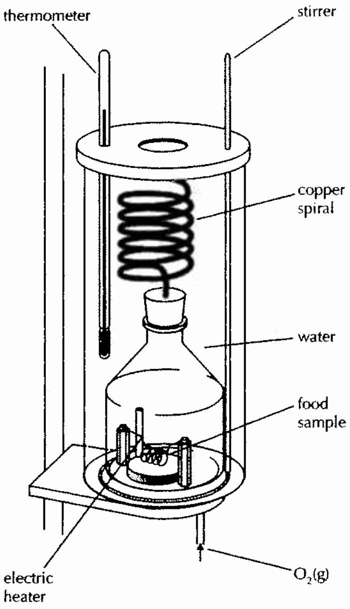
Energy and amino acids

ENERGY

The daily calorific intake for a moderately active woman is about 8400 kJ (2000 kcal) per day. For an adult male undertaking physical work this increases to about 14 700 kJ (3500 kcal). Energy is provided by fats, carbohydrates, and proteins. Carbohydrates provide the main source of energy but like proteins are already partially oxidized so do not provide as much energy weight-for-weight as fats which are used to store energy.

FOOD CALORIMETRY

The energy content of a food can be found by burning the food in a food calorimeter. A known mass of the food is heated electrically and burned in a supply of oxygen. The heat produced is transferred through a copper spiral to water and the temperature increase of the water recorded. The 'water equivalent' of the whole system is calibrated and the calorific value of the food determined. Calorific values are typically recorded as kcal per 100 g or more commonly as kJ per 100 g. 1 kcal = 4.18 kJ.



Sample calculation

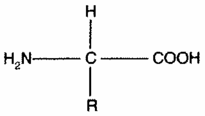
A recommended daily helping of a breakfast cereal is 37.5 g. 1.00 g of the dried breakfast cereal was combusted in a food calorimeter. The temperature rise was found to be 5.50 °C. The mass of water, stirrer, thermometer and glass surroundings had a water equivalent of 626 g. Calculate the energy content of the recommended amount of the cereal.

Heat evolved = mass of water x specific heat capacity of water x temperature rise

= 626 x 4.18 x 5.50 = 14400 J = 14.4 kJ per 1.00 g of cereal.

Energy content of recommended daily helping = 37.5 x 14.4 = 540 kJ

AMINO ACIDS AND POLYPEPTIDES



$$\mathrm{H_2N{-}\underset{R}{\overset{H}{C}}{-}COOH}$$

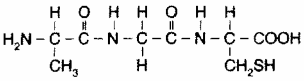
2-amino acid

Amino acids contain both an amine functional group and a carboxylic acid functional group. When they are both attached to the same carbon atom they are known as 2-amino acids (or α-amino acids).

There are about twenty 2-amino acids that occur naturally. They are the basic 'building blocks' of proteins in the body, which consist of long chains of polypeptides formed by condensation reactions between the amino acids.

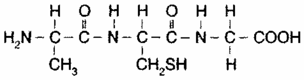
When two amino acids condense they form a **dipeptide and water**. A **tripeptide** is made up from three amino acids. If the three amino acids are all different then there are six possible ways in which they can combine.

Possible tripeptides formed from alanine $H_2N–CHCH_3–COOH$, glycine $H_2N–CH_2–COOH$, and cysteine $H_2N–CH(CH_2SH)–COOH$:



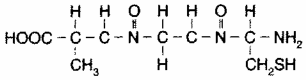
$$\mathrm{H_2N{-}\underset{CH_3}{\overset{H}{C}}{-}\overset{O}{C}{-}\overset{H}{N}{-}\underset{H}{\overset{H}{C}}{-}\overset{O}{C}{-}\overset{H}{N}{-}\underset{CH_2SH}{\overset{H}{C}}{-}COOH}$$

H_2N – Ala – Gly – Cys – COOH



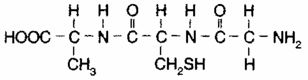
$$\mathrm{H_2N{-}\underset{CH_3}{\overset{H}{C}}{-}\overset{O}{C}{-}\overset{H}{N}{-}\underset{CH_2SH}{\overset{H}{C}}{-}\overset{O}{C}{-}\overset{H}{N}{-}\underset{H}{\overset{H}{C}}{-}COOH}$$

H_2N – Ala – Cys – Gly – COOH



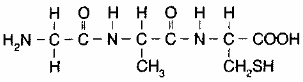
$$\mathrm{HOOC{-}\underset{CH_3}{\overset{H}{C}}{-}\overset{H}{C}{-}\overset{O}{N}{-}\underset{H}{\overset{H}{C}}{-}\overset{H}{C}{-}\overset{O}{N}{-}\underset{CH_2SH}{\overset{H}{C}}{-}NH_2}$$

HOOC – Ala – Gly – Cys – NH_2



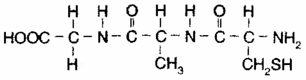
$$\mathrm{HOOC{-}\underset{CH_3}{\overset{H}{C}}{-}\overset{H}{N}{-}\overset{O}{C}{-}\underset{CH_2SH}{\overset{H}{C}}{-}\overset{H}{N}{-}\overset{O}{C}{-}\underset{H}{\overset{H}{C}}{-}NH_2}$$

