an unfertilised egg, then brought to birth in a surrogate mother. Dolly, the famous sheep, was cloned in this way from cells taken from the mammary glands of its genetic mother.

Unlike plant cells, animal cells can not be induced to grow into whole new organisms once they have begun the process of differentiation. The only time this is possible is before the onset of differentiation, in other words, at the egg or zygote stage.The possibilities for creating transgenic animals is therefore very much more limited in animals than in plants. Now that the procedures for embryo transfer and nuclear transfer are improving, the possibilities for genetic modification and the creation of transgenic animals are rapidly expanding. Pigs, genetically modified to reduce the rejection response from a human organ recipient, may soon be cloned successfully to provide kidneys and other organs for transplantation. This is another field of research which raises ethical issues of public concern over which much debate will surely follow.

◆ CHECKPOINT SUMMARY

- ◆ *Clones are genetically identical organisms*
- ◆ *Cloning may occur naturally, for example, identical twins in humans, binary fission in microorganisms and vegetative reproduction in plants, but it can also be achieved artificially*
- ◆ *The production of crops by vegetative propagation e.g. in which cuttings are taken from a desirable original is a form of cloning., some common crop plants, for example the banana, can only be grown by cloning*
- ◆ *Since members of a clone are genetically uniform under optimum conditions, they grow to the same height, flower at the same time, and produce fruit of a similar size, features which make them highly suitable for mechanised farming*
- ◆ *The uniformity which makes them so attractive to farmers applies also to their susceptibility to pests, diseases and other environmental hazards. An entire crop may be wiped out by a single type of pathogen*
- ◆ *It is now possible to clone plants from cell cultures through the techniques of micropropagation. In some cases, whole new plants can be propagated from single cells*
- ◆ *This is useful in producing virus free stock from apical meristem cells which viruses do not penetrate*
- ◆ *The cloning of animals is possible by new techniques for manipulating embryonic cells, e.g. by splitting apart the cells of developing embryos at a very early stage after fertilisation.*