HUMAN BIOCHEMISTRY

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The aim of this option is to give students an understanding of the chemistry of important molecules found in the human body, and the need for a balanced and healthy diet. Although the role that these molecules play in the body should be appreciated, the emphasis is placed on their chemistry, and students who have not followed a course in biology will not be at a disadvantage. Students will not be required to memorize complex structures, but they will be expected to recognize functional groups and types of bonding within and between molecules. Structures of some important biological molecules are given in the *Chemistry data booklet*.

The human body requires certain substances to function and grow and good diet is essential to staying healthy. Food provides energy and replaces molecules used up by bodily processes. Nutrients are the food components which provide growth, energy and replacement of body tissue. They are generally divided into six groups, namely proteins, carbohydrates, lipids, micro- and macro-nutrients (including vitamins and minerals) and water. The human body requires different amounts of each nutrient for good health.

Water comprises about 70% of our body mass and is responsible for dissolving most of the chemicals in our system and transporting **nutrients** and waste. In order to create a balance between our intake and the amount of water we release through sweat, urine, faeces and respiration, humans generally require about one to one and a half litres of water daily in addition to that obtained from the food consumed.

B1 ENERGY

B.1.1 Calculate the energy value of a food from enthalpy of combustion data.

The oxidation of macro-nutrients fats, carbohydrates and proteins produces carbon dioxide, water and energy. The energy requirements of human beings depend on age, size, sex and daily activity. For adults with low activity levels, the recommended daily energy intake is about 10500 kJ (2500 kcal) for males and about 8000 kJ (1900 kcal) for females. Metabolism, the chemical reactions in living organisms, converts the food we eat into energy and produces various materials that our bodies require. Fats are used to store energy and make available about 37 kJ g^{-1} of energy, more energy than carbohydrates (the main source of energy) and proteins which make available about 17 kJ g^{-1}. The ratio of H:O atoms in lipids is higher, for example in palmitic acid, $C_{16}H_{32}O_2$, it is 16:1 compared with a 2:1 ratio in carbohydrates. Thus carbohydrates (and proteins) are more oxidized and produce less energy per gram.

The amount of energy stored in food, its calorific or energy value, can be found experimentally by completely burning different foods and using the energy released to raise the temperature of a fixed mass of water using an insulated food calorimeter. The energy content or value of a large apple is about 420 kJ. This means that if the apple was burnt in a food calorimeter, the energy produced on combustion would raise the temperature of 1 kg water

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by 100°C, assuming the calorimeter does not absorb any heat.

$\Delta H = mc\Delta T = 1000 \text{ g} \times 4.18 \text{ J g}^{-1} \text{ °C}^{-1} \times 100 \times \text{°C} \approx 420 \text{ kJ}$

In calculations from experimental data however, any energy absorbed by the calorimeter must be taken into account.

Example

A large apple weighs 150 g. In a laboratory investigation, a 15.0 g sample of the apple, on complete combustion, raises the temperature of 200 g water in a glass container by 45.3 °C. Calculate the energy value of the whole apple. The heat capacity of the glass calorimeter = 89.1 J °C^{-1} and the specific heat of water = 4.18 J g^{-1} °C^{-1}.

Solution

Heat produced = heat absorbed by water + heat absorbed by calorimeter

$= (m \times c \times \Delta T)_{\text{water}} + (m \times c \times \Delta T)_{\text{calorimeter}}$

$= (200 \text{ g} \times 4.18 \text{ J g}^{-1} \text{ °C}^{-1} \times 45.3 \text{ °C}) + (89.1 \text{ J °C}^{-1} \times 45.3 \text{ °C})$

$= (37871 + 4036) \text{ J}$

$= 41907 \text{ J}$

$= 41.9 \text{ kJ}$ (produced by 15.0 g of apple)

Thus the energy value of the 150 g apple is 419 kJ.

Example

1.00 g cereal raises the temperature of 400 cm^3 water in an insulated food calorimeter from 23.7 °C to 33.4 °C. Calculate the energy value per gram of the cereal, assuming the heat capacity of the calorimeter is negligible and given the specific heat of water = 4.18 J g^{-1} C^{-1}.

Solution

Heat produced = heat absorbed by water

$= (m \times c \times \Delta T)_{\text{water}}$

$= [400 \text{ g} \times 4.18 \text{ J g}^{-1} \text{ C}^{-1} \times (33.4 - 23.7) \text{ °C}]$

$= 16.2 \text{ kJ}$ per gram of cereal.

The energy content of peanuts and proteins in eggs is about 24 kJ g^{-1}. A teaspoon of margarine releases about 420 kJ and the energy content of alcohol (ethanol) is about 29 kJ g^{-1}. The energy one consumes is expended through activity and the excess energy is stored as fat leading to weight gain. If a person eats less energy-containing food than the body needs, their stored fat is broken down for energy, resulting in weight loss.

B2 PROTEINS

Proteins are natural polymers, made up of C, H, O, N, (and sometimes S). Their primary use is to provide amino acids which are the building blocks of new proteins in the body. Amino acids are used to produce new proteins for growth and repair of body tissues as well as to make hormones, enzymes and antibodies. These can also be used as an energy source in the event of starvation. They make up 15% of our bodies and have molar masses of between 6000 to over 1,000,000 g mol^{-1}.

The variety of proteins that are important to human life are made of an assortment of some 20 α-amino acids. Out of some 20 amino acids, there are ten amino acids which our bodies cannot synthesize and cannot store in the same way they do fat, hence they must be found through food consumption. These are called **essential amino acids**. A protein which contains all ten of the amino acids we cannot synthesise and in a ratio similar to that which we need, is called a **complete protein**, such as casein from milk, cheese, eggs and soybeans. Most animal proteins such as those found in meat, fish and eggs are complete. Incomplete proteins include most plant proteins; wheat protein does not have lysine; rice protein does not contain lysine or threonine. Vegetarians can obtain all the proteins they require through a combination of legumes, such as peas, beans or corn, and grains as these serve to complement each other.

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B.2.1 Draw the general formula of 2-amino acids.

Proteins are polymeric substances made up of long molecules formed by sequences of smaller nitrogen–containing units called amino acids. Amino acids are molecules that contain both a carboxyl group (–COOH) and an amino group ($–NH_2$).

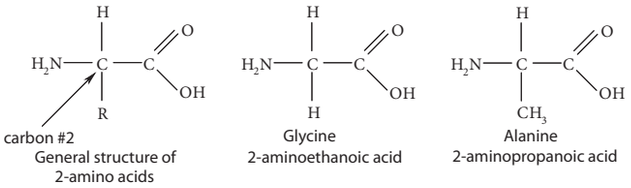

Figure 1301 General structural formula of 2-amino acid, glycine and alanine

The R group, called the side chain, can be hydrogen, an alkyl group or a complex substituent. All amino acids in natural proteins are **2-amino acids** (or **α-amino acids**), so called because the amino acid group is attached to the 2- or α–carbon atom, the one next to the carboxylic acid (–COOH) group. Amino acids have the ability to behave both as an acid and as a base in aqueous solution. In the simplest amino acid, glycine (IUPAC name 2-aminoethanoic acid), the methylene group ($–CH_2–$), is bonded to both an acidic carboxyl group (–COOH) and a basic amino group ($–NH_2$). If R is $–CH_3$, alanine is formed, (IUPAC name 2-aminopropanoic acid).

B.2.2 Describe the characteristic properties of 2-amino acids.

Amino acids are colorless, crystalline solids. These exist as **zwitterions** (dipolar ions) $H_3N^+CHRCOO^-$ which explains their relatively high melting points for organic compounds (for example, glycine has a melting point of 232 – 236°C), and why they are generally more soluble in water than in organic solvents.

Amino acids are amphoteric (capable of behaving as acids or bases); the majority of the amino acids contain one basic and one acidic group both of which can ionise in aqueous solution. As a base, the amine group accepts a proton from water:

$$H_2NCHRCOOH + H_2O \rightleftharpoons H_3N^+CHRCOOH + OH^-$$

At lower pH, the H^+ added reacts with OH^- present in the equilibrium and the forward reaction is favoured to replace some of the OH^- used up. Thus in a an acidic solution, the $–NH_2$ group is protonated.

As an acid, the carboxylic acid group donates a proton to water:

$$H_2NCHRCOOH + H_2O \rightleftharpoons H_2NCHRCOO^- + H_3O^+$$

At higher pH, the base added reacts with H_3O^+ present in the equilibrium and the forward reaction is favoured to replace some of the H_3O^+ used up. Thus in an alkaline solution, the carboxylic acid group donates a proton and is converted to the carboxylate ion.

A specific pH exists for each amino acid when the above two ionizations are identical (or balanced) and the amino acid exists only as zwitterions (dipolar ions): $H_3N^+CHRCOO^-$. This is called the **isoelectric point**, pI, of the amino acid, that is the pH at which the positive and negative charges are identical (or balanced), hence the molecule has no net charge and it shows no net migration in an electric field.

If the side chain, R also contains $–NH_2$ group, for example in lysine, then the number of $–NH_2$ groups > the number of –COOH groups, and it is a basic amino acid. If, on the other hand, the R group also contains a –COOH group, for example in aspartic acid, the number of –COOH groups > the number of $–NH_2$ groups, then it is an acidic amino acid. The presence of the basic side chains produces additional positively charged ions (NH_3^+) and the presence of acidic side chains produces additional negatively charged (COO^-) ions in the amino acids. Thus, how an amino acid behaves in the presence of an electric field depends very much on the relative numbers of these charged groups. This is affected by the acidity or basicity of the solution, that is, its pH.

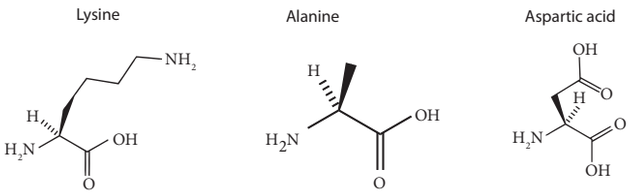

Figure 1302 The structures of some common amino acids

Their amphoteric nature makes it possible for the amino acids to act as buffers in aqueous solutions. Thus, when a strong acid, $H^+(aq)$, is added to an aqueous solution of an amino acid, the zwitterion accepts the proton, thus minimizing the effect of the acid added:

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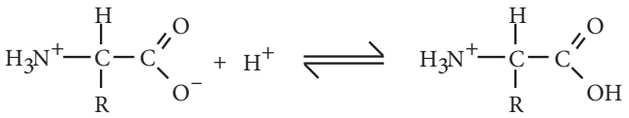
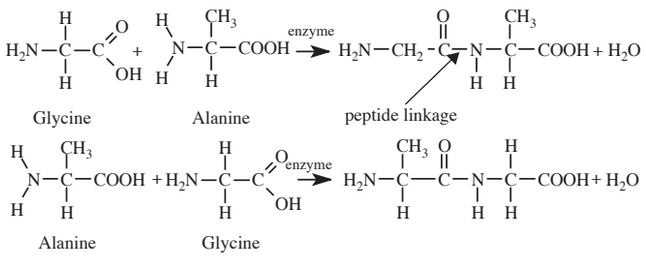


Similarly, if a strong base OH^- (aq) is added, the zwitterion donates H^+ to neutralize the base to form water:

Within the 2–amino acids, when R ≠ H, the 2–carbon atom is chiral, the molecule is asymmetric and gives rise to two possible stereoisomers. These non-superimposable mirror images are called **enantiomers** (and exhibit optical activity). Thus, all amino acids, except glycine where R = H, can exist as a pair of enantiomers (the D or L forms).

B.2.3 Describe the condensation reaction of 2-amino acids to form polypeptides.

Since all amino acids have both a carboxyl group and an amino group, they are able to undergo condensation reactions to form substituted amides. For example, glycine and alanine can combine to form two possible dipeptides:



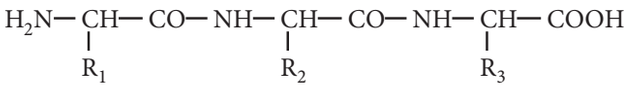
The product, a **dipeptide**, is a substituted amide made up of two amino acids joined by a peptide bond or peptide linkage. Water is the other product formed in the enzyme controlled process. Note that two amino acids can give two different products depending which has the free amino and which the free carboxyl end. Similarly six different tripeptides can be formed using three different amino acids, if each amino acid is used only once ($3 \times 2 \times 1$). If a compound contains many of these peptide bonds it is considered to be a polypeptide which, after folding, becomes a protein. The 20 naturally occurring amino acids can hence undergo condensation reactions to produce a huge variety of proteins.

B.2.4 Describe and explain the primary, secondary (α-helix and β-pleated sheets), tertiary and quaternary structure of proteins.

As described previously amino acids can undergo condensation reactions in any sequence (order), thus making it possible to form an extremely large number of proteins. The structure of proteins can be broken down to four levels: primary, secondary, tertiary and quaternary structure.

PRIMARY STRUCTURE

The **primary structure** is simply the sequence of amino acid residues that form the protein. This is indicated by using three–letter codes for the amino acids. A tripeptide containing the amino acids lysine, glycine and leucine would, for example, be lys–gly–leu in which, by convention, the terminal amino acid group of lysine is on the left and the terminal carboxylic acid group of leucine is on the right. Thus a different tripeptide leu–gly–lys consisting of the same three amino acids means the amino terminal group leucine is on the left and the acid terminal group lysine is on the right:



where R_1 is in leucine (containing the end group $-NH_2$), R_2 in glycine and R_3 in lysine (with the end group –COOH).

Each type of protein in a biological organism has its own unique primary sequence of amino acids. It is this sequence that gives the protein its ability to carry out its characteristic functions. Figure 1303 shows a typical primary structure with the amino end group tyrosine and carboxylic acid end group leucine.

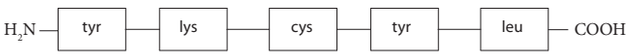


Figure 1303 A Primary structure

SECONDARY STRUCTURE

The **secondary structure** is the manner in which a polypeptide chain folds itself, due to intramolecular hydrogen bonding, in particular patterns that repeat themselves. This affects the arrangement in space of the polypeptide chain. Two features commonly found in a

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protein's secondary structure are referred to as an **α–helix** and a β-pleated sheet. In the α–helix structure, the peptide chain resembles a right handed spiral staircase or coiled spring; this shape is called a **helix**. This can make the protein elastic or sponge–like in fibrous proteins such as hair and wool. The α–helix maintains its shape through regular intramolecular hydrogen bonds. These are between the (δ–) oxygen of the carbonyl group (–C=O) and the (δ+) hydrogen of the $-NH_2$ group of the third peptide bond down the chain, that are in just the right position to interact. The H–bonding in the α–helix structure is parallel to the axis of the helix.

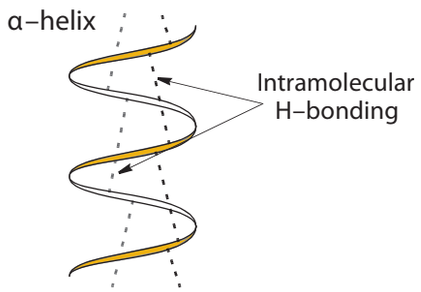

Figure 1304 The α-helix is a common secondary structure

In the **beta-pleated sheet** arrangement one, or more commonly several, different polypeptide chains are bound together by hydrogen bonds, (intramolecular if just one chain, or intermolecular if more than one) to create an orderly alignment of protein chains in which the direction of H–bonding is perpendicular to the sheet structure giving rise to a repeating, pleated pattern. Silk has this arrangement, making it flexible, but strong and resistant to stretching. If a particular part of a polypeptide chain does not exhibit a repeating pattern it is said to contain random coils.

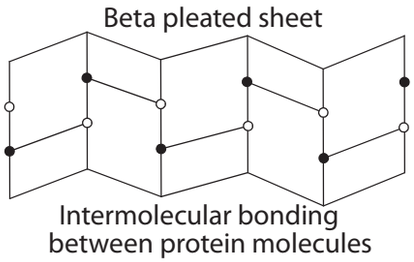

Figure 1305 The beta-pleated sheet is a secondary structures

TERTIARY STRUCTURE

This is the folding or curling due to the interaction between the sequence of amino acids that maintains the three dimensional shape of the protein. The amino acid secondary structure in the helical, pleated or random coil form arranges itself to form the unique twisted or folded three dimensional shape of the protein.

In myoglobin about 70% of the amino acid sequence is α-helical secondary structure. The non-helical regions form a random coil and are a major factor that determines its tertirary structure. Refer to Figure 1306.

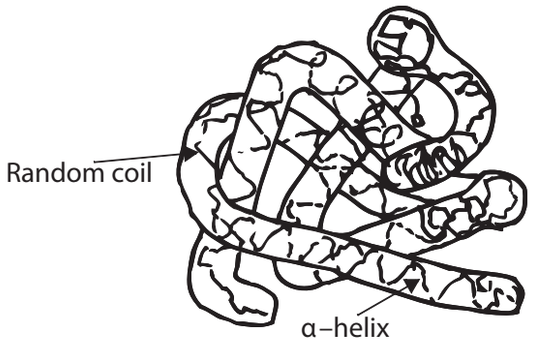

Figure 1306 The tertiary structure of myoglobin, a typical protein

There are four ways in which parts of the amino acid chains interact to stabilize their tertiary shapes.

These are:

(1) Covalent bonding, for example disulfide bridges can form between different parts of the protein when the –SH functional groups of two cysteine groups (cysteine is an aminoacid which has an -SH side-chain) link when oxidized under enzyme control:

$$\square\text{–S–H} + \text{H–S–}\square \xrightarrow{[O]} \square\text{–S–S–}\square + H_2O$$

For example, the **keratin proteins** in hair have a large number of cysteines connected by the disulfide bridges which hold the hair in its normal structure. In the artificial hair curling process, the –S–S– disulfide bridges on cysteine residues are reduced to the –S– H groups of cysteine:

$$\text{–S–S–} \xrightarrow{[2\ H]} \text{–S–H} + \text{H–S–}$$

On using a curler, hair is stretched and the –S–H groups are displaced. Then an oxidising agent is used to oxidize the curled hair to form new disulfide linkages to retain the new curls.

(2) Hydrogen bonding between $-NH_2$ and -COOH groups on the side chain.