HOOC – Ala – Cys – Gly – NH_2



$$\mathrm{H_2N{-}\underset{H}{\overset{H}{C}}{-}\overset{O}{C}{-}\overset{H}{N}{-}\underset{CH_3}{\overset{H}{C}}{-}\overset{O}{C}{-}\overset{H}{N}{-}\underset{CH_2SH}{\overset{H}{C}}{-}COOH}$$

H_2N – Gly – Ala – Cys – COOH



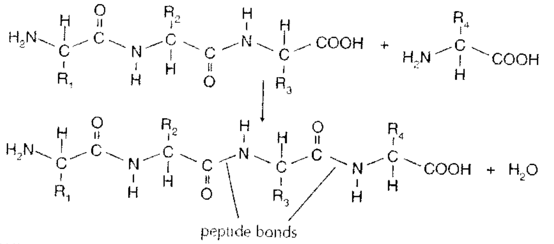
$$\mathrm{HOOC{-}\underset{H}{\overset{H}{C}}{-}\overset{H}{N}{-}\overset{O}{C}{-}\underset{CH_3}{\overset{H}{C}}{-}\overset{H}{N}{-}\overset{O}{C}{-}\underset{CH_2SH}{\overset{H}{C}}{-}NH_2}$$

HOOC – Gly – Ala – Cys – NH_2

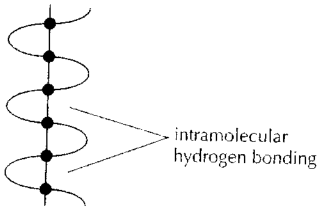
Structure and uses of proteins

STRUCTURE OF PROTEINS

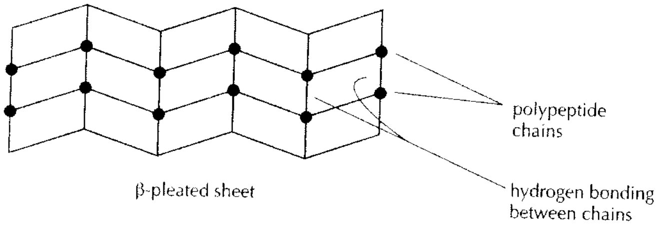
Proteins are large macromolecules made up of chains of 2-amino acids. About twenty 2-amino acids occur naturally. The amino acids bond to each other through condensation reactions resulting in the formation of a polypeptide, in which the amino acid residues are joined to each other by an amide link (peptide bond).



Each protein contains a fixed number of amino acid residues connected to each other in strict sequence. This sequence, e.g. gly-his-ala-ala-leu- ... is known as the primary structure of proteins. The secondary structure describes the way in which the chain of amino acids folds itself due to intramolecular hydrogen bonding. The folding can either be α-helix in which the protein twists in a spiralling manner rather like a coiled spring, or β-pleated to give a sheet-like structure.



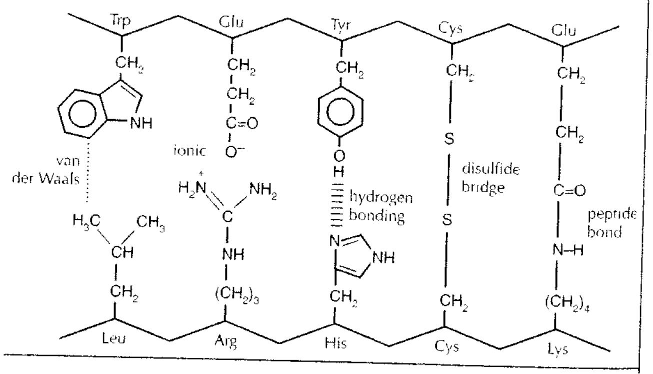
α-helix



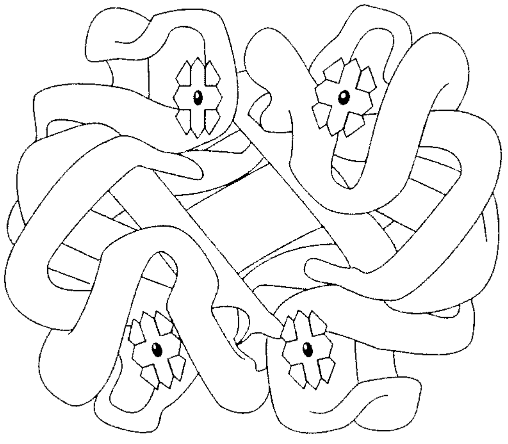
β-pleated sheet

The tertiary structure describes the overall folding of the chains by interactions between distant amino acids to give the protein its three-dimensional shape. These interactions may be due to hydrogen bonds, van der Waals' attraction between non-polar side groups, and ionic attractions between polar groups. In addition two cysteine residues can form **disulfide bridges** when their sulfur atoms undergo oxidation.

Examples of interactions between side groups on polypeptide chains:



Separate polypeptide chains can interact together to give a more complex structure – this is known as the quaternary structure. Haemoglobin has a quaternary structure that includes four protein chains (two α-chains and two β-chains) grouped together around four haem groups.



haemoglobin

USES OF PROTEINS

Proteins have many different functions in the body. They can act as biological catalysts for specific reactions (enzymes). They can give structure (e.g hair and nails consist almost entirely of polypeptides coiled into α-helices), and provide a source of energy. Some hormones are proteins, e.g. FSH (follicle stimulating hormone), responsible for triggering the monthly cycle in females.

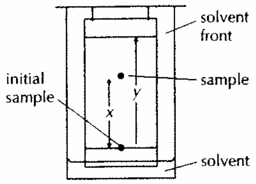
Analysis of proteins

The primary structure of proteins can be determined either by paper chromatography or by electrophoresis. In both cases the protein must first be hydrolysed by hydrochloric acid to successively release the amino acids. The three-dimensional structure of the complete protein can be confirmed by X-ray crystallography.

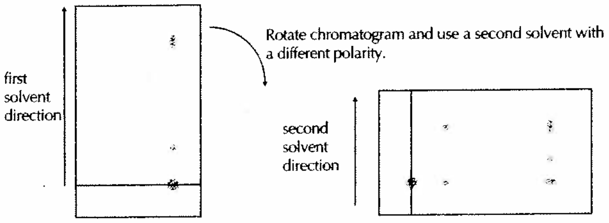
PAPER CHROMATOGRAPHY

A small spot of the unknown amino acid sample is placed near the bottom of a piece of chromatographic paper. Separate spots of known amino acids can be placed alongside. The paper is placed in a solvent (eluent), which then rises up the paper due to capillary action. As it meets the sample spots the different amino acids partition themselves between the eluent and the paper to different extents, and so move up the paper at different rates. When the eluent has nearly reached the top, the paper is removed from the tank, dried, and then sprayed with an organic dye (ninhydrin) to develop the chromatogram by colouring the acids. The positions of all the spots can then be compared.

If samples of known amino acids are not available the R_f value (retention factor) can be measured and compared with known values as each amino acid has a different R_f value. It is possible that two acids will have the same R_f value using the same solvent, but different values using a different solvent. If this is the case the chromatogram can be turned through 90° and run again using a second solvent.



$$R_f \text{ value} = \frac{\text{distance travelled by sample}}{\text{distance travelled by solvent}} = \frac{x}{y}$$



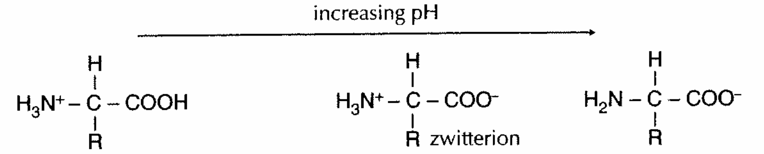
Three-component mixture split into two by first solvent.

Separation of the three components is achieved.

ELECTROPHORESIS

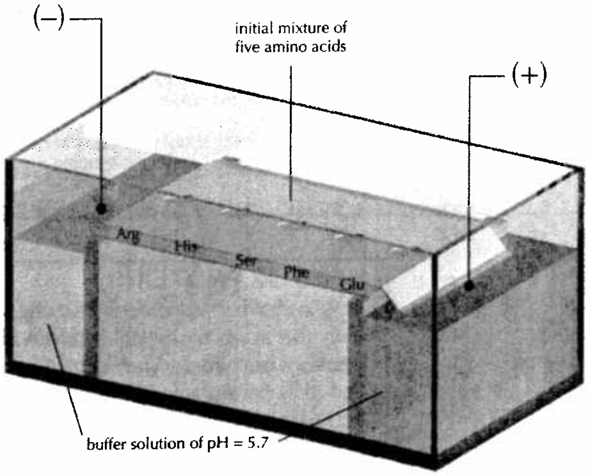
The structure of amino acids alters at different pH values. At low pH (acid medium) the amine group will be protonated. At high pH (alkaline medium) the carboxylic acid group will lose a proton. This explains why amino acids can function as buffers. If H^+ ions are added they are removed as $-NH_4^+$ and if OH^- ions are added the –COOH loses a proton to remove the OH^- ions as water. For each amino acid there is a unique pH value (known as the **isoelectric point**) where the acid will exist as the zwitterion.

The medium on which electrophoresis is carried out is usually a polyacrylamide gel. So the process is known as PAGE (polyacrylamide gel electrophoresis). The sample is placed in the centre of the gel and a potential difference applied across it. Depending on the pH of the buffer the different amino acids will move at different rates towards the positive and negative electrodes. At its isoelectric point a particular amino acid will not move as its charges are balanced. When separation is complete the acids can be sprayed with ninhydrin and identified by comparing the distance they have travelled with standard samples, or from a comparison of their isolelectric points.



$H_3N^+-CH(R)-COOH$ $H_3N^+-CH(R)-COO^-$ $H_2N-CH(R)-COO^-$

pH < isoelectric point isoelectric point pH > isoelectric point



2-amino acid	pH of isoelectric point
glutamic acid (Glu)	3.2
phenylalanine (Phe)	5.5
serine (Ser)	5.7
histidine (His)	7.6
arginine (Arg)	10.8

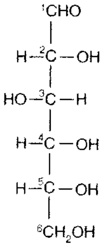
Separation of a mixture of five amino acids by electrophoresis
Serine does not move as its isoelectric point is the same pH as the buffer. Histidine and arginine contain $-NH_3^+$ at pH 5.7, so move towards the negative electrode. Glutamic acid and phenylalanine contain $-COO^-$ at pH 5.7, so move towards the positive electrode.