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(3) Salt bridges (electrostatic attraction) between $-N^{+}H_3$ and $-COO^{-}$ groups.

(4) The R group side chain affects the 3–D structure of the resulting proteins depending on whether it is non-polar containing mostly C–H bonds and therefore hydrophobic or polar containing N–H and O–H bonds and therefore hydrophilic. Hydrophobic interactions occur when non–polar, hydrophobic side groups tend to clump together on the inside, forcing the protein chain into a tertiary shape with the polar parts of the molecule on the outside. Hydrophobic interactions involve the exclusion of water from the non-polar interior of the protein.

QUATERNARY STRUCTURE

This occurs only in proteins that are composed of more than one polypeptide chain which are held together by non-covalent bonds; these consist of hydrophobic interactions, hydrogen bonding and ionic bonds. When a protein consists of more than one polypeptide chain, each is called a subunit. The quaternary structure is how the polypeptide subunits are held together in a precise, more complex three-dimensional structural arrangement. An example is haemoglobin, which consists of two slightly different pairs of polypeptide chains grouped together to form the quaternary structure together with the haem co-factor (this binds the oxygen molecules reversibly).

Proteins can be denatured, that is they can lose their three dimensional structure and hence biological activity, in a number of ways. Denaturation affects the functioning of a protein because the exact shape is the key to the function of each of the numerous proteins in the body. The most common cause of denaturing is heat, as is the case when an egg is fried or boiled, which cause the breaking of hydrogen bonds. Other ways in which denaturing can occur include high energy ionising radiation, strong acids, bases and concentrated salt solutions (which can disrupt salt bridges) and, organic solvents and detergents (which can disrupt hydrophobic interactions).

B.2.5 Explain how proteins can be analysed by chromatography and electrophoresis.

A protein can be analyzed, that is, its amino acid residue composition can be determined by hydrolyzing the peptide bonds as shown in Figure 1307. With the lone electron pair on the nitrogen, the peptide linkage is a strong bond and complete acid hydrolysis usually requires the use of 6 mol dm^{-3} HCl solution at 110°C for several hours.

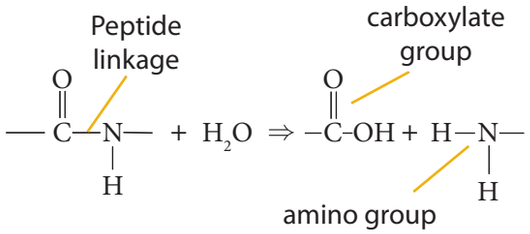

Figure 1307 Hydrolysis of a peptide linkage

Hydrolysis of the peptide linkages produces individual amino acids which can be identified, and the amounts determined, using **high performance liquid chromatography** (hplc) since amino acids are not very volatile. In the laboratory, it is possible to identify individual amino acids using paper chromatography or electrophoresis, using known samples for comparison.

CHROMATOGRAPHY

Chromatography is a very useful method for the separation of mixtures of substances which are otherwise not readily separated. Paper chromatography is suitable for the identification of components of a very small sample of mixture and is particularly suitable for separating hydrophilic substances such as amino acids. The relative solubility of different amino acids varies in the stationary phase (water, which is adsorbed on the cellulose paper), and the mobile phase (solvent). The principle of paper chromatography is the partition of solutes (the amino acids) between the two phases. Thus, amino acids with greater solubility in the eluting solvent will travel further in the direction of the solvent flow. Experimentally, a solution of the sample of the mixture of amino acids to be analyzed is placed as a spot on the surface of the chromatographic paper a couple of centimetres from the bottom, marked in pencil, and allowed to dry.

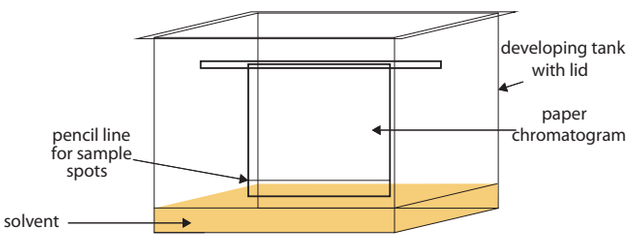

Figure 1308 One possible experimental arrangement for chromatography

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The paper is placed vertically in a developing tank, the bottom of which contains about a ½ cm depth of the solvent. This is then covered and the solvent allowed to rise by capillary action. The components of the mixture move with the solvent at different rates depending on their solubility in the stationary and moving phases. Once the solvent nears the top of the paper it is removed, the solvent front is marked and the solvent allowed to evaporate. As the amino acids are colourless the plate must be developed by spraying it with a solution of ninhydrin. Glycine, for example, forms a blue/purple compound with ninhydrin, as do 19 of the 20 protein-derived α-amino acids (proline gives an orange color).

A number of spots will be found corresponding to the different amino acids in the protein. The amino acids can be identified by comparing the R_f values of the spots with those for pure amino acids developed at the same time, under the same conditions of solvent and temperature. R_f represents the 'Ratio of fronts' and refers to the ratio of the distance traveled by a compound (d_c) divided by the distance traveled by the solvent (d_s):

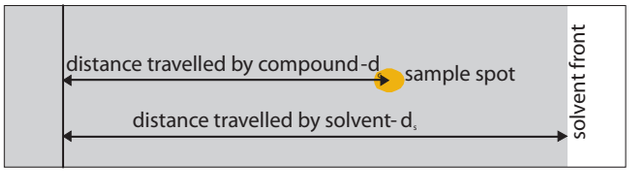

Figure 1309 Calculating the 'ratio of fronts' (R_f)

$$R_f = \frac{d_c}{d_s} = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$$

Different substances have different R_f values under identical experimental conditions, so comparison of R_f values allows for the components of a mixture to be identified. If several components of a mixture have similar R_f values using a particular solvent, thus leading to incomplete separation, it is possible to repeat the experiment with a different solvent. One development of this is two–dimensional chromatography in which the sample spot is placed in one corner of a square piece of chromatography paper, the chromatogram is developed by eluting with one solvent system to allow partial separation. The paper is dried, turned at right angles from its original position and developed using a second solvent system (such as basic butan-2-ol/ammonia mixture if acidic butan-1-ol/ ethanoic acid was the first solvent) to achieve a more complete separation.

Electrophoresis and Isoelectric Points

Electrophoresis is the method of separating (similar sized) molecules on the basis of their electric charges. In order to analyse a protein using electrophoresis, the peptide bonds in the protein must first be hydrolysed to release the individual amino acids.

After a protein has been hydrolysed, each amino acid in the mixture produced has a different isoelectric point. This means that they can be separated using electrophoresis. Such a separation can be carried out using paper, cellulose acetate or other appropriate solid supports. The solid support is saturated with a buffer solution of known pH.

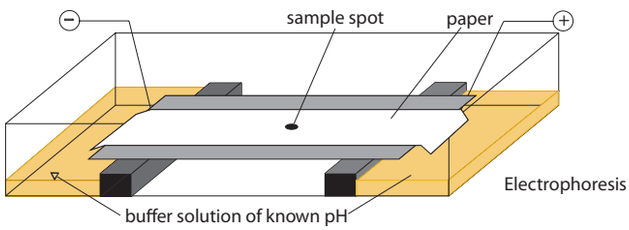

Figure 1310 Electrophoresis equipment

The sample consisting of a mixture of amino acids is applied to the centre of the paper. The electrodes and ends of the paper are placed in the buffered solution and a high electric potential is applied to the electrodes. Any amino acid at its isoelectric point does not move in either direction. However, amino acids with positive charges at that pH move to the cathode and amino acids with negative charges at that pH move to the anode. After sufficient separation is achieved, the paper strip is dried and sprayed with ninhydrin solution to make the components visible. The paper can then be compared with known samples. By repeating the process at different pH values the isoelectric points of the components can be determined, helping to identify individual amino acids. Figure 1311 lists the isoelectric points, pI, of some amino acids (see also the IB Chemistry Data Booklet Table 19, page 26):

Amino acid	Symbol	pI (pH units)
Cysteine	Cys	5.1
Glutamine	Gln	5.7
Glycine	Gly	6.0
Histidine	His	7.6
Lysine	Lys	9.7

Figure 1311 The iso-electric points of some common amino acids

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At pH 6.0, glycine exists as the dipolar ion, $H_3N^+–CH_2–COO^-$ and hence will not move if electrophoresis is carried out at this pH. If electrophoresis is carried out with glycine at pH = 7.0, the pH of the buffer is more basic than the isoelectric point of glycine (7.0 compared to 6.0), glycine will have a net negative charge because it largely exists as $H_2N\text{-}CH_2\text{-}COO^-$ and it will migrate toward the positive electrode. However, if the electrophoresis is carried out at pH = 5.0, the pH of the buffer is more acidic than the isoelectric point of glycine (5.0 compared to 6.0), glycine will have a net positive charge ($H_3N^+\text{-}CH_2\text{-}COOH$) and will migrate towards the negative pole.

If electrophoresis of a mixture of the five amino acids listed in the previous table is carried out at a pH of 6.0, glycine will not move from the point of origin as it has a net charge of zero at its isoelectric point of 6.0.

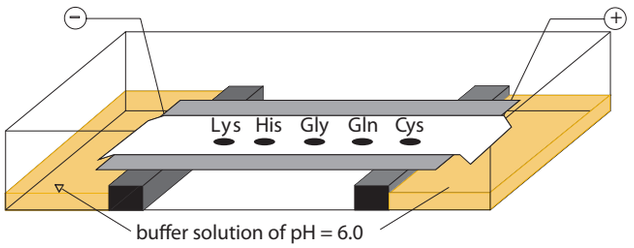

Figure 1312 Electrophoresis of a mixture of some common amino acids

Cysteine (pI = 5.1) and glutamine (pI = 5.7) will have negative charges because the buffer pH of 6.0 is more basic and will move to the positive pole. Histidine (pI = 7.6) and lysine (pI = 9.7) will have positive charges since the buffer of 6.0 is more acidic and they will move to the negative pole. Also, generally, the greater the difference between the pI and the pH, the faster the migration.

B.2.6 List the major functions of proteins in the body.

Proteins carry out many important functions in the body such as providing structure, and acting as enzymes (biological catalysts), hormones, immunoproteins (as antibodies in providing protection) and transportation proteins as well as acting as an energy source if carbohydrates and fats are not available.

STRUCTURAL

Proteins such as collagen (found under the skin) and keratin (found in hair and nails) provide structure and strength.

BIOLOGICAL CATALYSTS

Enzymes, which are proteins, catalyze almost every chemical reaction in the human body without which reactions would occur much too slowly. Enzymes provide an alternate pathway for the reaction, thus lowering the activation energy and speeding up the reaction.

HORMONES

Hormones such as insulin are important proteins in humans and animals.

ANTIBODIES

Proteins that are produced as a result of the presence of foreign materials in the body which provide immunity to diseases, for example, interferons provide protection against viral infection.

TRANSPORT

Haemoglobin in the red blood cells carries oxygen from the lungs to the cells and to some extent, carbon dioxide from the cells to the lungs.

ENERGY

Proteins in the human body can be used to provide energy. Under starvation conditions, when supplies of carbohydrates and fats are insufficient, protein, from muscle, can be metabolised to provide energy.

B3 CARBOHYDRATES

Carbohydrates, empirical formula CH_2O, are the main energy source for our bodies and are vital to the synthesis of cells. They serve as food sources for living organisms and provide the structural support for plants. Many of the carbohydrates are large polymeric molecules made of simple sugars. Only plants synthesise carbohydrates. Important carbohydrates are: starch (a polysaccharide), lactose and sucrose (disaccharides) and glucose and fructose (monosaccharides). Glucose, molecular formula $C_6H_{12}O_6$, is found in all body cells and fructose, which has the same molecular formula as glucose, is found in fruits and honey.

Most carbohydrates (but not cellulose in humans) are changed to glucose, a simple sugar, as a result of digestion. Glucose is then carried by the blood to body cells, where it is oxidised during respiration. The energy available goes to physical activities, keeps the body warm and is used for repair and growth of cells. Excess carbohydrates are converted to fats and stored in the body.

Cellulose, another polysaccharide, is the major component of plant cells. It cannot be digested by humans who lack the enzyme cellulase to hydrolyse it. Cellulose though not a source of energy, is an important part of diet and is called fibre or roughage.

B.3.1 Describe the structural features of monosaccharides.

Monosaccharides, literally meaning 'one sugar', are the smallest molecular units of carbohydrates with the general formula $(CH_2O)_n$ where n = 3 to 9 and hence have the empirical formula CH_2O. Monosaccharides are aldehydes (alkanals) or ketones (alkanones) containing a carbonyl (>C=O) group and at least two hydroxyl (–OH) groups. Examples of monosaccharides are glucose, galactose and fructose. These three simple sugars contain six carbon atoms and are all examples of hexoses. Monosaccharides with five carbon atoms (such as ribose) are given the general name of pentoses. Pentoses and hexoses are the most common monosaccharides found in nature.

Monosaccharides and disaccharides have low molar masses, are sweet and readily soluble in water due to hydrogen bonding between water and the –OH groups. They form crystalline solids, due to intramolecular hydrogen bonding between the –OH groups. Since aldehydes are easily oxidized (to carboxylic acids), monosaccharides with aldehyde groups such as glucose are called reducing sugars.

B.3.2 Draw the straight–chain and ring structural formulas of glucose and fructose.

The straight chain formula of D–glucose containing the aldehyde group at C-1 is shown in the structure below:

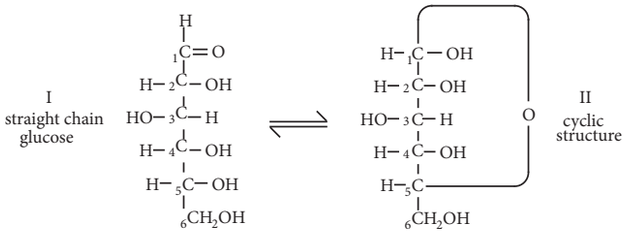

Figure 1313 Structures of glucose

The carbon atoms in glucose are numbered, starting with 1 at the carbonyl group. Note that there are four similar chiral (asymmetric) carbon atoms (C_2, C_3, C_4 and C_5) in the glucose molecule and thus several stereoisomers exist; C_1 and C_6 are not chiral.

Glucose is found almost exclusively in a ring or cyclic (hemiacetal) structure in aqueous solution (structure II). Because the intramolecular reaction between the aldehyde group on C-1 and the OH group on the C-5 atom produces a chiral carbon C-1 there are two possible ring structure isomers of glucose called α–D–glucose (where the –OH on C-1 is below the ring) and β–D–glucose (where the –OH group on C-1 is above the ring). (*Refer to Chapter 17, Option F Food Chemistry, for an explanation of D (and L) notation*). The only difference between the two is which side of the C-1 atom the –H and – OH are on (giving them different physical and chemical properties):

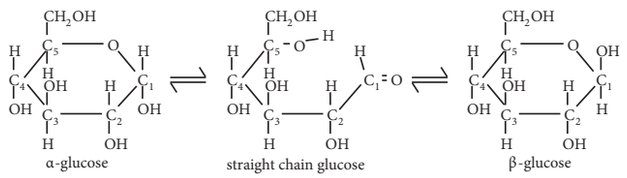

Figure 1314 The interconversion of α- and β-glucose

Fructose is an isomer of glucose but it is a ketose containing C=O in the C-2 position. It has the same configuration as straight chain glucose at C-3 to C-6 and differs only in the arrangement at C-1 and C-2. Fructose is capable of forming a six or a five membered ring:

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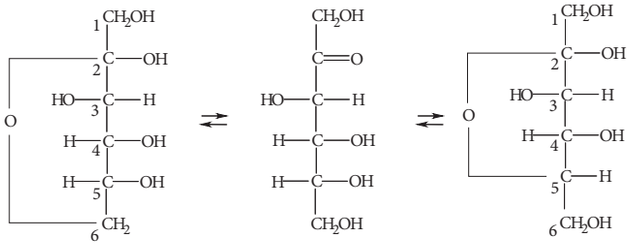
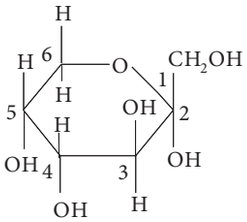


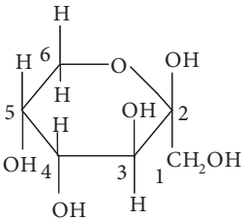
Figure 1315 Straight chain and cyclic structures of D- fructose

Similar to α- and β-glucose the two six-and five-membered ring structures of D-fructose, the only difference is which side of the C-2 atom the $-CH_2OH$ and – OH are on.



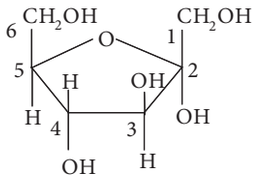
α-D-fructose

(pyranose ring)



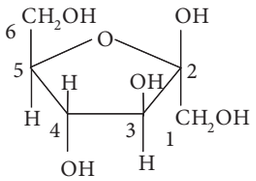
β-D-fructose

(pyranose ring)



α-D-fructose

(furanose ring)



β-D-fructose

(furanose ring)

Figure 1316 Six-and five-membered D-fructose ring structures

B.3.3 Describe the condensation of monosaccharides to form disaccharides and polysaccharides.

When monosaccharides form disaccharides or when smaller polysaccharides form larger polysaccharides they do so through condensation reactions by eliminating a water molecule to form a C–O–C bond between the rings; this is called the glucoside (glycosidic) linkage. This normally forms between carbon atoms 1 and 4 of neighbouring units and is called a 1,4 or 1→ 4 bond. When a person digests sucrose, hydrolysis of the glucoside linkage takes place, (the reverse of the condensation reaction) and produces the two monosaccharides.