Carbohydrates

MONOSACCHARIDES

All monosaccharides have the empirical formula CH_2O. In addition they contain a carbonyl group ($>$C=O) and at least two -OH groups. They have between three and six carbon atoms. Monosaccharides with the general formula $C_5H_{10}O_5$ are known as pentoses (e.g. ribose) and monosaccharides with the general formula $C_6H_{12}O_6$ are known as hexoses (e.g. glucose).

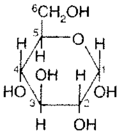
Many structural isomers of monosaccharides are possible. In addition several carbon atoms are chiral (asymmetric) and give rise to optical isomerism. To make matters worse open chain structures and ring structures are possible. The form of glucose that is found in nature is known as D-glucose.



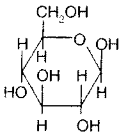
D-glucose

Straight chain formula of D-glucose

The ring structure of D-glucose can exist in two separate crystalline forms known as α-D-glucose and β-D-glucose. Note: the only difference is that the -OH group on the first carbon atom is inverted.



α-D-glucose



β-D-glucose

Six-membered ring monosaccharides are known as pyranoses. Hexoses can also have a furanose structure where they have a five-membered ring containing an oxygen atom.

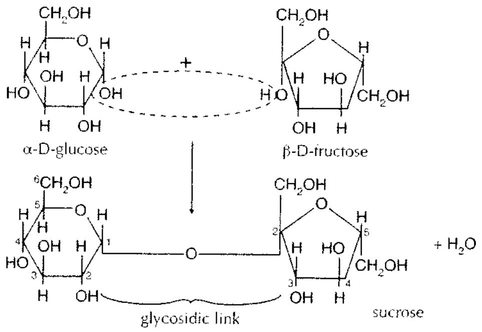
MAJOR FUNCTIONS OF POLYSACCHARIDES IN THE BODY

Carbohydrates are used by humans:

- **to provide energy:** foods such as bread, biscuits, cakes, potatoes, and cereals are all high in carbohydrates
- **to store energy:** starch is stored in the livers of animals in the form of glycogen – also known as animal starch. Glycogen has almost the same chemical structure as amylopectin
- **as precursors** for other important biological molecules, e.g. they are components of nucleic acids and thus play an important role in the biosynthesis of proteins.
- **as dietary fibre:** Dietary fibre is mainly plant material that is not hydrolysed by enzymes secreted by the human digestive tract but may be digested by microflora in the gut. Examples include cellulose, hemicellulose, lignin and pectin. It may be helpful in preventing conditions such as diverticulosis, irritable bowel syndrome, obesity, Crohn's disease, haemerrhoids and diabetes mellitus.

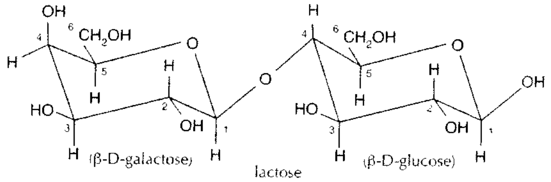
POLYSACCHARIDES

Monosaccharides can undergo condensation reactions to form disaccharides and eventually polysaccharides. For example, sucrose, a disaccharide formed from the condensation of α-D-glucose in the pyranose form and β-D-fructose in the furanose form.



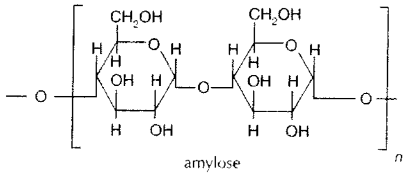
formation of sucrose by a condensation reaction

The link between the two sugars is known as a glycosidic link. In the case of sucrose the link is between the C-1 atom of glucose in the α-configuration and the C-2 atom of fructose. The link is known as an β-1,2 bond. Maltose, another disaccharide is formed from two glucose molecules condensing to form an α–1,4 bond. Lactose is a disaccharide in which the β-D-galactose is linked at the C-1 atom to the C-4 atom of β-D-glucose. This is called a β-1,4 bond.



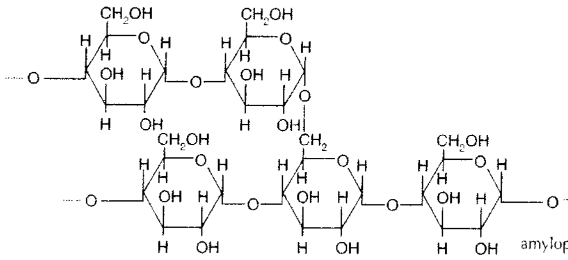
lactose

One of the most important polysaccharides is starch. Starch exists in two forms: amylose, which is water soluble, and amylopectin, which is insoluble in water.



amylose

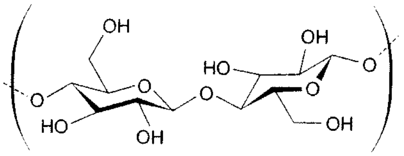
Amylose is a straight chain polymer of α-D-glucose units with α-1,4 bonds:



amylopectin

Amylopectin also consists of α-D-glucose units but it has a branched structure with both α-1,4 and α-1,6 bonds:

Most plants use starch as a store of carbohydrates and thus energy.



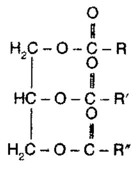
Cellulose, a polymer of β-D-glucose contains β–1,4 linkages. Cellulose, together with lignin, provides the structure to the cell walls of green plants. Most animals, including all mammals, do not have the enzyme cellulase so are unable to digest cellulose or other dietary fibre polysaccharides.

Lipids (1)

Lipids are organic molecules with long hydrocarbon chains that are soluble in non- polar solvents. They are mainly used for energy storage, insulating and protecting vital organs, forming cell membranes and, in some cases, acting as hormones. Three important types of lipids are triglycerides (fats and oils), phospholipids (lecithin) and steroids (cholesterol).

FATS AND OILS

Fats and oils are triesters (triglycerides) formed from the condensation reaction of propane-1,2,3-triol (glycerol) with long chain carboxylic acids (fatty acids).



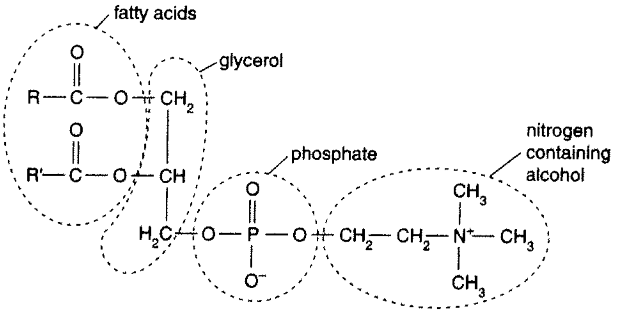
R, R', and R'' are long chain hydrocarbons formed from carboxylic acids which may be the same or different.

General formula of a fat or oil.

Fats are solid triglycerides, examples include butter, lard and tallow. Oils are liquid at room temperature and include castor oil, olive oil and linseed oil. The essential chemical difference between them is that fats contain saturated carboxylic acid groups (i.e. they do not contain C=C double bonds). Oils contain at least one C=C double bond and are said to be unsaturated. Most oils contain several C=C double bonds and are known as polyunsaturated.

PHOSPHOLIPIDS

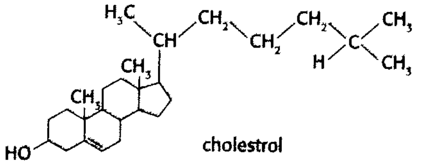
Phospholipids form an integral part of all cell membranes. They are essentially made of four components. A backbone such as propane-1,2,3-triol (glycerol), linked by esterification to two fatty acids and a phosphate group which is itself condensed to a nitrogen containing alcohol. There are many different phospholipids. They can be exemplified by phosphatidyl choline – the major component of lecithin, present in egg yolk.



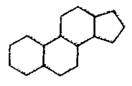
The structure of phosphatidyl choline showing the origins of the four distinct components.

CHOLESTEROL

Cholesterol has the characteristic four ring structure possessed by all steroids.



cholestrol

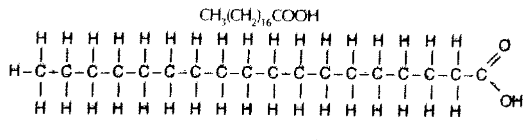


steroid 'backbone'

It is transported around the body by lipoproteins. **Low density lipoproteins** (LDL) are in the order of 18-25 nm and transport cholesterol to the arteries where it can line the walls of the arteries leading to cardiovascular diseases. The major source of these low density lipoproteins are saturated fats, in particular those derived from lauric (C_{12}), myristic (C_{14}) and palmitic (C_{16}) acids. Smaller lipoproteins, in the order of 8-11 nm, known as **high density lipoproteins** (HDL) can remove the cholesterol from the arteries and transport it back to the liver.

FATTY ACIDS

Stearic acid (m.pt 69.6 °C) and linoleic acid (m.pt –5.0 °C) both contain the same number of carbon atoms and have similar molar masses. However, linoleic acid contains two double bonds. Generally the more unsaturated the fatty acid the lower its melting point. The regular tetrahedral arrangement of saturated acids means that they can pack together closely, so the van der Waals' forces holding molecules together are stronger as the surface area between them is greater. As the bond angle at the C=C double bonds changes from 109.5° to 120° in unsaturated acids it produces a 'kink' in the chain. They are unable to pack so closely and the van der Waals' forces between the molecules become weaker, which results in lower melting points. This packing arrangement is similar in fats and explains why unsaturated fats (oils) have lower melting points.