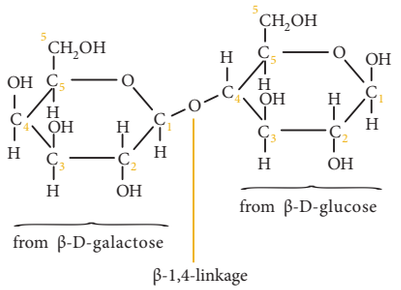
The following are some disaccharides formed from monosaccharides:

(i) β–glucose + β–galactose → lactose

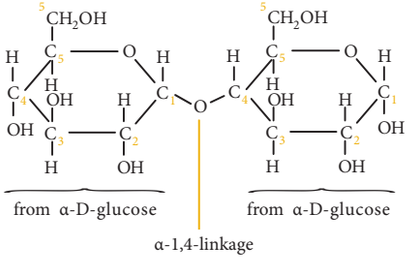
(ii) α–glucose + α–glucose → maltose

(iii) α–glucose + β–fructose → sucrose (table sugar)

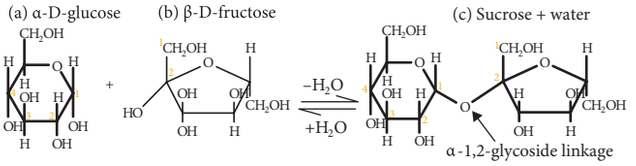
(i) Lactose contains β-1,4-glycoside linkages:



(ii) Maltose contains α-1,4-glycoside linkages:



(iii) Sucrose contains α-1,2 -glycoside linkages:



Polysaccharides are complex carbohydrates consisting of numerous monosaccharide units. These have large molar masses, are not sweet, are non–reducing and are generally insoluble or only slightly soluble in water. Starch is the polysaccharide by which plants store glucose for energy. Polysaccharides differ in the nature of their recurring monosaccharide units, the bonds connecting these, the length of their chains and the degree of branching. There are two forms of starch; both are polymers of α–glucose. Water soluble amylose (M_r = 10 000 – 50 000) has a straight chain structure consisting of long chains (thousands of monosaccharide units long) glucose joined by α–1,4 glycosidic linkages. Water insoluble amylopectin (M_r = 50 000 – 100 million) has these linkages as well as branches formed by α–1,6 glycosidic linkages on the glucose at which the branching occurs.

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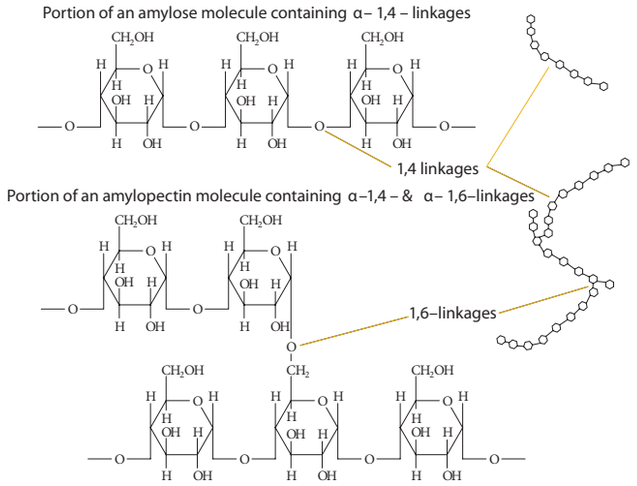

Figure 1317 Structure of amylose and amylopectin

Glycogen is made up of α-glucose and contains straight-chain α-1,4 glycosidic linkages as well as α-1,6 branching similar to amylopectin. The difference between glycogen and amylopectin is in the degree of branching: glycogen is more highly branched as the straight-chain component contains a smaller number of glucose units and branching occurs via α-1,6 linkage on the average every 8 to 12 glucose units.

Cellulose is made up of β-glucose units with all glycosidic linkages in the 1,4-positions, making it a linear polymer. This allows side by side alignment of cellulose chains resulting in extensive hydrogen bonding between them which gives it strength and rigidity, hence it is used as a structural component in plants. The extensive intermolecular hydrogen bonding also makes cellulose insoluble in water (as not enough water molecules can hydrogen bond with cellulose to separate the chains) and the length of the chains (M_r ~ 1 million) contributes to the lack of solubility.

B.3.4 List the major functions of carbohydrates in the human body.

Functions of carbohydrates include:

- **Energy source:** Potatoes, bread, corn, rice and fruits contain carbohydrates, as do snack foods such as sweets, chips and soft drinks.
- **Energy reserve:** Animals use glycogen stored in the liver as their energy storage polysaccharide. Glycogen can be broken down by enzymes into glucose which can be transported by the blood to cells. Glucose and oxygen are the reactants necessary for aerobic cellular respiration which releases energy (see B9).
- **Precursor of other biologically important molecules:** Heparin which occurs in intestinal walls and is used as an anticoagulant is formed from carbohydrates. Carbohydrates are also found as components of nucleic acids.

B.3.5 Compare the structural properties of starch and cellulose and explain why humans can digest starch but not cellulose.

As described earlier, both starch and cellulose are polymers of glucose units. Starch exists in two forms: amylose, which is a straight-chain polymer (α-1,4 linkage), and amylopectin, which is a branched structure with both α-1,4 and α-1,6 linkages. Cellulose on the other hand contains only β-1,4 linkage, which can be hydrolysed to glucose by the enzyme cellulase, which is absent in most animals, including mammals. Thus humans cannot digest cellulose. Cows and many other animals are able to digest cellulose in plants such as grass to produce glucose as a source of energy since they have bacteria in their alimentary canal that produce the cellulase enzyme.

B3.6 State what is meant by the term dietary fibre.

Dietary fibre is mainly plant material that is part of fruits, grains and vegetables that the human body cannot digest as it is not hydrolysed by enzymes produced by the human digestive tract (but may be digested by bacteria in the gut). Examples include cellulose, hemicellulose (made of different sugar monomers unlike cellulose) lignin and pectin.

B. 3.7 Describe the importance of a diet high in dietary fibre.

There are two types of dietary fibre. Water insoluble fibre, cellulose and lignin, present mainly in whole grain foods, fruits and vegetables. They absorb water, thus providing bulk and moving food through the digestive system.

Water soluble fibre includes pectins which undergo fermentation in the large intestine by bacteria to produce

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short-chain fatty acids such as propanoic and butanoic acids. These both have positive health effects such as stabilizing lipid and blood glucose levels (thus water soluble fibre may be helpful in the prevention of diabetes), and provide immune protection through stimulating the production of antibodies. Dietary fibre may be helpful in the prevention of other conditions as follows.

CONSTIPATION AND DIVERTICULOSIS

The large intestine (colon) makes, stores and eliminates stool (waste products). Low fibre diets lead to constipation due to the presence of hard stool that does not pass easily or frequently through the colon and requires effort. Pressure applied to move a stool along causes diverticulosis, the presence of bulges in the colon at weak places leading to abdominal pain. Diverticulosis is quite common in the western world where some diets consist of too much processed foods, which often lack fibre.

IRRITABLE BOWEL SYNDROME

Irritable Bowel Syndrome (IBS) refers to symptoms arising from the bowel not working as it normally should and includes constipation, bloating (feeling full), abdominal pain, etc. One way to decrease symptoms of IBS is to include more dietary fibre in the diet.

OBESITY

Regular intake of excess food leads to storage of energy in the fatty tissues. **Obesity** is excess body mass and leads to problems such as cardiovascular disease (involving heart and/or blood vessels), obesity related diabetes, breathing difficulties during sleep, etc. A high fibre diet leads to feeling full on a diet with reduced carbohydrates and fats, which then reduces weight gain.

CROHN'S DISEASE

This is an inflammatory bowel disease of the lower part small intestine and/or the large intestine. The cause of the disease is unknown; dietary fibre may be helpful in its prevention.

HAEMORRHOIDS

In this condition there are enlarged blood vessels in and around the rectum and anus that are swollen at weak points and can burst causing bleeding; these can also occur when the blood vessels get infected. Haemorrhoids can be caused by pressure in the abdomen as a result of constipation which can cause strain during bowel movements and by being obese or overweight. High fibre diet makes the bulk move through the large intestine more easily.

B4 LIPIDS

The term **lipid** does not suggest a particular chemical structure (unlike for example amino acids) but includes different structures. Lipids are substances found in living organisms that are defined in terms of their solubility: in general these are poorly soluble in water, but soluble in organic solvents which are non-polar or of low polarity. The very low solubility of lipids in water is due to large non–polar, hydrophobic ('water–hating') hydrocarbon chains present in such molecules with only a few hydrophilic polar groups. For this reason, lipids cannot dissolve in the bulk fluids of animal bodies whose chemistry is based upon water (the human body is around 70% water).

Lipids can be divided into several groups: triglycerides (fats and oils) such as tristearin, phospholipids (complex lipids containing phosphorus) such as lecithin and steroids such as cholesterol. 98% of lipids are triglycerides (glycerol esters) and 2% are complex lipids and cholesterol. Triglycerides (which are present in butter, cheese, meats, milk and nuts) contain carbon, hydrogen and oxygen (in a reduced form) with a low proportion of oxygen. For example, tristearin, a fat has the molecular formula $C_{57}H_{110}O_6$. Thus they are little oxidised and hence the most concentrated energy source (providing over twice as much energy per gram as carbohydrates).

Linoleic and linoeic acids are the two most vital fats since they cannot be synthesized in our bodies, but are necessary for its correct functioning.

B.4.1 Compare the composition of the three types of lipids found in the human body.

TRIGLYCERIDES (FATS AND OILS)

A triglyceride is formed from one molecule of glycerol (propan-1,2,3-triol, $CH_2(OH)CH(OH)CH_2OH$) and three fatty aliphatic acids (R–COOH, which can be the same or different fatty acids) which are carboxylic acids with long, straight, hydrocarbon chains. The fatty acids contain between 16 and 22 carbon atoms. If the triglyceride

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contains the same fatty acid (that is, $R_1 = R_2 = R_3$), then it is called a **simple triglyceride**. If the fatty acids are different (that is, $R_1 \neq R_2 \neq R_3$), then it is a **mixed triglyceride**.

Fats such as butter contain no unsaturation in the hydrocarbon chain whereas oils such as olive oil contain at least one C=C and are thus unsaturated. These are mono-unsaturated if there is one C=C and poly-unsaturated if there is more than one C=C bond. The arrangement around the double bonds is *cis,* meaning the alkyl groups across the C=C bond are on the same side. Triglycerides are un-ionised lipids (unlike phospholids that are ionised):

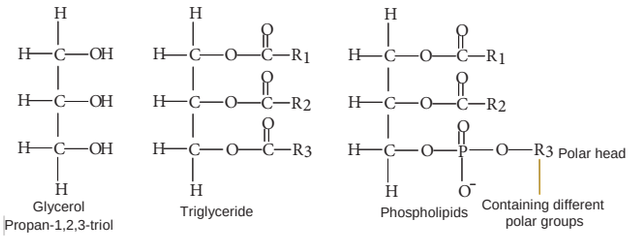

Figure 1318 Structures of glycerol, triglycerides and phospholipids

PHOSPHOLIPIDS

These are lipids that contain the phosphate group. They are formed from a backbone, such as glycerol with two fatty acids esterified onto it. The third carbon of glycerol is esterified with a phosphate group bonded to an alcohol derived from different polar groups (such as choline, ethanolamine). Thus, phospholipids are similar to triglycerides in their structure except that one fatty acid is replaced by a phosphate that contains a polar group. One example is lecithin which contains choline ($(CH_3)_3N^+CH_2CH_2OH$) as the side chain. The phospholipids are thus a source of choline for the production of acetylcoline, a nerve stimulus chemical transmitter:

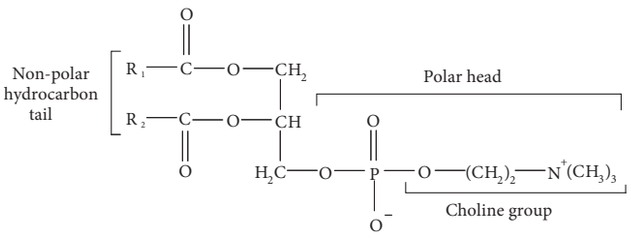

Figure 1319 Structure of lecithin

The R groups of the fatty acids are non-polar tails whereas the phosphate group is the polar head of the molecule. Such molecules are called **amphipathic**.

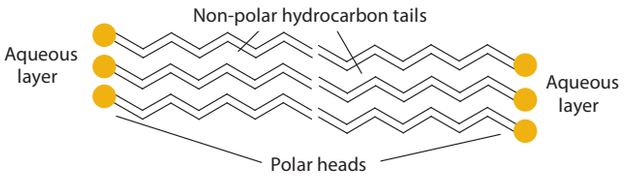

Figure 1320 Phospholipid bilayer structure

In water, phospholipids form a **bilayer** with the polar, hydrophilic heads facing the aqueous layer and the non-polar hydrocarbon tails lining up with each other. Phospholipids are a major part of all biomembranes that act as barriers around and inside a cell. Differences in the polar group and hydrocarbon tail (length of carbon chain and degree of unsaturation) determine the function and shape of the phospholipid.

Steroids (for example, cholesterol): The steroid skeleton is a characteristic molecular tetracyclic fused carbon ring structure consisting of three cyclohexane rings (labelled A, B and C) and a cyclopentane ring (labelled D), hence it is not related to the structures of fatty acids. The steroid skeleton can be modified in a large number of ways by attaching functional groups to it, one important example being **cholesterol** shown in Figure 1321.

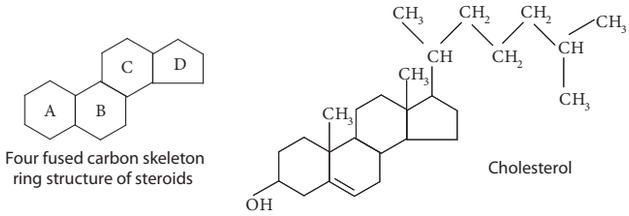

Figure 1321 The steroid structure showing cholsterol as a typical example

Cholesterol, $C_{27}H_{45}OH$, is the most abundant and important steroid in the body and belongs to the group of steroids called **sterols**. The steroid ring skeleton and hydrocarbon tail region are hydrophobic and hence fat-soluble, whilst the presence of the –OH group means it also has a hydrophilic, water-soluble, region and it is thus amphipathic. Cholesterol is produced primarily in the liver and is found in the bloodstream and body cells. It is present in foods like dairy products, eggs and meats but not in foods from plants. Steroids, such as sex hormones, and some vitamins, such as vitamin D, are synthesised from cholesterol.

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B.4.2 Outline the difference between HDL and LDL cholesterol and outline its importance.

HDL is high-density lipoprotein; LDL is low-density lipoprotein. **Lipoproteins** are not different types of cholesterol but rather contain different proportions of lipid and proteins which transport cholesterol through the blood around the body. Lipoproteins are amphipathic: the protein part of a lipoprotein is water soluble whereas lipids such as cholesterol are not soluble in water. Since blood is mostly water, it is the protein part of a lipoprotein that forms an outer layer around cholesterol allowing it to be transported in the blood.

There are two main types of lipoproteins, HDL and LDL, which differ in composition and function. Their density is determined by the amount of proteins present in the molecule. HDLs consist of 40-55% protein and 45 to 60% lipid, whereas LDLs consist of 20-25% protein and 75 – 80% lipid, that is HDLs have a much higher percentage of proteins, about twice as much, as LDLs. HDLs thus prevent the build-up of cholesterol in the arteries. The lower percentage of lipids in HDLs means these can absorb more cholesterol and hence carry it away from the arteries, where it can build up narrowing the artery, to the liver where it is broken down for recycling and elimination.

LDLs carry cholesterol through the blood to the body to build and repair damaged tissues. However, LDLs contain a higher percentage of lipid cholesterol which tends to accumulate around damaged tissues. LDLs are thus associated with depositing cholesterol on arteries which leads to the formation of cholesterol plaque, a hard, thick substance. With time, the plaque leads to thickening of the arterial walls which narrow, leading to atherosclerosis and cardiovascular diseases.

B.4.3 Describe the difference in structure between saturated and unsaturated fats

Saturated and unsaturated fats are terms most commonly used in the context of nutrition. If the hydrocarbon chain has no double bonds present between carbon atoms (thus consisting of a maximum number of hydrogens bonded to the carbon atoms in the R groups) it is called a **saturated fat**. These are common in most animal fats (for example butter) and are usually solids at room temperature. The regular tetrahedral arrangement of carbon atoms in a saturated fat makes it possible for it to pack fairly closely together with parallel chains, (note that these are in fact triglycerides and not isolated single chains). Although weak van der Waals' forces are involved, the large surface area in the long R groups produce forces strong enough to make these solids at room temperature.

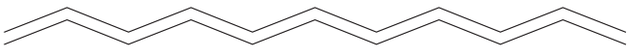

Figure 1322 Closely packed parallel chains of tetrahedral carbon atoms

An unsaturated fatty acid has one or more double bonds formed by the removal of hydrogen atoms. These include vegetable oils and are found to be liquids at room temperature. Most C=C double bonds in biological systems tend to have the *cis* arrangement. *Cis* and *trans* classification is based on the arrangement of the H atoms around the C=C double bond: *cis* contains the H on each carbon across the double bond on the same side; *trans* involves the H on the opposite side. The change in the bond angle to 120° at the C=C double bonds and the *cis* configuration prevents the oil molecules from packing closely together to solidify. The greater the number of C=C double bonds, the more difficult the close packing and the lower the melting points. Oils with one C=C double bond per fatty acid chain are called 'mono-unsaturated oils' and with more than one C=C double bond per fatty acid chain are called 'poly-unsaturated oils'. Most naturally occuring fats and oils have side chains containing a

Name	Formula	Structural Formula	Number of C atoms	Number of C=C bonds (degree of saturation)	Melting Point (°C)
Lauric acid	$C_{11}H_{23}COOH$	$CH_3(CH_2)_{10}COOH$	12	0 saturated	44
Myristic acid	$C_{13}H_{27}COOH$	$CH_3(CH_2)_{12}COOH$	14	0 saturated	58
Palmitic acid	$C_{15}H_{31}COOH$	$CH_3(CH_2)_{14}COOH$	16	0 saturated	63
Stearic acid	$C_{17}H_{35}COOH$	$CH_3(CH_2)_{16}COOH$	18	0 saturated	71
Oleic acid	$C_{17}H_{33}COOH$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	18	1 mono-unsaturated	16
Linoleic acid	$C_{17}H_{31}COOH$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	18	2 poly-unsaturated	5

Figure 1323 Some Fatty Acids found in Dietary Fats and Oils

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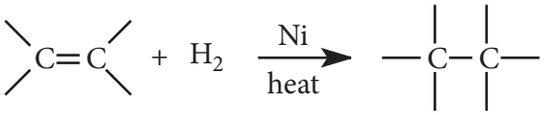
mixture of saturated, mono- and poly-unsaturated acids, which are classified according to the predominant type of unsaturation present.