stearic acid – a saturated acid

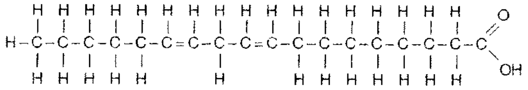
Name		Number of C atoms per molecule	Number of C=C bonds	Melting point / °C
saturated fatty acids				
lauric acid	$CH_3(CH_2)_{10}COOH$	12	0	44.2
myristic acid	$CH_3(CH_2)_{12}COOH$	14	0	54.1
palmitic acid	$CH_3(CH_2)_{14}COOH$	16	0	62.7
stearic acid	$CH_3(CH_2)_{16}COOH$	18	0	69.6
unsaturated fatty acids				
oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	18	1	10.5
linoleic acid	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	18	2	–5.0

Lipids (2)

ESSENTIAL FATTY ACIDS

Most naturally occurring fats contain a mixture of saturated, mono-unsaturated and poly-unsaturated fatty acids and are classified according to the predominant type. Essential fatty acids are fatty acids that the body is unable to synthesize. Two essential unsaturated fatty acids are ω-6 linoleic acid and ω-3 linolenic acid. From these the body is able to synthesize longer and more unsaturated fatty acids. Green leaves are a good source of ω-3 fatty acids whereas most seeds and vegetable oils are a good source of ω-6 fatty acids.

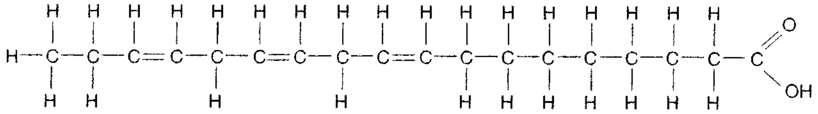
$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7COOH$



The structure of linoleic acid (*cis,cis*-9,12-octadecadienoic acid)

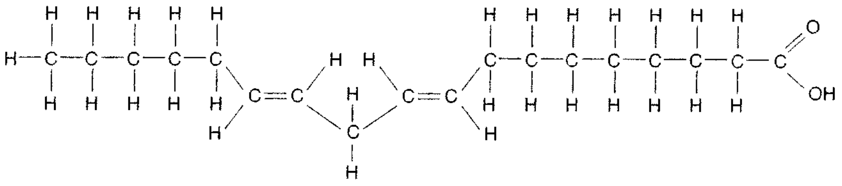
The double bonds in linoleic acid are on the ninth and twelfth carbon atoms of the acid but it is known as an omega(ω)-six fatty acid as the first double bond is on the sixth carbon atom from the end of the hydrocarbon chain (i.e. counting from the other end).

ω-3 fatty acids, such as linolenic acid, have the first double bond on the third carbon atom from the end of the hydrocarbon chain.



The structure of linolenic acid (*cis,cis,cis*-9,12,15-octadecatrienoic acid)

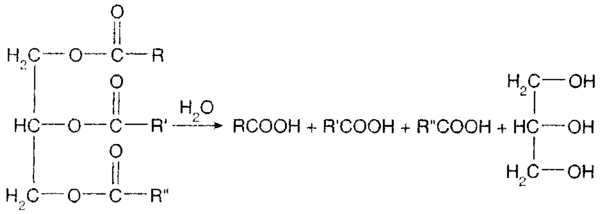
When fatty acids are made synthetically by partially hydrogenating other polyunsaturated fatty acids then the *trans*-isomers may be formed. *Trans*-fatty acids are present in fried foods such as French fries and some margarines. They increase the formation of LDL cholesterol and thus increase the risk of heart disease.



The structure of the *trans,trans*- form of linoleic acid

HYDROLYSIS OF FATS

In the body, fats and oils are hydrolyzed by enzymes, known as lipases, to glycerol and fatty acids.

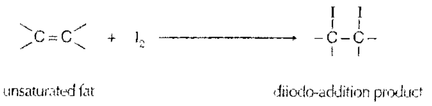


The hydrolysis of fats. (This is the reverse of the formation of fats from glycerol and fatty acids)

These in turn are broken down by a series of redox reactions to produce ultimately carbon dioxide, water and energy. Because they are essentially long chain hydrocarbons with only two oxygen atoms each on the three carboxyl atoms fats are in a less oxidized form than carbohydrates so weight for weight produce more energy.

DETERMINING THE NUMBER OF C=C BONDS IN AN UNSATURATED FAT

Unsaturated fats can undergo addition reactions. Margarine is made by the hydrogenation of vegetable oils so that it is a solid at room temperature. The addition of iodine to unsaturated fats can be used to determine the number of C=C double bonds since one mole of iodine will react quantitatively with one mole of double bonds. Iodine is coloured. As the iodine is added to the unsaturated fat the purple colour of the iodine will disappear as the addition reaction takes place. Often fats are described by their iodine number, which is the number of grams of iodine that add to 100 g of the fat.



THE ROLES OF LIPIDS IN THE BODY

- Energy storage. Because they contain proportionately less oxygen than carbohydrates they release more energy when oxidized.
- Insulation and protection of organs. Fats are stored in adipose tissue which provides both insulation and protection to parts of the body.
- Steroid hormones. Examples include progesterone and testosterone.
- Cell membranes. Lipids provide the structural component of cell membranes.