Note the trend in the melting point of the first four acids listed, namely an increase with an increase in the length of the hydrocarbon chain (thus experiencing greater van der Waals' forces). The presence of one C=C double bond in oleic acid and two double bonds in linoleic acid, correspondingly decreases the melting points of these fatty acids due to the prevention of the close packing possible in the saturated fatty acids.

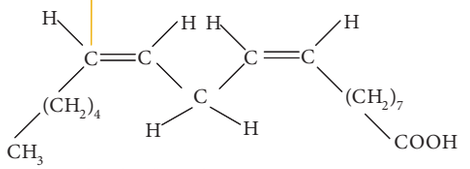
Unsaturated oils can be hydrogenated to solid, saturated fats by the reaction with hydrogen gas in the presence of nickel or platinum as a catalyst and heat.



B.4.4 Compare the structures of the two essential fatty acids linoleic (omega-6 fatty acid) and linolenic (omega-3 fatty acid) and state their importance.

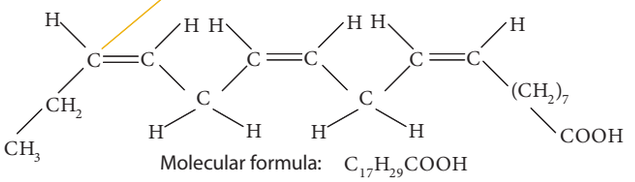
A **fatty acid** is a long hydrocarbon chain carboxylic acid which has an acid group at one end and a methyl group at the other end. The human body is able to synthesize most of the fatty acids it requires, saturated or unsaturated. However, there are two essential fatty acids (EFAs) that our bodies cannot make and these must be present in our diets in order for the body to function properly. These are linoleic (omega-6 fatty acid) and linolenic (omega-3 fatty acid). Both contain 18-carbon chains. Linoleic acid contains two C=C double bonds in which the closest C=C bond to the end methyl group (labelled omega, the last letter of the Greek alphabet) is 6 carbon atoms away. Thus it is called omega-6 fatty acid. Linolenic acid contains 3 C=C double bonds with the closest C=C bond to the end methyl group 3 carbon atoms away and is called omega-3 fatty acid.

Linoleic acid: C #6 from methyl group, thus omega-6 fatty acid



Molecular formula: $C_{17}H_{31}COOH$

Linolenic acid: C #3 from methyl group, thus omega-3 fatty acid



Molecular formula: $C_{17}H_{29}COOH$

Figure 1324 Structures of Linoleic and Linolenic acids

IMPORTANCE OF ESSENTIAL FATTY ACIDS

Lipids, including triglycerides and phospholipids are the primary structural components of body cell walls and membranes. Since most cells break down and are replaced, the **essential fatty acids** (EFAs) need to be part of the diet to manufacture the lipids. EFAs are precursors for important hormone-like chemicals, the prostaglandins and leukotrienes.

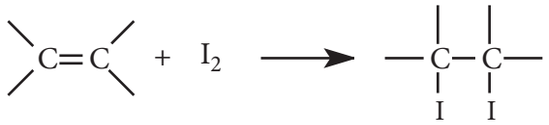
EFAs provide energy during lipid oxidation. Omega-3 is important for brain growth and development during pregnancy; EFAs promote good health, maintain healthy cholesterol levels, promote good circulation and support a healthy immune system. EFAs also elevate mood and reduce depression (a reason why people eat often when depressed). Lack of EFAs leads to lethargy, irritability, dry skin and reduced brain function amongst other symptoms.

B.4.5 Define the term iodine number and calculate the number of C=C double bonds in an unsaturated fat/oil using addition reactions.

OPTION

The **iodine index** or **iodine number** is the number of grams of iodine (in solution) that adds to 100 g of a triacylglyceride. Addition of iodine solution to an unsaturated molecule will cause the double bonds to break to form single–bonded carbon atoms. Since saturated and unsaturated fats and their products are colorless and iodine is colored, the reaction mixture of an unsaturated fat and iodine will turn from a red–violet to a colourless solution as the iodine is used up in the addition reaction. If

a fat contains no double bonds and is therefore a saturated fat, it will not react with iodine. The number of moles of iodine reacting with one mole of fat hence indicates the number of double bonds present in the fat since each mole of double bond requires one mole of I_2:



Example

0.010 mol of linoleic acid reacts with 5.1 g iodine. Determine the number of double bonds present in the acid.

Solution

$$n_{I_2} = \frac{5.1\ \text{g}}{254\ \text{g mol}^{-1}} = 0.020\ \text{mol}\ I_2.$$

Therefore one mol of linoleic acid reacts with two moles of I_2 and it therefore contains two C=C double bonds.

Sunflower oil requires more drops of iodine solution for a red-violet colour to remain than peanut oil does. This is because the sunflower oil is more highly unsaturated (that is, it contains more C=C bonds per molecule of oil) than peanut oil.

Oil or Fat	Saturated fats	Mono Unsaturated fats	Poly Unsaturated fats	Iodine Number*
Butter fat	67%	29%	4%	30-38
Olive oil	15%	75%	10%	79-95
Peanut oil	18%	49%	33%	85-100
Sunflower oil	10%	13%	77%	119-138

Figure 1325 Iodine numbers and percentage fatty acid composition of common fats and oils

* Note that iodine numbers are often quoted as ranges because the fats and oils listed contain variable mixtures of triacylglycerols, whose composition varies according to the mixture of fats and oils in different samples of the foods. Thus, the more unsaturated oil has a higher iodine number.

Example

Calculate the iodine number of linoleic acid, $C_{17}H_{31}COOH$

Solution

$M_{(\text{acid})} = 18(12.0) + 32(1.0) + 2(16.0) = 280\ \text{g mol}^{-1}$

$M_{(\text{iodine})} = 2 \times 126.9 = 253.8\ \text{g mol}^{-1}$

Linoleic acid has 2 double bonds because its formula would require four more hydrogens to be that of the corresponding saturated fatty acid ($C_{17}H_{35}COOH$) and hence it reacts with 2 moles of I_2.

$\therefore$ 280 g of fat reacts with 2×253.8 g of I_2.

$\therefore$ 100 g of fat reacts with

$$\frac{2 \times 253.8}{280} \times 100 = 181\ \text{g of}\ I_2.$$

$\therefore$ I_2 number = 181

B.4.6 Describe the condensation of glycerol and three fatty acid molecules to make a fat.

In a **condensation reaction** such as **esterification**, two molecules react (or link) together with the elimination of a small molecule such as water. Esters are formed from the condensation reaction of an organic acid and an alcohol in which the ester group (or linkage) connects the two molecules together. Fats and oils are a special type of ester in which the alcohol is glycerol (1,2,3–propantriol or propan-1,2,3-triol). Fats and oils are therefore esterified glycerol molecules:

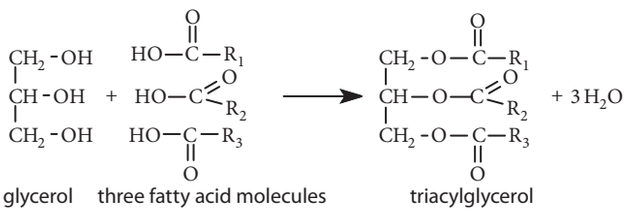


Figure 1326 Formation of triacylglycerol

Since glycerol has three –OH groups, a single molecule of glycerol can have three acid molecules attached to it through ester bonds. Compounds with three acids attached to the glycerol are known as triglycerides or triacylglycerols where R_1, R_2 and R_3 are three fatty acid side chains.

Although the R group of the acid component varies, fats and oils have some features in common. They are almost always straight hydrocarbon chains without any branching present; they contain an even number of carbon atoms (as they are synthesised from a series of ethanoate ions by an enzyme catalysed reaction); they usually contain between 10 and 20 carbon atoms in the R group; besides the C=C bonds present in unsaturated fats, no other functional groups are present. Unsaturated fats almost invariably contain double bonds with the *cis* arrangement.

B.4.7 Describe the enzyme-catalysed hydrolysis of fats during digestion.

Hydrolysis of fats is the reverse of the esterification reaction. Hydrolysis is the splitting of a covalent bond by reaction with water. Hydrolysis of fats during digestion involves splitting the fats into their carboxylic acids and glycerol (propan-1,2,3-triol) catalysed by the enzyme lipase (since the uncatalysed reaction is too slow). The reaction tends to take place in steps via the formation of diglyceride and monoglyceride:

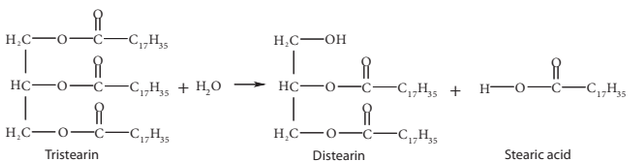


Figure 1327 The hydrolysis of Tristearin

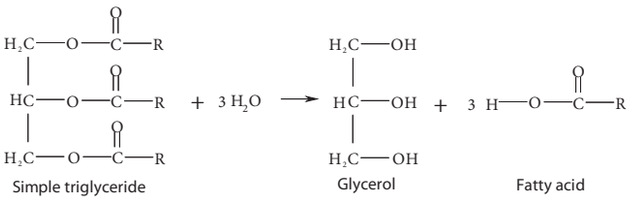


Figure 1328 Overall hydrolysis of a simple triglyceride

B.4.8 Explain the higher energy value of fats as compared to carbohydrates.

Fats have fewer oxygen atoms than carbohydrate molecules of corresponding molar masses, that is, these are less oxidized and thus more oxidation can take place. Therefore, more energy is released from the oxidation of fats compared with carbohydrates. Hence fats are much better biological fuels. Fats provide nearly twice as much energy (more kJ/g) as carbohydrates.

This can be illustrated by comparing the oxidation state of carbon in a unsaturated fatty acid (closely related to fats and oils) and a trisaccharide with the same number of carbon atoms, namely $C_{17}H_{31}COOH$ ($C_{18}H_{32}O_2$) and $C_{18}H_{32}O_{16}$: The 'average' oxidation number of carbon in the two compounds can be calculated since the sum of the oxidation numbers of the elements in a compound is equal to zero. Thus, if x is the oxidation of C, then:

(i) In the fatty acid: $18x + 32(+1) + 2(-2) = 0$ and $x = -28/18$

(ii) In the trisaccharide: $18x + 32(+1) + 16(-2) = 0$ and $x = 0$

Thus carbohydrates (polysaccharides) are in a more oxidized form compared with fats, where the carbon atoms are in a more reduced form. Oxidation (for example combustion) reactions are exothermic and release energy. A more oxidized molecule such as a carbohydrate will therefore produce less energy (as it is at a lower energy level) than fats (made up of fatty acids and glycerol) that are in a less oxidized state (or more reduced state) and produce more energy.

For the above two examples, the oxidation products would be:

$$C_{18}H_{32}O_2 + 25\ O_2 \rightarrow 18\ CO_2 + 16\ H_2O$$

$$C_{18}H_{32}O_{16} + 18\ O_2 \rightarrow 18\ CO_2 + 16\ H_2O$$

The two reactions form the same products in the same amounts meaning the same number of bonds are formed (bond formation is exothermic). The difference in the change in energy for the two reactions must then depend on the energy required to break the bonds in the two reactants.

Although fewer oxygen molecules are being broken in the second reaction, more strong O–H bonds are present in

OPTION

$C_{18}H_{32}O_{16}$ (requiring more energy; in the gaseous state it would be 463 kJ mol^{-1}) compared with $C_{18}H_{32}O_2$ that has many more weak C–H bonds (requiring less energy; in the gaseous state it would be 412 kJ mol^{-1}). The net result is that fats produce more energy than carbohydrates.

B.4.9 Describe the important roles of lipids in the body and the negative effects that they can have on health.

IMPORTANT ROLES OF LIPIDS IN THE BODY INCLUDE

Energy storage

Lipids, such as fats are a very efficient way for the body to store energy. Hydrolysis of a fat produces glycerol plus the corresponding fatty acids present in the fat. The fatty acids are oxidized to produce large amounts of energy. Lipids provide energy storage primarily in the form of triacylglycerols in which fatty acids are the major components.

Insulation and protection of organs

Fats are stored in adipose tissue which provides insulation that is important for regulating the internal temperature of the body as well as providing a protective covering for some parts of the body.

Steroid hormones

Lipids serve as precursors to steroids (such as estrogen and testosterone), cholesterol and vitamins (such as vitamin D).

Structural components of cell membranes

Phospholipids arranged in a bilayer form a stable boundary between two aqueous components, such that the hydrophilic parts face water molecules and protect the hydrophobic parts that face towards each other and away from the aqueous environment. Cell membranes are made mostly of phospholipids.

Omega-3 poly-unsaturated fatty acids

These fatty acids are present in fish oil and are thought to reduce the risk of cardiovascular disease.

Poly-unsaturated fats

These may lower levels of LDL, the low density lipoproteins associated with cholesterol deposits on artery walls.

NEGATIVE EFFECTS INCLUDE

Increased risk of heart disease

From elevated levels of LDL cholesterol and *trans*-fatty acids; the major source of LDL cholesterol is saturated fats, in particular lauric (containing 12 carbon atoms, $CH_3(CH_2)_{10}COOH$), myristic (C_{14}; $CH_3(CH_2)_{12}COOH$) and palmitic (C_{16}; $CH_3(CH_2)_{14}COOH$) acids.

Obesity

When we eat more than we metabolise, this leads to fat being deposited on the abdomen, hips and buttocks, eventually leading to obesity.

OPTION

B5 MICRONUTRIENTS AND MACRONUTRIENTS

Nutrients in food have specific roles in metabolism in the human body. **Micronutrients** such as vitamins and trace minerals are required in very small amounts. Vitamins are organic compounds required for metabolism, to protect health and for proper growth in children. Vitamins also assist in the formation of hormones, blood cells, nervous system chemicals and genetic material. They generally act as catalysts, combining with proteins to create enzymes that regulate changes inside body cells and produce hundreds of important chemical reactions throughout the body. Without vitamins, many of these reactions would slow down or cease.

Minerals are the very small amounts of inorganic ions required by the body for strong bones and teeth (Ca, Mg and P) and for the formation of hormones, enzymes and in the maintaining of fluid levels in the body.

B.5.1 Outline the difference between micronutrients and macronutrients.

Micronutrients are substances required in very small daily amounts (mg or μg), that mainly function as a co-factor of enzymes (<0.005% body weight) and are essential for proper cell function. Like water, micronutrients do not produce energy. Examples include vitamins and trace minerals (Fe, Cu, F, Zn, I, Se, Mn, Mo, Cr, Co, B). Minerals and most vitamins except vitamin D are not synthesized by the body and must be taken in the diet.

Macronutrients are chemical substances that are required in relatively large amounts (>0.005% body weight). Examples include proteins, fats, carbohydrates and minerals (Na, Mg, K, Ca, P, S, Cl). Macronutrients such as carbohydrates, fats and proteins supply energy and are required for growth and maintainance of the body. They are therefore required in the diet in large amounts as are some minerals.

B.5.2 Compare the structures of retinol (vitamin A), calciferol (vitamin D) and ascorbic acid (vitamin C).

B.5.3 Deduce whether a vitamin is water or fat soluble from its structure.

Various vitamins are not chemically related and most differ in their structures and physiological actions. Vitamins such as A, D, E and K contain long hydrocarbon groups which make these vitamins more fat-soluble in organic non-polar or slightly polar solvents. The fat–soluble vitamins are generally consumed along with fat–containing foods and because they can be stored in the body's fat, they do not have to be consumed every day. The water–soluble vitamins, the eight B vitamins and vitamin C, cannot be stored and must be consumed frequently, preferably every day (with the exception of some B vitamins). Vitamin C, for example, contains highly polar OH groups which are capable of extensive hydrogen bonding with water, making it water soluble (see Figure 1331).

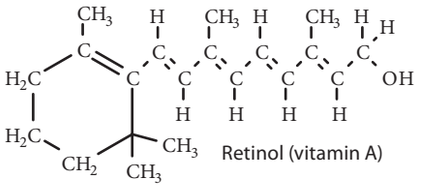


Figure 1329 Structure of Vitamin A

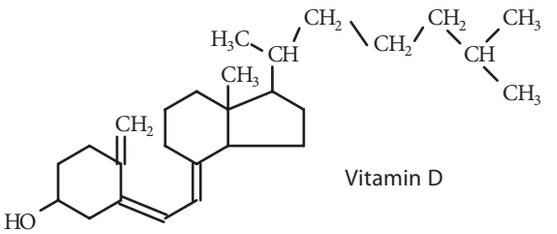


Figure 1330 Structure of Vitamin D

Vitamin A (retinol) contains a long carbon chain, with a conjugated system of alternate double and single bonds, and one –OH group (see Figure 1329). It is a fat soluble primary alcohol and is light sensitive because it contains many conjugated double bonds. Vitamin A is required for the production of rhodopsin (light sensitive material in the rods of the retina). It is the active material in the process where light impulses change the conformations of the molecules. This is the specific arrangement of atoms, assuming that there is free rotation around a single bond. **Conformers** are isomers that differ only in rotation around a single bond. The changed conformation of the molecules

OPTION

eventually results in a nerve impulse, which is translated in the cortex of the brain as vision.

Vitamin C (ascorbic acid) has a five-membered ring containing an oxygen atom and overall contains 4 hydroxyl groups (-OH), a C=C alkene and a C=O carbonyl group (see Figure 1331).

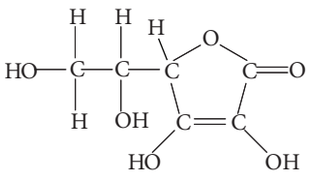


Figure 1331 Structure of vitamin C

B.5.4 Discuss the causes and effects of nutrient deficiencies in different countries and suggest solutions.

CAUSES OF NUTRIENT DEFICIENCIES

Deficiences in micro- and macronutrients in the diet such as carbohydrates, proteins, vitamins and minerals that are essential for growth and development leads to malnutrition. In the developed world, income disparities, lack of economic opportunities and poor eating habits (particularly in children and the aged) are important reasons for malnutrition. In the developing and third world, poverty, limited food supply and famine are the key reasons. Lack of safe drinking water, proper sanitation and lack of good hygiene lead to the spread of infectious diseases leading to malnutrition. Also, lack of health services and poor literacy rates contribute to the problem. It is estimated that one third of African children and about half of South Asian children are under-weight or malnourished.

Examples of micro-nutrient deficiencies and their effects

- Iron deficiency causes anaemia, a blood disorder, characterised by a deficiency of red blood cells. Heme is a complex of iron in haemoglobin, the oxygen transport protein. Due to anaemia, insufficient oxygen is carried to the cells, leading to fatigue.
- Iodine deficiency causes goitre which is swelling in the neck due to enlarged thyroid gland.
- Retinol (vitamin A) deficiency may cause xerophthalmia (dry eyes) due to failure to produce tears; an early deficiency symptom of vitamin A is night blindness (difficulty in adapting to darkness).
- Niacin (vitamin B3) deficiency causes pellagra; deficiency symptoms include diarrhea, dementia (mental disorder) and skin rash (dermatitis).
- Thiamin (vitamin B1) deficiency causes beriberi which affects the muscles, heart, nerves etc.
- Ascorbic acid (vitamin C) deficiency causes scurvy, first noticed in sailors on long voyages. Scurvy is identified by bleeding lesions on the legs and thighs and soft, rotten gums.
- Calciferol (vitamin D) deficiency causes rickets, the malformation and softening of bones. Vitamin D is necessary for normal bone formation and for the retention of calcium and phosphorus in the body. Vitamin D absorbs calcium ions into the blood stream and, in the presence of phosphorus, makes it possible for the calcium ions to be added to the bones and teeth. Thus vitamin D protects the teeth and bones against the effects of low calcium intake by making effective use of calcium and phosphorus.

Examples of macronutrient deficiencies and their effects

- **Protein deficiency** produces marasmus and kwashiorkor. Marasmus is severe protein and calorie deficiency in children under one year of age. It results in growth retardation and wasting; victims are emaciated (gaunt) and body mass may be as little as 20% of normal mass. Kwashiorkor is malnutrition from insufficient protein in the diet and occurs in children over 18 months old.

OPTION

Protein deficiency leads to edema (swelling), fatigue, decreased immunity, lethargy, muscle weakness, slow growth and development, weight loss, etc.
- Lack of calcium produces osteoporosis.
- Lack of sodium ions produces cramps.

Solutions to micronutrient deficiencies

A balanced diet provides all the vitamin and micro-nutrient requirements of humans; however it is common to add vitamins to foods, such as vitamin A to margarine, vitamin B to flour, vitamin C to juices and vitamin D to milk. Diets high in fruits and vegetables tend to meet many of the requirements. Balanced vegetarian diets should include essential nutrients. Such diets tend to be lower in cholesterol and saturated fats with lower risks of diabetes, coronary disease, high blood pressure and obesity.

Solutions to vitamin deficiency include:

- providing food rations that are composed of fresh vitamin- and mineral-rich foods (brown rice, whole grains, fruits and vegetables for vitamin B1, fish, poultry, nuts, cereals for vitamin B3, fruits and green vegetables for Vitamin A and C, milk and milk products for calcium, etc.)
- adding nutrients missing in commonly consumed foods, such as salt fortified with iodine
- genetic modification of food so that foods contain essential nutrients that would not be naturally present
- providing nutritional supplements for many of the vitamins and minerals
- providing selenium supplements to people eating foods grown in selenium-poor soil
- eating iron rich foods such as green leafy vegetables, red meat, whole grain, etc. or taking iron containing supplements.

B6 HORMONES

B.6.1 Outline the production and function of hormones in the body.

Hormones are chemical messengers that are produced by the body's endocrine glands (many of which are controlled by the pituitary gland which in turn is controlled by the hypothalamus). Hormones are released directly into the bloodstream, and then pass to distant receptor sites such as an organ, tissue or cells where they are absorbed and exert a specific effect. Hormones perform a variety of different functions and vary greatly in their chemical composition and structure. Hormones generally have a negative feedback mechanism whereby a high level of the hormone inhibits its own production. Hormones are effective in minute amounts and only target cells that are equipped to respond to them. Hence, a hormone can affect different target cells in different ways.

Negative feedback mechanism – an example

The increased blood glucose level caused by eating and digesting a chocolate bar results in the production of insulin. This stimulates the body cells to take up glucose, as does the liver, which stores it as glycogen and the blood glucose level is lowered. Skipping a meal lowers blood glucose level and the cells in the pancreas produce glucagon. This stimulates the liver to convert glycogen to glucose and release it into blood. The blood glucose level increases. As a result of this **negative feedback mechanism**, the blood glucose level remains around the optimum level (controlled by the hypothalamus).

B.6.2 Compare the structures of cholesterol and the sex hormones.

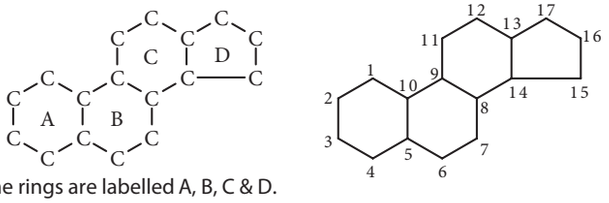
OPTION

Steroids are a family of polycyclic ring structure chemicals containing a common carbon molecular framework (or backbone). The ring structure consists of 17 carbon atoms arranged as 3 cyclohexane rings fused together with a cyclopentane ring on one extremity. The presence of various functional groups such as the methyl and hydroxyl groups results in hormones that give rise to a variety of physiological functions ranging from sexual

Hormone	Production Location	Derived from	Role in the Body
ADH, anti-diuretic hormone (note: caffeine and alcohol reduce ADH production and are diuretics)	Pituitary gland located at the base of the brain	Amino acids; 9 amino acid peptide	ADH is released when the body has lost water. It stimulates the absorption of water back into the blood in the kidneys and preserves water in the body through a reduction in the formation of urine and subsequent loss of water.
Aldosterone, a steroid hormone	Adrenal gland which rests on top of both kidneys	Cholesterol	Regulates the concentration of electrolyte ions (mostly Na^+ and K^+) in the blood and body fluids.
Adrenalin or epinephrine; a stimulant related to amphetamine	Adrenal gland which rests on top of both kidneys	The amino acid tyrosine	Responsible for flight or fight response characterized by goose bumps, increased heart rate/output and blood pressure. Affects rate of glucagon release into the liver and release of glucose by liver into the blood.
Thyroxine, an iodine containing amino acid.	Thyroid gland, located in the neck	Small molecule from amino acids	Responsible for basal metabolic rate in vertebrates: essential for regulating metabolism.
Insulin, a protein of 51 amino acids	Pancreas, located at back of abdomen	Protein	Regulates blood sugar levels. Decreases blood glucose level; increases glucose and amino acid uptake and use by cells.
Sex hormones 1. Androgens, principally testosterone	Principally the testes (the ovaries produce very small amounts)	Cholesterol	Responsible for development and maintenance of the male reproductive system as well as secondary sexual characteristics.
2. Estrogen, principally estradiol	Principally the ovaries		Play a similar role in females; responsible for sexual development; regulate changes in uterus and ovaries for menstrual and reproductive cycles.
3. Progestins including progesterone	Principally the ovaries		

Figure 1332 Hormones and their roles

characteristics (by estrogen and testosterone) to cell membrane components (by cholesterol).



The rings are labelled A, B, C & D.

Figure 1333 Polycyclic ring structure of steroids

Sex hormones such as **androgens** (male sex hormones), estrogens (female sex hormones) and progestins (present in the male and female body) have a similar polycyclic structure to cholesterol. However, the functional groups on the sex hormones are different from those found on cholesterol and these slight differences in their molecular structures give rise to totally different functions.

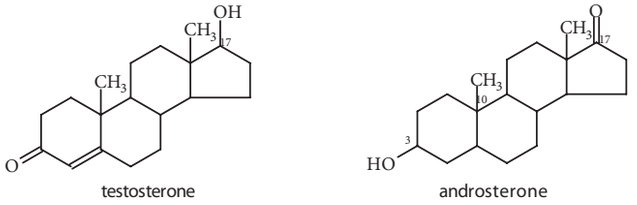


Figure 1334 Structures of testosterone and androsterone

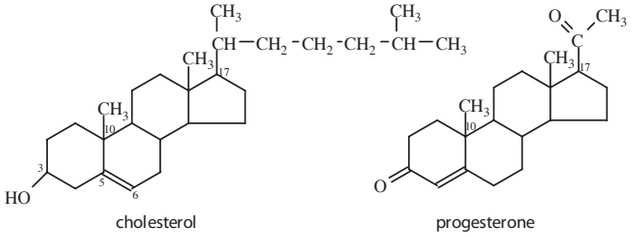


Figure 1335 Structures of cholesterol and progesterone

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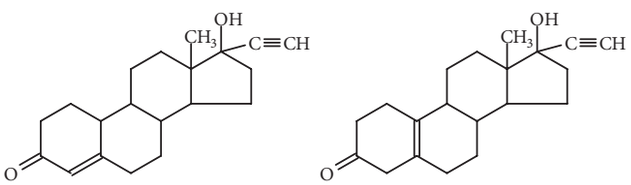
For example, the only difference between testosterone and progesterone is the presence of the –OH group on C–17 of the steroid framework for testosterone, compared to the presence of the methyl alkanone on progesterone. Similarly, the difference between cholesterol and androsterone is the presence of a C=C bond between C–5 and C–6 (in the second ring) and a longer chain R group on C–17 in cholesterol, compared to just the C=O on C–17 of the framework. Note that besides the common framework, both these molecules contain the secondary –OH group on C–3 as well as the methyl groups on C–10 and C–13 atoms.

B.6.3 Describe the mode of action of oral contraceptives.

During pregnancy, progesterone prevents ovulation; estrogen prevents irregular flow during a menstrual cycle. Thus, the most popular version of **oral contraceptive** (the pill) combines a synthetic progesterone and estrogen. The two hormones act to stop the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary. This results in the ovaries not being stimulated and thus inhibits ovulation. In effect, the female reproductive system is fooled because the drug mimics the action of progesterone in a pregnant woman and ovulation is stopped.

Problems with the use of progesterone such as rapid breakdown by the liver and side effects has led to the use of progesterone-like synthetic chemicals such as norethynodrel and norethindrone combined with an estrogen-like compound to prevent irregular menstrual flow. The molecular framework of the synthetic chemicals in such pills is the same as progesterone, but the $-COCH_3$ group on the D ring is replaced by the –OH and –C≡CH on C17. The presence of these groups seems to cause the synthetic steroids to tightly bind to their receptor sites. Thus, the rapid breakdown by the liver no longer takes place, making it possible to administer the pill orally.



Norethindrone Norethynodrel

Figure 1336 Structures of norethindrone and norethynodrel

A second type of oral contraceptive is called the minipill. It contains progestin only (a progesterone–like synthetic chemical). It changes the composition of the cervical mucous from the mucous membrane, thereby preventing the sperm from entering the uterus. This variation of the pill can be inserted underneath the skin and will slow-release the progestin for a period of up to 5 years.

B.6.4 Outline the use and abuse of steroids.

Besides the development of male secondary sexual characteristics, testosterone promotes muscle growth. Such anabolic (meaning 'building up') steroids are effective ways to increase muscle mass. Thus for patients suffering from long, debilitating illnesses such as cancer, testosterone-like steroids, which cause minimal side effects, can be used to stimulate muscle growth and increase muscle mass and help such patients recover their body weight. However, athletes have been known to abuse such drugs. Both male and female athletes stand to improve their performances by using these substances. Testosterone is more prevalent in men and is principally responsible for muscle build-up. Women who use anabolic steroids have much to gain because, initially, there is a low concentration of testosterone present in their bodies.

Taking large doses of anabolic steroids causes harmful side effects. In males, the effects of aging are observed including impotence, baldness, problems in urinating, smaller testes, etc. In women, steroids affect secondary sex characteristics, build up of muscles and the growth of facial hair. Both men and women can also develop violent tempers, increased aggressive behavior as well as an increased risk of diseases such as liver tumors, high blood pressure and heart attacks. As a result, anabolic steroids are strictly forbidden at international athletic competitions. Competitors are given random urine tests (to detect steroids and other banned drugs) and winners are often required to undergo compulsory urine tests for such banned substances.

OPTION

B7 ENZYMES (HL)

B.7.1 Describe the characteristics of biological catalysts (enzymes).

B.7.2 Compare inorganic catalysts and biological catalysts (enzymes).

Enzymes are protein biological catalysts that speed up the rate of reactions in the body. A **catalyst** does not alter the enthalpy or free energy change of a reaction. Thus it does not change the position of an equilibrium reaction or the equilibrium constant; it only speeds up the reaction so the equilibrium is reached faster. Thus, a catalyst cannot make a reaction take place that would not usually take place without the catalyst. Rather the same equilibrium position is reached but much faster with an enzyme. A catalyst increases the rate of a reaction by providing an alternate reaction pathway with a lower activation energy without being used up or chemically changed. See Figure 1337. Catalysts can be divided into inorganic and biological (or organic) catalysts called **enzymes**. See also Chapter 6.

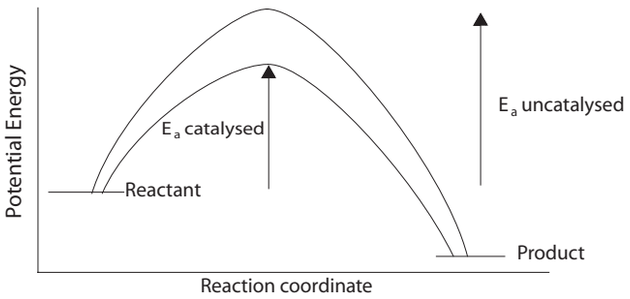


Figure 1337 Activation energy

Enzymes are proteins which have four levels of structure. It is their tertiary and quaternary structures which determine if the enzyme can act properly. This is because of the 'lock & key' nature of their action – the shape of the enzyme allows the substrate (i.e. the substance on which it acts) to bond to its surface, but will not bond to, or affect, other substances that have a different shape. When a protein denatures due to its environment, the tertiary and quaternary structures are altered. For example, the hydrophobic regions in the core of the tertiary structure may be affected when the protein is placed in an organic solvent such as ether. Chemicals can also disrupt the hydrogen bonds, ionic bonds and the disulfide bridges in the tertiary and quarternary structures.

The active site is usually a pocket or a grove on the surface of the protein, formed by only a few of the enzyme's amino-acid residues, into which the substrate molecule fits. An enzyme can distinguish its substrate from closely related compounds. Hence, enzymes are very specific, each of them catalyzing only one particular reaction. The active site is not rigid as it can change its shape slightly so as to allow a better fit for the substrate. This is called an '**induced fit**'. The enzyme E reacts with the substrate at the active site by weak interactions such as hydrogen bonds and ionic interactions to form the enzyme-substrate complex, ES, which then decomposes to form the product and the free enzyme is regenerated. Thus, when bound, the catalytic action of the enzyme converts the substrate to the product of the reaction which is then released by the enzyme, leaving the active site free for another substrate molecule to bind to it.

For example, the enzyme urease catalyzes only the hydrolysis of urea but not other amides. Where stereoisomers exist, an enzyme that is effective with one enantiomer is found to be ineffective with the mirror image enantiomer. Sometimes a reaction can be catalysed by an enzyme and by inorganic catalysts. For example the decomposition of hydrogen peroxide can be brought about by the enzyme catalase, or by various inorganic catalysts such as manganese(IV) oxide.

An enzyme is highly efficient. Enzymes operate under fairly mild conditions of temperature and pH. The efficiency of an enzyme can be seen from the biological synthesis of ammonia that requires only the enzyme **nitrogenase**. The industrial synthesis of ammonia by the Haber process requires the inorganic catalyst Fe as well as pressures of 200 to 1000 atmospheres and a temperature of about 500°C. Similarly, the enzyme catalase decomposes hydrogen peroxide much more efficiently than inorganic catalysts such as MnO_2. Enzymes are extremely effective biological catalysts increasing some rates of reactions by factors as much as 10^8 to 10^{20}.

Most enzymes are protein molecules and enzymatic reactions take place in aqueous solution in biological organisms. Inorganic catalysts can be surface metal catalysts such as Ni or Fe where gas phase heterogeneous reactions occur or these can be homogeneous catalysts where the catalyst and the reactant are in the same phase, as in the case of sulfuric acid in esterification reactions.

Inorganic catalysts are not temperature sensitive; enzymes are. Like all reactions, the rate of an enzyme catalyzed reaction increases with temperature, but excess heating denatures the enzymes, that is, the complex enzyme molecules can be easily destroyed by heat and this leads to a

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decrease in the reaction rate as the active sites are no longer available. Hence proteins have an optimum temperature at which they operate most efficiently. Enzymes also work optimally in a narrow pH range.

This topic can lead to a good investigation to compare the effect of inorganic catalysts such as MnO_2, $FeCl_3$ and Zn with enzyme catalase from pieces of apple, banana, beef liver or potato on the decomposition of hydrogen peroxide. This can be done by observing the rate of production of oxygen gas compared with a standard where no catalyst is added.

B.7.3 Describe the relationship between substrate concentration and enzyme activity.

If the concentration of an enzyme is increased, while all other factors such as temperature, pH and substrate concentration are held constant, the reaction rate (velocity) is found in most cases to be first order with respect to enzyme concentration. That is, the rate is directly proportional to the enzyme concentration when the concentration of the enzyme is much less than the substrate concentration.