In addition omega-3-poly-unsaturated fatty acids are thought to reduce the risk of heart disease and polyunsaturated fats may lower the level of LDL (bad) cholesterol. The negative effects of lipids include the increase in LDL cholesterol from *trans*- fatty acids and saturated fatty acids such as lauric (C_{12}), myristic (C_{14}) and palmitic (C_{16}) acids.

Micro- and macro-nutrients

DEFINITIONS

Micro-nutrients are substances required in very small amounts (mg or μg). They mainly function as a co-factor of enzymes and include vitamins and trace minerals such as Fe, Cu, F, Zn, I, Se, Mn, Mo, Cr, Co and B.

Macro-nutrients are substances that are required in relatively large amounts (> 0.005% of body mass) and include proteins, fats, carbohydrates and minerals (Na, Mg, K, Ca, P, S and Cl).

MALNUTRITION

Malnutrition occurs when either too much food is consumed which leads to obesity or the diet is lacking in one or more essential micro- and macro-nutrients. Specific micro-nutrient deficiencies include:

- Fe – anaemia
- I – goiter
- vitamin A (retinol) – xerophthalmia, night blindness
- vitamin B_3 (niacin) – pellagra
- vitamin B_1 (thiamin) – beriberi
- vitamin C (ascorbic acid) – scurvy
- Vitamin D (calciferol) – rickets

Deficiencies in macronutrients such as protein include marasmus and kwashiorkor.

Solutions to combat malnutrition include

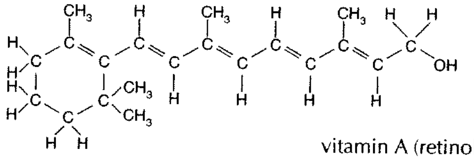
- eating fresh food rich in vitamins and minerals
- adding nutrients that are missing in commonly consumed foods
- genetic modification of food
- providing nutritional supplements

VITAMINS

Vitamins can be classified as fat soluble or water soluble. The structure of fat soluble vitamins is characterised by long, non-polar hydrocarbon chains or rings. These include vitamins A, D, E, F and K. They can accumulate in the fatty tissues of the body. In some cases an excess of fat soluble vitamins can be as serious as a deficiency. The molecules of water soluble vitamins, such as vitamin C and the eight B-group vitamins, contain hydrogen attached directly to electronegative oxygen or nitrogen atoms that can hydrogen bond with water molecules. They do not accumulate in the body so a regular intake is required. Vitamins containing C=C double bonds and –OH groups are readily oxidized and keeping food refrigerated slows down this process.

VITAMIN A (RETINOL)

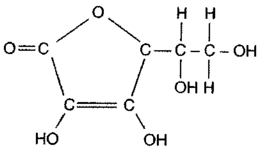
Although it does contain one -OH group, vitamin A is fat soluble due to the long non-polar hydrocarbon chain. Unlike most other vitamins it is not broken down readily by cooking. Vitamin A is an aid to night vision.



vitamin A (retinol)

VITAMIN C (ASCORBIC ACID)

Due to the large number of polar -OH groups vitamin C is soluble in water so is not retained for long by the body. The most famous disease associated with a lack of vitamin C is scorbutus ('scurvy'). The symptoms are swollen legs, rotten gums and bloody lesions. It was a common disease in sailors, who spent long periods without fresh food, until the cause was recognised.

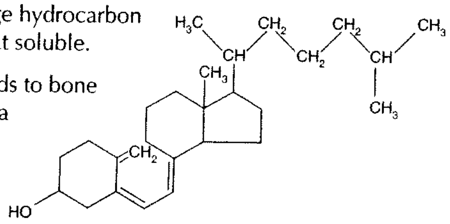


vitamin C (ascorbic acid)

VITAMIN D (CALCIFEROL)

Vitamin D is essentially a large hydrocarbon with one –OH group and is fat soluble.

A deficiency of vitamin D leads to bone softening and malformation - a condition known as rickets.



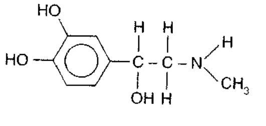
vitamin D

Hormones

Hormones are chemicals produced in endocrine glands and transported to the site of action by the blood stream. Hormones act as chemical messengers and perform a variety of different functions. Examples of specific hormones include antidiuretic hormone (ADH) which controls body water content, aldosterone (a steroid which regulates the sodium and potassium balance in the blood), adrenaline, thyroxin, insulin and the sex hormones.

ADRENALINE

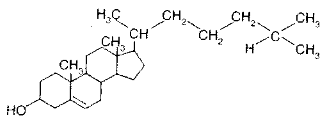
Adrenaline (epinephrine) is produced in the adrenal glands – two small organs located above the kidneys. It is a stimulant closely related to the amphetamine drugs. It is released in times of excitement and causes a rapid dilation of the pupils and airways and increases heartbeat and the rate of release of sugar into the bloodstream. It is sometimes known as the 'fight or flight' hormone.