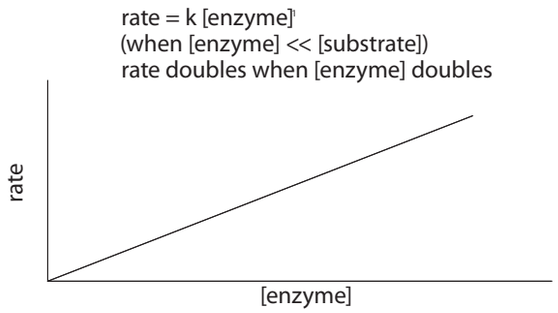

Figure 1338 The effect of enzyme concentration on reaction rate

Substrates bind to an enzyme's active site to form an enzyme–substrate complex. Once the reaction is finished, the product leaves and the enzyme binds to another substrate molecule. At very low substrate concentrations, the rate generally increases in a linear fashion because the active sites of the enzyme molecules have not been used up. Increasing substrate concentration involves more enzyme molecules and the conversion of substrate to product proceeds at a faster rate (velocity).

However, a hyperbolic dependence is observed at higher substrate concentrations as shown in Figure 1339. This is because, once the concentration reaches a particular point, all the active sites are engaged and the rate will not speed up anymore at that enzyme concentration. Eventually the enzyme saturation point is reached by the substrate at V_{max}, the maximum rate (velocity) and the reaction becomes zero order with respect to the substrate concentration. At this point, the enzyme is saturated and the rate (velocity) of the reaction is dependent on the rate at which the substrate is converted to the product and leaves the active site. Here, the only method of increasing the rate (velocity) is to increase the enzyme concentration. The variation of rate with substrate concentration when temperature and pH are constant are shown in Figure 1339.

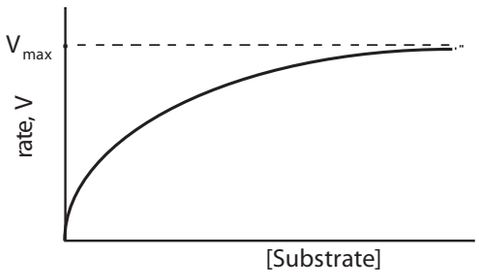

Figure 1339 Variation of rate with [substrate]

B. 7.4 Determine V_{max} and the value of the Michaelis constant (K_m) by graphical means and explain its significance.

The saturation effect led two scientists, Michaelis and Menten, to derive a theory of enzyme kinetics. Here the enzyme E reacts with the substrate S to form ES, the enzyme-substrate complex, which then decomposes to form the product P and the free enzyme E is generated.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} P + E$$

In the first equilibrium equation, k_1 is the rate constant of the forward reaction and k_2 is the rate constant of the reverse reaction. k_3 is the rate constant for the second forward reaction. The rate constant of the second reverse reaction is ignored since it is usually too small compared with k_1, k_2 and k_3.

Applying the principles of kinetics, the **Michaelis–Menten equation** can be derived which provides a means of analyzing enzyme-catalyzed reactions in terms of their rate constants:

V_{max} is the maximum velocity (reaction rate) of the enzyme reaction, that is when the enzyme is fully saturated. [S] is

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$$\text{Rate} = \frac{V_{max}[S]}{K_m + [S]}$$

the substrate concentration and K_m, the Michaelis constant (a function of three or more rate constants), in the above equation it is equal to:

$$\frac{k_2 + k_3}{k_1}$$

The Michaelis–Menten equation accounts for the hyperbolic relationship between the rate, v, and the substrate concentration. Note that, in that curve, it is difficult to obtain an accurate value of V_{max} by extrapolation due to the hyperbolic nature of the line. Also, if [S] is much less than K_m, then in the equation the denominator $\approx K_m$ and the equation reduces to $v \approx V_{max}[S]/K_m$ or $v \propto [S]$. This accounts for the linear dependence of velocity (rate), v, on [S] at very low substrate concentrations. If $[S] >> K_m$. Then the denominator in the rate equation $\approx [S]$, and $v = V_{max}$.

The Michaelis–Menten equation gives the quantitative relationship between the rate of enzyme reaction, v, and the substrate concentration, [S], if either V_{max}, the maximum velocity, or K_m is known. To understand the significance of the constant, K_m, consider half the maximum velocity, that is, $v = \frac{1}{2}V_{max}$:

According to the Michaelis–Menten equation:

$$\text{rate} = v = \frac{V_{max}[S]}{K_m + [S]}; \text{ if } v = \frac{1}{2}V_{max}, \text{ then}$$

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

$$K_m + [S] = 2[S]$$

$$\therefore K_m = [S] \text{ when } v = \frac{1}{2}V_{max}$$

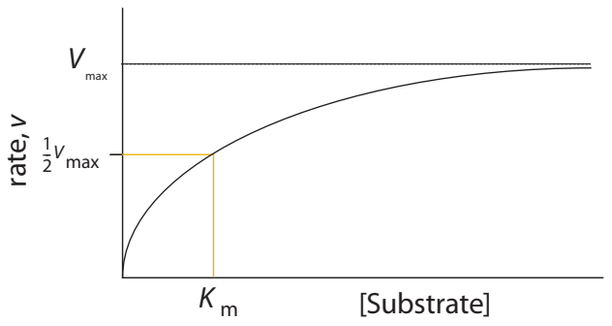

Figure 1340 Determination of K_m from rate versus [substrate] graph

Thus K_m is equal to the substrate concentration when the velocity (rate) is equal to half the maximal value. Units of K_m are the same as the units of [S], namely mol dm^{-3}. K_m is an experimentally determined quantity; it is independent of the enzyme concentration [E], though its value varies with the chosen substrate, temperature and pH. Note that the higher the K_m value, the lower the enzyme activity, i.e. K_m is inversely proportional to enzyme activity because a lower value of K_m means a more efficient enzyme. That is, with a given substrate concentration, there is a higher reaction velocity (rate) relative to V_{max}.

The 'double reciprocal' (**Lineweaver–Burke**) plot of reciprocal of substrate concentration versus reciprocal of initial velocity (rate) allows the accurate determination of V_{max}, unlike the velocity (rate) versus [substrate] plot where the determination of the V_{max} value is uncertain due to its asymptotic approach. This involves the rearrangement of the Michaelis–Menten Equation:

$$\text{Rate} = \frac{V_{max}[S]}{K_m + [S]}$$

Taking the inverse of both sides:

$$\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]}$$

$$= \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$= \left[\frac{K_m}{V_{max}} \times \frac{1}{[S]}\right] + \frac{1}{V_{max}}$$

(***y*** = ***mx*** + ***c*** is a straight line equation)

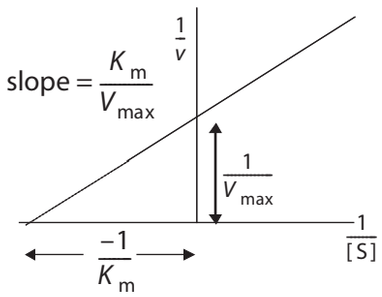

Figure 1341 The Lineweaver-Burke plot

Thus a graph of $\frac{1}{v}$ against $\frac{1}{[S]}$ is a straight line with slope $= \frac{K_m}{V_{max}}$ and its intercept is equal to $\frac{1}{V_{max}}$.
Also, extrapolating the line, when $\frac{1}{v} = 0$, then $\frac{1}{[S]} = \frac{-1}{K_m}$.

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B.7.5 Describe the mechanism of enzyme action, including enzyme substrate complex, active site and induced fit model.

The complex structure of enzymes requires specific shape with active sites for interaction with a reactant called a substrate. The active site is usually a pocket or a groove on the surface of the protein, formed by only a few of the enzyme's amino-acid residues, into which the substrate molecule fits. This site is not rigid and it can change its shape slightly so as to allow a better fit for the substrate. This is called an **induced fit**. The enzyme E reacts with the substrate at the active site by weak interactions such as hydrogen bonds and ionic interactions to form the enzyme-substrate complex, ES, which then decomposes to form the product and the free enzyme is regenerated. Thus, when bound, the catalytic action of the enzyme converts the substrate to the product of the reaction which is then released by the enzyme, leaving the active site free for another substrate molecule to bind to it. Refer back to 7.1 and 7.2.

B.7.6 Compare competitive inhibition and non-competitive inhibition.

Particular chemicals inhibit the action of specific enzymes. If the **inhibitor** attaches to the enzyme by covalent bonds, inhibition is irreversible and usually involves the destruction or permanent modification of the enzyme structure. If it attaches by weak interactions, the inhibition is reversible and can be treated quantitatively by using the Michaelis–Menten equation.

There are two major types of reversible inhibition: competitive and non–competitive. Inhibitors that resemble the normal substrate molecule and compete for the enzyme's active site are called **competitive inhibitors**. These reduce the activity of the enzyme because they block the substrate from entering the active site. If inhibition is competitive, an increase of substrate concentration can reduce the impact of the inhibitor.

Consider the enzyme succinate dehydrogenase which catalyzes the reduction (namely the removal of two H atoms) from the two $-CH_2$ groups of the succinate ion:

$$^{-}OOC{-}CH_2{-}CH_2{-}COO^{-} + X \underset{\text{succinate ion}}{\overset{\text{enzyme}}{\rightleftharpoons}} {}^{-}OOC{-}CH{=}CH{-}COO^{-} + H_2X$$

The malonate ion, $^{-}OOC{-}CH_2{-}COO^{-}$, which also has two ionized groups, resembles the succinate ion in structure and hence inhibits the action of the enzyme because both compete for the same site. If the concentration of the succinate ion is increased, the amount of inhibition by the malonate ion is reduced.

Competitive inhibition is recognized in the Lineweaver–Burke double reciprocal plots of $1/v$ versus $1/[S]$ at various inhibitor concentrations. In such a case, V_{max} remains the same and is not affected by the competitive inhibitor, I. This is because at any inhibitor concentration, it is still possible to reach the same maximum velocity (that is full enzyme activity) at a substrate concentration, however high.

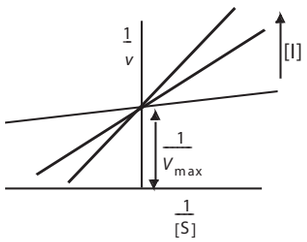

Figure 1342 Competitive inhibition

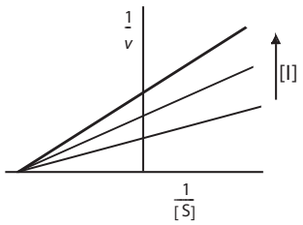

Figure 1343 Non-competitive inhibition

Non–competitive inhibitors impede enzymatic reactions by binding to a part of the enzyme away from the active site, which causes the enzyme to alter its shape, reducing its effectiveness. The inhibitor, I, may bind to the free enzyme, E (to give EI), to the enzyme–substrate complex ES (to give ESI) or to both, making the enzyme inactive.

$$E + I \; EI; \; ES + I \rightleftharpoons ESI$$

Consider the reversible action of a heavy metal ion Hg^{2+} on the –SH group of the cysteine residue which is essential for enzyme catalytic activity:

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$$2\ E–S–H + Hg^{2+} \rightleftharpoons E–S–Hg–S–E + 2H^{+}$$

The enzyme is inhibited non–competitively by the formation of the –S–Hg–S– linkage. Thus, one would expect V_{max} to be decreased by the inhibitor as the active enzyme concentration is decreased and the velocity cannot be increased by increasing the substrate concentration. Thus in the double reciprocal graphs of $1/v$ against $1/[S]$, the slopes do not have the same intercept on the $1/v$ axis. The higher the inhibitor concentration, the lower the velocity v, the larger the $1/v$ value and therefore the greater the intercept as shown above. Thus the hyperbolic curves for the rate of reaction versus substrate concentration are shown in Figure 1344.

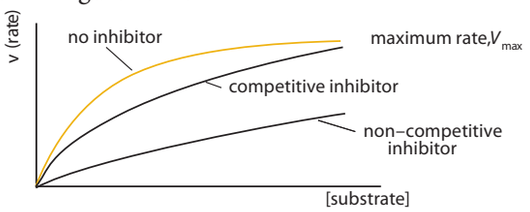

Figure 1344 Effect of substrate concentration on inhibitors

B.7.7 State and explain the effects of heavy metal ions, temperature changes and pH changes on enzyme activity.

If in any way the enzyme changes its shape or arrangement, the substrate will no longer be able to bind to the active site and the enzyme is rendered non-functional. This **denaturation** can take place when the surrounding environment changes even slightly. This may be brought about in several ways such as a variation in temperature or pH of the solution, or by the presence of heavy metal ions such as Hg^{2+}.

Each enzyme has conditions under which it works optimally as that environment favours the most active conformation for the enzyme. Temperature increases enzymatic reaction rates up to a certain point as the substrates collide with active sites more frequently as the molecules move faster. However, the speed of the reaction drops sharply when the temperature reaches a certain point. Here, the thermal agitation of the enzyme disrupts the hydrogen bonds, ionic bonds and other non–covalent interactions that stabilize its active structure. If the three-dimensional structure is changed as a result of temperature, the enzyme activity is affected. All enzymes have an optimum temperature at which they are not yet denatured and the substrates collide fastest with the enzyme. In humans, enzymes have an optimum temperature of about 37°C, about the same as the internal body temperature. Above this temperature, the change in the enzyme structure affects the active site (usually irreversibly) and the rate drops sharply. Refer to Figure 1345. The optimum temperature is often called **critical temperature** in industrial applications of enzymes.

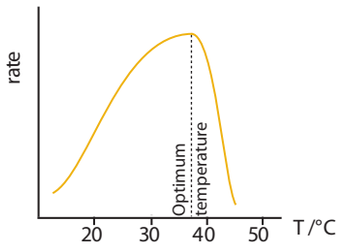

Figure 1345 Rate of reaction versus temperature

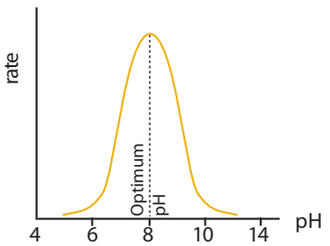

Figure 1346 Rate of reaction versus pH

Proteins contain groups such as $–NH_2$ and –COOH and are susceptible to pH changes. Extreme changes in pH values denature such ionisable enzymes rendering them ineffective, hence at low or high pH values, the enzyme is irreversibly denatured and the rate (v) drops sharply. Within a narrow pH range, the enzyme structure changes reversibly and each such enzyme works optimally at a particular pH. Thus the maximal rate for the enzyme chymotrypsin occurs around pH 8 and for pepsin this occurs at pH 2. If however an enzyme is acting on an electrically neutral substrate molecule such as sucrose, or where the charge plays no role in the catalyzed reaction, changes in pH have little effect on the rate (v) of the reaction. For example the enzyme invertase, which catalyzes the hydrolysis of the sucrose molecule, has a constant rate (v) in the pH range 3.3 to 7.5.

Heavy metal ions can also disrupt the active sites of some enzymes. When a heavy metal ion is present at the active site, substitution of a different metal ion for the original ion can cause the enzyme to malfunction and lose its activity. This is particularly evident where heavy metal ions can bind or chelate to the –S–H groups in proteins to form a –S–M–S– type arrangement.

Uses of enzymes in biotechnology

Biotechnology is the application and harnessing of microorganisms (such as bacteria, viruses and fungi) or biological processes to produce desired and useful substances (such as insulin) and facilitate industrial processes. **Fermentation** is an example of microbial biotechnology. Brewing, baking and the manufacture of cheese all involve the fermentation process. The manufacture of wine, for example, involves the fermentation of grape juice, a rich source of glucose, by wild yeasts present on grape skin. The process produces an alcohol content of 8 to 15% by volume (which is high enough to kill the yeast).

Similarly, the fermentation of sugar by yeast is the basis for the production of other alcoholic drinks:

$$\underset{\text{Starch}}{2\,(C_6H_{10}O_5)_n} + n\,H_2O \xrightarrow{\text{diatstase in malt}} \underset{\text{maltose}}{n\,C_{12}H_{22}O_{11}}$$

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{maltase}} \underset{\text{glucose}}{2\,C_6H_{12}O_6}$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{zymase}} \underset{\text{ethanol}}{2\,C_2H_5OH} + 2\,CO_2$$

Better brewing through improving yeast and large scale production are the results of biotechnology. The genes responsible for the yeast enzymes have been cloned and bacteria have been used in the large scale production of the yeast enzymes needed.

Genetic engineering involves the manipulation of genes. It is the term used to describe the modern techniques in molecular biology where genes can be removed from one type of cell and placed in another, altering a cell's genetic content and potential. **Genetic engineering** has revolutionized the process of biotechnology and has given rise to the manufacture of important products such as new antibiotics, insulin and biological detergents. Transfer of human insulin genes into yeast or bacteria has made large scale production of human insulin possible in bioreactor vessels (much as in brewing).

Lipolase, an enzyme which is a constituent of biological detergents, consists of fat-digesting (splitting) enzymes. Scientists at Novo Nordisk in Japan have created lipolase by taking a gene coding from a particular species of fungus and transferring it to another microbe, *aspergillus*, that produces enzymes in very high yields. As a result, enzymes are widely used in the soap and detergent industry. Such biological detergents' main environmental benefits are that they save energy (a reduction in washing temperature from 60°C to 40°C). They are rapidly biodegradable, thus leaving no harmful residues, and produce no negative impact on sewage treatment processes and pose no risk to aquatic life.

Similarly scientists working in South America have discovered an enzyme which breaks the glucose chains in cellulose by hydrolysis only when a strand of cellulose comes loose. Thus this has found ready application in detergents used to clean cellulose cotton in which the enzyme is able to break bonds holding loose fibers, making the fabric appear new.

Other applications of genetic engineering have found their way into medicine. **Interferons** are natural anti-viral proteins that can be used against viral infections. Genes cloned in yeasts are used to produce such proteins and extensive research is under way in this area. The Hepatitis B vaccine has been genetically engineered and research is continuing into finding vaccines for AIDS and malaria.

B8 NUCLEIC ACIDS

B.8.1 Describe the structure of nucleotides and their condensation polymers (nucleic acids or polynucleotides).

B.8.2 Distinguish between the structures of DNA and RNA.

A **nucleic acid** is a polymer chain of repeating nucleotides which consist of a phosphate group, a pentose sugar and an organic nitrogenous base. Thus, nucleic acids are high molar mass polymers of fairly simple composition involving only a few different nucleotide bases compared with proteins which are made of some 20 different amino acids. Genetically, the most important components of nucleotides are the nitrogenous bases, since the sequence of the bases in DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) polymer molecules are the key to the storage of genetic information.

There are five nitrogenous bases that are incorporated as part of naturally occurring nucleotides. These may be classified into two groups, the purines and the pyrimidines. Adenine (A) and guanine (G) are purines, which have a double ringed structure and cytosine (C), thymine (T) and uracil (U) are pyrimidines with a single ringed structure. The base thymine is present predominantly in DNA nucleotides (and rarely in RNA, for example, in transfer RNA) while uracil, rather than thymine, is found

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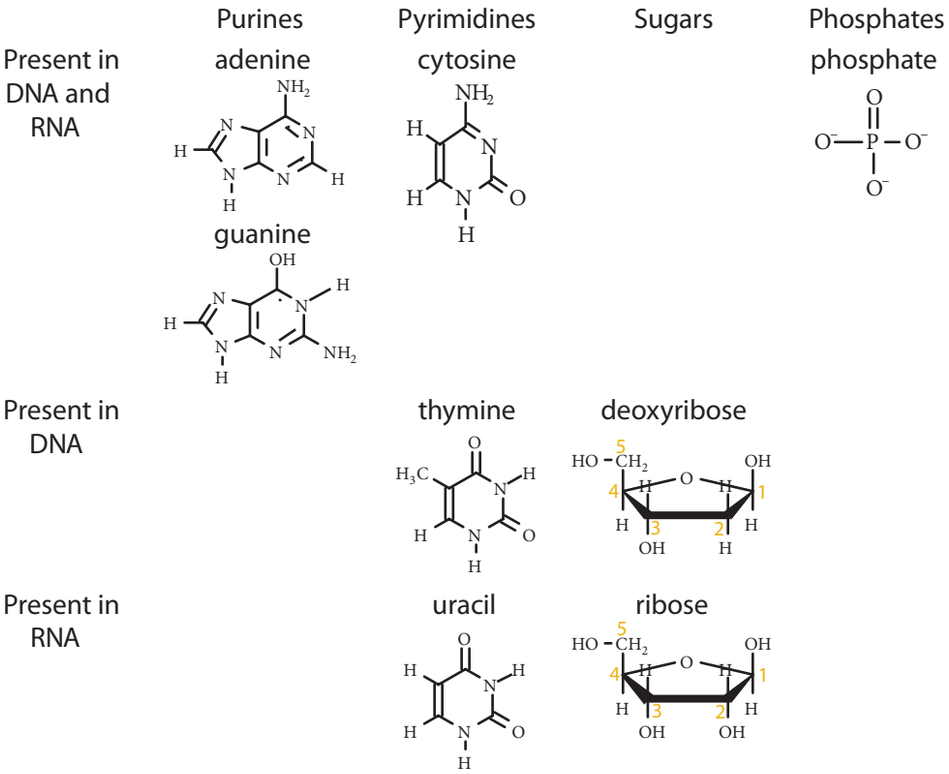

Figure 1347 Components of nucleic acids

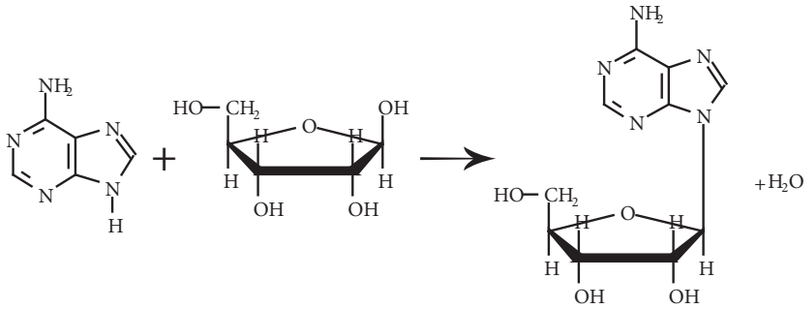

Figure 1348 Condensation reaction of adenine with ribose

in RNA nucleotides. Thymine differs from uracil only in the presence of a $-CH_3$ group on the C–5 position.

There are two forms of pentose that may make up a nucleoside. As the name implies, the sugars are either ribose (corectly called D-ribose) found in RNA or deoxyribose (2-deoxy-D-ribose) found in DNA nucleotides (stereochemistry is crucial to nucleic acid structures). These sugars differ only in that the hydroxyl group on the C–2 of ribose is replaced by a hydrogen atom in deoxyribose and lacks an oxygen atom on C–2. Thus deoxysugars, like deoxyribose, do not fit the empirical formula, CH_2O, generally given for carbohydrates.

A nitrogenous base together with a pentose sugar form a nucleoside. The condensation reaction occurs through the hydrogen atom present on a N atom of the base combining with the hydroxyl group on the C–1 of the sugar to release water and form a covalent N–C bond between the sugar and the base.

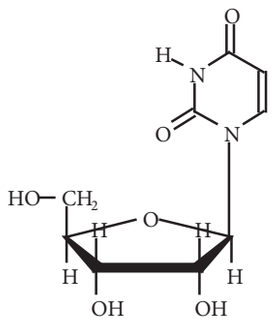

Figure 1349 Nucleoside from uracil and ribose

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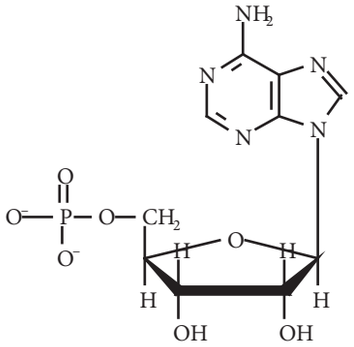


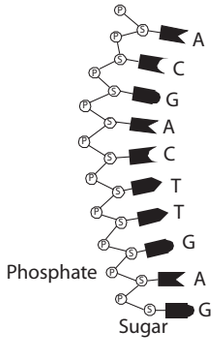
Figure 1350 A nucleotide

A nucleoside combines with a phosphate group to produce a nucleotide. The phosphate group HPO_4^{2-} is an ionized form of phosphoric acid, H_3PO_4 - the reason both DNA and RNA are acidic in aqueous solution. Again, a condensation reaction bonds the $-CH_2OH$ of a nucleoside to the phosphate group, with the hydroxyl group on the C–5 of the sugar reacting with a hydrogen from the phosphate group to release water and form a covalent bond. The bonded phosphate group has a charge of negative two, making the entire nucleotide negatively charged.

Note that a nucleoside is made up from a base and a sugar molecule, a nucleotide from a base, a sugar and an ionised phosphate group and a nucleic acid is a polymer of nucleotides.

A nucleic acid polymer consists of a chain of nucleotides formed by enzyme catalysed condensation reactions. The phosphate of one nucleotide combines with the hydroxyl group on the C–3 of the sugar on another nucleotide, releasing water and forming a bond. As the polymerization continues, a backbone of alternating sugar and phosphate groups is formed with the nitrogenous bases emerging from this backbone.

The sequence of these bases is important in the storage of genetic information. At one end of a nucleic acid chain is a nucleotide that does not have another nucleotide attached to its phosphate group and at the other an unbonded sugar. Figure 1351 shows the regularity of the sugar phosphate 'backbone' of DNA.



Section of DNA, deoxyribonucleic acid

Figure 1351 Section of DNA

B.8.3 Explain the double helical structure of DNA.

DNA has a secondary structure that results in the formation of a **double helix** that consists of two strands of nucleic acid that interact through intermolecular hydrogen bonding between the bases attached to the strands to form a double helix. The organic bases are located between the two backbones of sugar and phosphate groups. The structure of DNA shows that adenine (A) and thymine (T) only occur opposite each other and the same applies to cytosine (C) and guanine (G), see Figure 1353. Part of the reason for this is that only the combination of a purine and a pyrimidine give a similar distance between the two backbones of DNA, since a purine is double ringed and a pyrimidine is single ringed, as shown in Figure 1353.

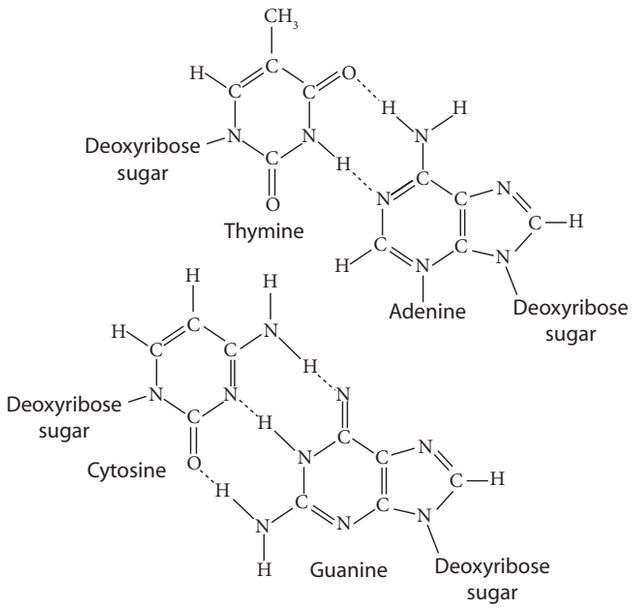


Figure 1353 Hydrogen bonding in Thymine-Adenine and Cytosine-Guanine base pairs

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The major reason why each base combines only with one other type of base is due to the intermolecular hydrogen bonding that occurs between them and this is the major factor holding the double-stranded DNA molecule together. Adenine forms two hydrogen bonds with thymine because of their molecular geometry and cytosine and guanine form three hydrogen bonds. The pairing of adenine with cytosine and thymine and guanine do not form hydrogen bonds strong enough to hold DNA together. The double helix is also stabilised by other interactions such as dipole-dipole hydrophobic interactions and van der Waals' forces between the base pairs. In order to minimize the electrostatic repulsions between negatively charged phosphate residues, the sugar-phosphate backbone adopts a helical configuration.

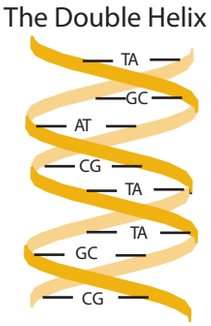

Figure 1354 The DNA double helix

The hydrogen bonding between the bases and the twisting of the sugar–phosphate backbone result in DNA's secondary structure taking the form of a double stranded, helical shape with a 'ladder' of bases spanning the gap between the two strands. The fact that each organic base on a strand has only one possible complement on the other (A & T and C & G; called **complementary base pairs**) is essential to the passing on of genetic information from one cell to the next. This pairing is based entirely on intermolecular hydrogen bonding.

B.8.4 Describe the role of DNA as the repository of genetic information, and explain its role in protein synthesis.

The role of DNA is to reproduce itself and carry the information which encodes the proteins (including the enzymes) in any organism. DNA carries the genetic information that defines any organism. A gene is a section of a DNA molecule that codes for a protein. A gene contains many nucleotides with a specific sequence of the four bases A, C, G and T in the required order to produce a specific protein. That is, the sequence of the organic bases in each gene specifies the linear amino acid sequence of a polypeptide chain (or of an RNA molecule). An organism's characteristic properties are determined by its DNA. All cells in one organism have exactly the same DNA with

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TOK What are the implications of the molecular basis of life?

Now this one probably deserves a book, or even a series of books, with one volume devoted to each field of knowledge. About the best I can do in the confines of this tiny box that I've been given is generate a few random thoughts, mainly related to our mental pre-programming, assuming that our brains are as different as our bodies and that this affects the way we think about things (are these both fair assumptions?).

- Suppose we know somebody has a genetic tendency to violence or criminal acts, will we keep that person under extra surveillance? If your answer is "No", then what are your reasons? What if you could detect this during the first weeks of pregnancy?
- Should we allow people to choose their own jobs? If I have an excellent mind for a teacher, wouldn't it be a waste to allow me to work as a check-out operative at a supermarket (and *vice-versa* - equally culpable). Does this mean that we would get paid different amounts on the basis of our mental construction?
- Suppose two artists produce equally brilliant work, but we know one has the perfect brain for artistic creativity (are either of these two assumptions possible?), whereas the other has a brain totally unsuited to this, but ideal for flipping burgers. Would knowing this affect the way in which we viewed their work?
- What about religions that postulate a soul or reincarnation? Do they assume that there is a part of DNA responsible for this (maybe divine influence affects which bits come from each parent and hence constructs the required physical/mental incarnation) or do they assume that it is passed on in some other way?

the same sequence of base pairs; different species contain different DNA molecules. Also, genetic information is passed on to the offspring of an organism through the transfer of DNA. Therefore, when new organisms are produced or cells divide, DNA must be accurately copied or replicated. The complementary base pairs allow this process to occur easily.

If a DNA sequence is damaged, for example by ultraviolet (UV) light or X-rays, it may produce little or no protein, or a different mutant protein or damaged DNA may be produced. The production of damaged DNA can lead to disease, for example, the uncontrolled growth of cells (cancer). In other cases, a changed base sequence may give rise to a non-harmful genetic change such as different hair or eye color.

Instructions for protein synthesis are encoded in DNA. DNA contains four nucleotides, but some twenty amino acids which are involved in the synthesis of proteins. Clearly one nucleotide base could not denote a particular amino acid as this would only specify four of the twenty amino acids. Similarly, two nucleotides would not be sufficient as the four bases would specify only $4^2 = 16$ amino acids (e.g. AA, AG, AC, AT, GC, GT, GA, GG, etc.). A three nucleotide sequence with four bases can produce a total of $4^3 = 64$ combinations of triplets to specify all the amino acids, where many of the amino acids are encoded by more than one triplet. For example, AAA and AAG both specify the amino acid lysine. The triplet code AUG signals the start of a protein chain whereas three other triplets specify the end of the protein chain. A three nucleotide sequence, called a triplet code or codon, codes for a specific amino acid in the polypeptide chain. Thus the sequence of nucleotides in DNA determines the precise arrangement of amino acids in proteins. Proteins are not made directly from the genetic information stored in the DNA in the cell nucleus. The transfer of genetic information involves transcription from DNA to messenger RNA (mRNA) and translation from mRNA to protein synthesis by transfer RNA (tRNA).

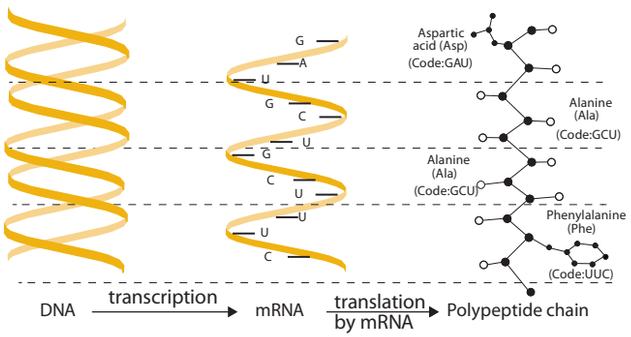

Figure 1355 Transcription and translation

B.8.5 Outline the steps involved in DNA profiling and state its use.

One of the many useful applications of DNA technology is **DNA profiling**, which involves the production of a genetic 'fingerprint'. The key to DNA profiling is that all cells from an organism must create the same DNA profile. If the process is carried out extensively enough it is possible to produce a profile of sufficient detail that would make it almost impossible for any other organisms to have the same profile. A DNA profile may be obtained by two methods, both of which require a small amount of cellular material and involve observing the number of different pieces a molecule of DNA can be split into and the sizes of these pieces.

The first method depends on the use of **restriction enzymes**, which have the ability to find a certain sequence of usually four to eight base-pairs and to cut the molecule of DNA at or near to those sequences. Therefore, if a section of DNA is chosen that varies considerably from person to person, then each person will have this section of DNA cut into different lengths as the restriction sequences will occur at different points in each person's DNA. This method has low sensitivity.

Another more sensitive method of obtaining a unique combination of DNA sizes is through examining a section of DNA called VNTR (variable number of tandem repeats). The exact function of these parts of DNA is not known, but they consist of a short sequence of base pairs repeated many times, with the number of repeats varying from person to person. These fragments of DNA can be analysed through the use of an enzyme catalyzed reaction which copies the required section of DNA millions of times, even if the initial sample is very small. Depending on the number of repeats on the VNTR site, different sizes of DNA fragments are produced. Many people will have the same number of repeats at one VNTR site, but if numerous sites are chosen then some of these will vary in length and a DNA profile can be created that is unique.

Once a unique combination of DNA fragments of different sizes is created, **gel electrophoresis** is used to make an observable DNA profile. A thin plate of electrically conducting gel is set up, a negative potential is applied at one end of the plate and a positive potential at the other. A solution of the various DNA fragments is placed on the plate near to the negative end. Since DNA has negatively charged phosphate groups it is attracted to the positively charged end. As the fragments of DNA move, the smaller fragments move more quickly through the gel than the

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larger ones. A fluorescent dye is added which makes the DNA glow in UV light. A photograph can then be taken of the number and position of the bands of DNA that appear in the gel.

DNA profiling is mainly used to identify people. This is especially useful in helping to solve crimes, as any cells left behind at the scene of a crime in the form of blood, semen, saliva or hair roots can be used to provide a DNA sample. DNA profiles can be made from the suspect's DNA and compared to the DNA profile connected with the scene of the crime. DNA profiling can also be used in paternity cases, especially the VNTR method, since all of the child's different VNTR lengths must come only from those that are present in the mother or the father.

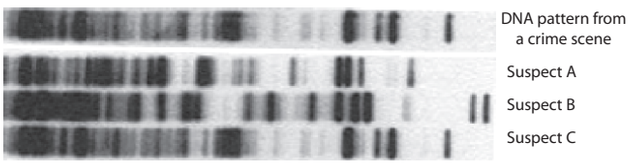

Figure 1356 Using DNA to solve a crime and implicate suspect C

B9 RESPIRATION (HL)

B.9.1 Compare aerobic and anaerobic respiration of glucose in terms of oxidation/reduction and energy released.

Digestion breaks down proteins to amino acids, fats to fatty acids (and glycerol) and carbohydrates to simple sugars such as glucose. Cellular respiration involves complex sequence of metabolic chemical reactions in a cell involving enzymes that convert energy from glucose (but occasionally also amino acids and fatty acids) into chemical energy. Sugars undergo glycolysis (breakdown of glucose) as the first stage in cellular respiration in which a glucose molecule, $C_6H_{12}O_6$ splits into two pyruvic acid molecules each with three carbon atoms. Pyruvic acid is a weak acid and dissociates to form pyruvate ions:

$C_3H_4O_3 \rightleftarrows C_3H_3O_3^- + H^+$ (*Note: some sources use the term pyruvic acid and pyruvate interchangeably).*

Figure 1357 Structures of pyruvic acid and pyruvate ions

The formation of smaller molecules from larger ones during metabolism is also called **catabolism**. In glucose this involves removal of hydrogen atoms and is achieved by hydrogen carrier species such as nicotinamide adenine dinucleotide, NAD^+ to form NADH: Thus the conversion of glucose to pyruvate is summarised by the equation:

$$C_6H_{12}O_6 + 2\ NAD^+ \rightarrow 2\ C_3H_3O_3^- + 2\ NADH + 4\ H^+ + \text{energy}$$

This process is **anaerobic** (the opposite of **aerobic**) respiration since it occurs in the absence of air, meaning 'without oxygen'. In this reaction, there are now fewer H atoms present per carbon in the product compared with the reactant; thus carbon experiences an increase in oxidation number and is oxidized. NAD^+, on the other hand, gains electrons and is reduced:

$$NAD^+ + H^+ + 2e^- \rightarrow NADH$$

Aerobic (also called oxidative) respiration requires air in which molecular oxygen is the oxidizing agent (electron acceptor) and a fuel molecule is the reducing agent (the electron donor). Once glucose is converted into the pyruvate ion, aerobic oxidation takes place in which oxygen converts pyruvate ion to carbon dioxide and water, reforming the NAD^+:

$$2CH_3COCOO^- + 2NADH + 4H^+ + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2NAD^+$$

Overall redox reaction: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$; $\Delta H^\circ = -2820\ kJ\ mol^{-1}$

Overall, in aerobic oxidation, the carbon atoms in glucose are oxidized to form CO_2 and oxygen is reduced to form water. The two redox half equations for the reactions are:

Oxidation ½-reaction (Ox. No. of C increases):

$$C_6H_{12}O_6 + 6\ H_2O \rightarrow 6\ CO_2 + 24\ H^+ + 24\ e^-$$

Reduction ½-reaction (Ox. No. of O decreases):

$$6\ O_2 + 24H^+ + 24\ e^- \rightarrow 12\ H_2O$$

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In this reaction the 'average' oxidation number of C in glucose ($6x + 12(+1) + 6(-2) = 0$; $x = 0$) increases from 0 to + 4 in CO_2. That is, there is a loss of 4 e^- per carbon atom which is oxidized. The molecular oxygen is the electron acceptor; it accepts 2 e^- per oxygen atom and is reduced; in H_2O. Its oxidation number is –2. Overall, energy is produced by the oxidation reaction since glucose (and also amino acids and fatty acids) contain a larger number of C–C and C–H bonds which are not as strong (bond enthalpies respectively of 348 and 412 kJ mol^{-1} in the gaseous state) compared with C=O and O–H bonds (743 and 463 kJ mol^{-1} respectively) in the products. Thus less energy is required to break the weaker reactant bonds and more energy is produced in making stronger product bonds; the net result is an exothermic reaction.

When the supply of oxygen is insufficent, aerobic respiration cannot occur and under such circumstances, the pyruvate ion is reduced to the lactate ion:

$$CH_3COCOO^- + NADH + H^+ \rightarrow CH_3CHOHCOO^- + NAD^+ + \text{energy}$$

The respective reduction and oxidation half-reactions are:

$$2e^- + 2H^+ + CH_3COCOO^- \rightarrow CH_3CHOHCOO^-$$

$$NADH + H^+ \rightarrow NAD^+ + 2e^- + 2H^+$$

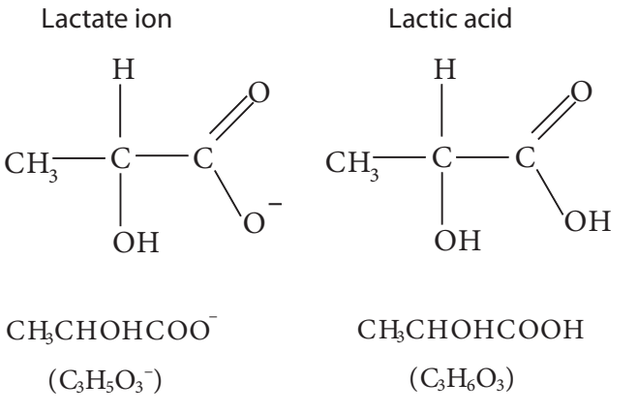

Fig 1358 Structures of lactate ion and lactic acid

This is known as **anaerobic respiration** and during strenuous exercise, which requires oxygen faster than it can be delivered by the blood stream, anaerobic respiration occurs, causing the build up of lactic acid in muscle cells. This happens for a limited time only in the human body as the increase in lactic acid decreases the pH in the muscle causing pain. If oxygen is again available, lactic acid can be oxidized to carbon dioxide and water. The overall reaction and the respective reduction and oxidation half-reactions are:

$$C_3H_6O_3 + 3\ O_2 \rightarrow 3\ CO_2 + 3\ H_2O + \text{energy}$$

$$3\ O_2 + 12\ e^- + 12\ H^+ \rightarrow 6\ H_2O$$

$$C_3H_6O_3 + 3\ H_2O \rightarrow 3\ CO_2 + 12\ e^- + 12\ H^+$$

Whereas pyruvate ions are converted to lactate ions in anaerobic respiration in humans, yeast converts pyruvate ions to ethanol and carbon dioxide.

$$2\ CH_3COCOO^- + 2\ NADH + 4\ H^+ \xrightarrow{Yeast} 2\ C_2H_5OH + 2\ CO_2 + 2\ NAD^+$$

Thus, in the presence of yeast as catalyst, overall conversion of sugar takes place to alcohol.

$$\text{Overall reaction: } C_6H_{12}O_6 \xrightarrow{Yeast} 2C_2H_5OH + 2CO_2$$

In this reaction, the 'average' oxidation number of carbon in the reactant decreases from 0 ($6x + 12(+1) + 6(-2) = 0$; $x = 0$) to –2 in the reactant ethanol ($2x + 6(+1) + (-2) = 0$; $x = -2$) and increases to +4 in CO_2. Thus the glucose is reduced to ethanol and oxidized to carbon dioxide.

B.9.2: Outline the role of copper ions in electron transport and iron ions in oxygen transport.

METAL IONS IN BIOLOGICAL SYSTEMS

Metal ions found in the diet are essential to biological systems. Different metal ions fulfil different roles in the body due to their differences in charge density, redox properties and complex ion formation. Iron was the first trace metal ion found to be essential in the human diet. Other first row transition metals such as copper and cobalt are also required in trace amounts by the human body. Iron is present in the haemoglobin molecule of red blood cells so that iron deficiency produces anemia and causes fatigue as cells are deprived of oxygen. Copper deficiencies have been known to give rise to bone disease and cobalt is a vital component of vitamin B12. Transition metal ions are important in biological systems because they have the ability to form complex ions and also to exist in multiple oxidation states and hence catalyse redox reactions.

In haemoglobin for example coordinate covalent bonding takes place between the lone electron pairs on the nitrogen containing bases and the iron ion, which has a charge because of the presence of lone electron pairs on the nitrogen of the bases and the high charge density (charge

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to size ratio) of the transition metal ions. Consequently coordinate covalent bonding takes place where the metal ion is the electron pair acceptor (Lewis base) and the nitrogen on the base, the electron pair donor (the Lewis base). This is an example of a complex ion formation in which the species containing the nitrogen donor atoms surrounding the metal ion is called a **multidentate ligand**:

structure of heme group of haemoglobin

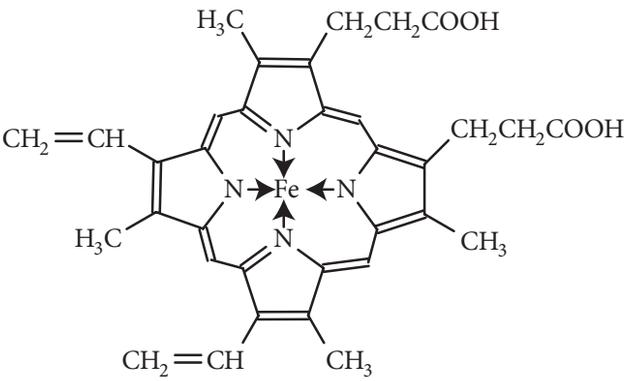

Figure 1359 The structure of the heme group of hemoglobin

Macro-nutrients such as proteins, fats and carbohydrates undergo a series of redox reactions involving gain and loss of electrons in the mitochondria inside cells. **Cytochromes** are part of the electron transfer chain which generates **adenosine triphosphate** (ATP), a form of short term stored chemical energy. Cytochromes are iron and copper containing proteins that carry energetic electrons to produce ATP. Living organisms use ATP to transfer useful energy ($\Delta G°$ is negative) from exothermic reactions, such as oxidation of carbohydrates and fats to biosynthetic reactions and other endothermic processes that require energy. The energy produced in the oxidation of food is stored in the form of ATP (the 'energy currency'). ATP (and water) is made by the addition of **adenosine diphosphate** (ADP) to phosphate ion and this reaction is endothermic. The reverse reaction, hydrolysis of ATP to ADP is exothermic and provides the energy for the cell to function. The sequence of such reactions in the oxidation of glucose ensures there is controlled slow release of energy.

$$ATP \rightleftharpoons ADP + P + \Delta G$$

The iron atoms of the cytochromes undergo one-electron oxidation-reduction reactions during aerobic respiration between iron(II) and iron(III) oxidation states and the copper between copper(I) and copper(II):

$$Fe^{2+} \rightleftharpoons Fe^{3+} + e^-; \quad Cu^{1+} \rightleftharpoons Cu^{2+} + e^-$$

Reduced co-enzymes such as NADH (nicotinamide adenine dinucleotide) carry hydrogen ions and electrons from the metal ions which serve as intermediates to form water and produce energy:

$$4e^- + 4\,H^+_{(aq)} + O_{2\,(g)} \rightleftharpoons 2\,H_2O_{(l)} + \text{energy}$$

This exothermic reaction is carried out in many steps involving a number of enzymes. Copper in the cytochrome oxidase is the terminal electron carrier in the **electron transport chain** (also called the respiratory chain) which converts oxygen to water and the energy produced is used to form ATP.

Haemoglobin is the best known oxygen transport protein. It bonds to oxygen in the lungs in order to transport it via the blood to tissues throughout the body. Heme is a complex of iron in a hydrophobic environment due to the non-polar side chains that surround it. This environment makes it possible for the oxygen to bind with the Fe^{2+} reversibly without oxidising the metal to Fe^{3+}.

The iron in haemoglobin bonds to oxygen to form oxy-haemoglobin. This carries oxygen to the cells where it releases the oxygen, picks up some of the carbon dioxide and returns the CO_2 to the lungs where it is released. Most of the carbon dioxide in the circulatory system is transported as carbonic acid, i.e. as hydrogen carbonate and hydrogen ions, and is eventually exhaled through the lungs:

$$CO_2\,(g) + H_2O\,(l) \rightleftharpoons H^+(aq) + HCO_3^-(aq)$$

Iron deficiency causes anemia (shortage of red blood cells) and, as a result, insufficient oxygen is carried to the cells, leading to fatigue. Species such as carbon monoxide, CO, bind tightly with Fe(II) and block its ability to pick up oxygen. This interferes with oxygen transport, depriving the body cells of vital oxygen. The heart must pump at a greater rate and if sufficient oxygen cannot be supplied, the animal can die by asphyxiation. Since the reactions of haemoglobin with both O_2 and CO are reversible, excess amounts of oxygen can eventually displace the CO and the effect of CO metabolic poisoning can be reversed to some extent.

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OPTION B: HUMAN BIOCHEMISTRY

QUESTIONS

B1 Energy

1. 1,00 g of roasted peanut is heated electrically and burned in a supply of oxygen in a food calorimeter. The heat produced increases the temperature of the water and calorimeter by 6.60°C. Given the 'water equivalent' of the calorimeter and water is equal to 532 g, calculate the energy content of the peanut in kJ per g.

B2 Proteins

2. Describe the structural feature that distinguishes glycine from alanine.

3. Draw the condensation reaction of two molecules of alanine to form the dipeptide. Name and describe the covalent bond (linkage) that connects the two amino acids.

4. (i) Using symbols, write the combinations of tripeptides that can be formed from alanine (Ala) and glycine (Gly).

(ii) Thus deduce the number of different tripeptides that can be formed using three different amino acids.

(iii) Determine the number of tripeptides if three different amino acids are present only once in each tripeptide.

5. Outline two experimental procedures that can be used to analyse the amino acids present in a tripeptide. Describe and explain how each method can identify the individual amino acids.

6. Draw the structure of aspartic acid from the Data Booklet. Given that the isoelectric point of aspartic acid = 2.8, draw the structure of the species present in solution and explain your reasoning. Deduce the structure of the species at pH = 1.0 and 11.0 and explain your reasoning.

7. Describe and explain how the primary, secondary (α-helix and β-pleated sheets), tertiary and quaternary structure of proteins differ.

8. List the major functions of proteins in the body.

B3 Carbohydrates

9. State the empirical formula of a monosaccharide and identify two functional groups present in monosaccharides.

10. Identify the structural characteristics present in the formula of straight chain glucose, and state the type of isomerism it exhibits.

11. Two common ring structures of glucose are called α-glucose and β-glucose. Describe any differences between the two structures.

12. Maltose is a disaccharide that contains two glucose rings in a 1→ 4 linkage. Describe the type of reaction and the linkage that leads to the formation of maltose, and meaning of the expression: 1→ 4. Write a balanced equation for the reaction, given glucose has the formula: $C_6H_{12}O_6$. Describe what happens when one consumes maltose.

13. Describe the similarity and difference(s) between the structure of amylose and amylopectin.

14. Based on the structures of the disaccharides maltose and sucrose and the polysaccharide starch, deduce the names of the monosaccharides they are formed from.

15. Explain why monosaccharides and disaccharides are water soluble.

16. Describe the difference between amylopectin and glycogen.

B4 Lipids

17. Explain the relationship between iodine number and unsaturation in a fat or an oil.

18. Linolenic acid has the formula: $C_{17}H_{29}COOH$. 2.78 g of the fatty acid requires 7.62 g iodine. Determine the number of double bonds present in the acid.

19. Compare the composition of the three types of lipids found in the human body, triglycerides (fats and oils), phospholipid (lecithin) and steroids (cholesterol).

20. Describe the structural similarities and any differences between a fat and an oil and explain why they differ in their melting points.

21. (a) Predict and explain whether stearic acid, $C_{17}H_{35}COOH$, relative molecular mass 284, is a solid or liquid at room temperature.

(b) Oleic acid, $C_{17}H_{33}COOH$, of a similar relative molecular mass, is a liquid at room temperature. Suggest a reason why this is the case.

(c) Linoleic acid, $C_{17}H_{31}COOH$, of a similar relative molecular mass, is a liquid with a lower melting point than oleic acid. Suggest a reason why this is the case.

22. Explain why triglycerides are a concentrated energy source providing more energy per gram than carbohydrates.

B5 Micro- and macro-nutrients

23. Define the term 'micronutrient' and give two examples.

24. Define the term 'macronutrient' and give two examples.

25. Study the structure of vitamin D in the Data Booklet. Identify two functional groups present in the molecule. Deduce whether it is fat or water soluble and explain your reasoning.

26. Study the structure of vitamin C in the Data Booklet. Identify two functional groups present in the molecule. Deduce whether it is fat or water soluble and explain your reasoning.

B6 Hormones

27. Outline the production and function of hormones in the body.

28. Study the structures of the two sex hormones oestradiol and testosterone given in the Data Booklet. List the differences between the two structures and suggest how the two hormones could be distinguished in a school laboratory.

[HL] B7 Enzymes

29. List the function of an enzyme and describe the characteristics of enzymes.

30. The enzyme urease decomposes urea in living systems.

(i) Outline the mechanism of the reaction of the enzyme, E with the substrate, S.

(ii) Sketch a graph of the velocity, *V,* of the reaction (on the *y*-axis) against the concentration of urea (on the *x*-axis).

(iii) Describe and explain the shape of the graph. Clearly show the value of V_{max} on the sketch and explain its significance.

(iv) From the sketch you have drawn, show the value of the Michaelis constant (K_m) and explain its significance.

(v) Describe the relationship between K_m value and enzyme activity.

31. Explain why enzyme activity is affected even when there is a slight change in its shape.

32. State and explain the effect of the following factors on enzyme activity:

(i) the presence of heavy-metal ions

(ii) temperature changes, and

(iii) pH changes.

[HL] B8Nucleic acids

33. (i) Describe the structure of nucleotides and list the bases involved.

(ii) State the reaction that leads to the formation of nucleic acids from nucleotides.

(iii) Describe how nucleotides link to form polynucleotides.

(iv) Describe the structure of nucleic acids (polynucleotides).

(v) State two differences between the chemical composition of the nucleic acids DNA and RNA.

34. The structure of DNA shows that adenine (A) and thymine (T) only occur opposite each other and the same applies to cytosine (C) and guanine (G). Use the structures of the bases in the Data Booklet to illustrate why this is the case, and identify the force of attraction that leads to it.

35. (i) Describe the basis of DNA profiling.

(ii) Outline the steps involved in DNA profiling.

(iii) Describe one method used to obtain a genetic fringerprint.

(iv) State and explain the uses of DNA profiling.

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[HL] B9 Respiration

36. (a) Distinguish between aerobic and anaerobic respiration.

(b) Identify the oxidizing and reducing agent in the aerobic oxidation of glucose

(c) Write the oxidation and reduction half-reactions and the overall reaction for the aerobic oxidation of glucose.

(d) In terms of bond energies, explain why the aerobic oxidation of glucose is an exothermic process.

(e) Deduce the type of respiration that takes place during strenuous exercise. Explain your reasoning and describe its consequence. Describe the products formed if additional oxygen is then available.

37. Outline the role of copper ions in electron transport and iron ions in oxygen transport.

Outline the role d-block metal ions play in:

(i) electron transport and

(ii) oxygen transport.

For each process, state the biological molecule, the metal ion present in it and describe how the molecule functions giving relevant equation(s).