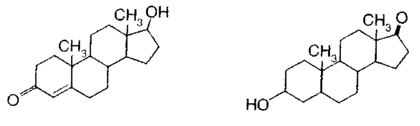
adrenaline

SEX HORMONES

The sex hormones are all steroids. Steroids contain a characteristic four-ring structure. The basic building block for the other steroids is cholesterol formed in the liver and found in all tissues, the blood, brain, and spinal cord. The male sex hormones are produced in the testes and comprise mainly testosterone and androsterone. They are anabolic – encouraging tissue, muscle, and bone growth – and androgenic – conferring the male sexual characteristics. The female sex hormones are structurally very similar with just small changes in the functional groups attached to the steroid framework. They are produced in the ovaries from puberty until menopause. The two main female sex hormones are oestradiol and progesterone. They are responsible for sexual development and for the menstrual and reproductive cycles in women.

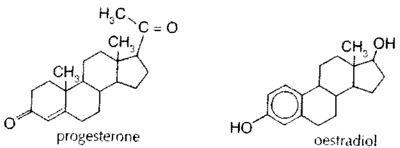


cholesterol



testosterone androsterone

male sex hormones

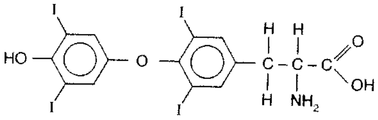


progesterone oestradiol

female sex hormones

THYROXINE

Thyroxine is produced in the thyroid gland located in the neck. It is unusual in that it contains iodine. A lack of iodine in the diet can cause the thyroid gland to swell to produce the condition known as goitre. Thyroxine regulates the body's metabolism. Low levels of thyroxine cause hypothyroidism, characterized by lethargy as well as sensitivity to cold and a dry skin. An overactive thyroid gland can cause the opposite effect. This is known as hyperthyroidism with the symptoms of anxiety, weight loss, intolerance to heat, and protruding eyes.



thyroxine

INSULIN

Insulin is a protein containing fifty-one amino acid residues. It is formed in the pancreas – an organ located at the back of the abdomen – and regulates blood sugar levels. In diabetics the levels of insulin are low or absent and glucose is not transferred sufficiently from the bloodstream to the tissues. This is known as hyperglycaemia and results in thirst, weight loss, lethargy, coma, and circulation problems. Long term sufferers of diabetes can suffer blindness, kidney failure, and need limbs amputated due to poor circulation. It is treated by reducing sugar intake and taking daily insulin injections. Too much insulin can cause hypoglycaemia, where the blood sugar level falls resulting in dizziness and fainting.

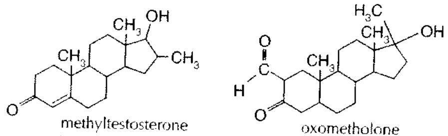
ORAL CONTRACEPTIVES

At the beginning of the menstrual cycle the pituitary releases follicle stimulating hormone (FSH). FSH travels to the ovaries causing the release of oestradiol, which prepares for the release of the ovum or egg and the build up of the uterine wall. After about two weeks a feedback system stops the release of FSH and triggers the release of the luteinizing hormone (LH). This travels to the ovaries and releases progesterone. The progesterone causes the egg to be transported to the uterus as well as continuing to build up the uterine wall. If the egg is fertilized it embeds itself into the uterine wall and hormone levels rise dramatically, otherwise hormone levels fall and menstruation begins.

The most common 'pill' contains a mixture of oestradiol and progesterone and mimics pregnancy by intentionally keeping the hormones at a high levels so that no more eggs are released. It is usual to take the pill for 21 days and then a placebo for 7 days so that a mild period will result, but without the risk that the hormone levels will fall and allow the unexpected release of an egg. Oestradiol and progesterone may also be given to post menopausal women as hormone replacement therapy (HRT) partly to prevent brittle bone disease (osteoporosis).

ANABOLIC STEROIDS

The anabolic steroids have similar structures to testosterone and build up muscle. They may be given to someone recuperating from a serious illness to build up muscles weakened by inactivity. Some athletes have abused these drugs as they can enhance athletic performance. Competitors are given random urine tests to detect these and other banned substances.



methyltestosterone oxometholone

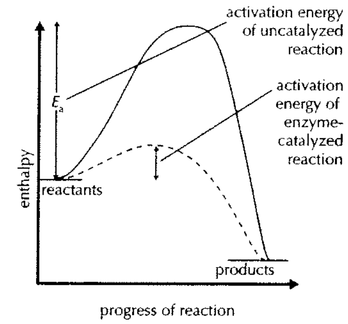
examples of anabolic steroids

Enzymes

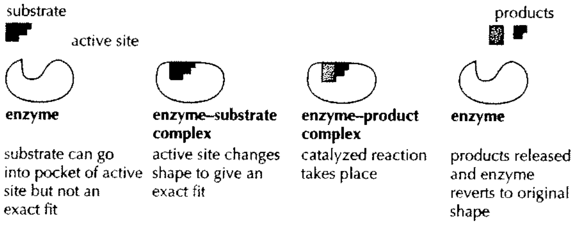
CATALYTIC ACTIVITY AND ACTIVE SITE

Enzymes are protein molecules that catalyse biological reactions. Each enzyme is highly specific for a particular reaction, and extremely efficient, often being able to increase the rate of reaction by a factor greater than 10^8. Enzymes work by providing an alternative pathway for the reaction with a lower activation energy, so that more of the reactant particles (substrate) will possess the necessary minimum activation energy.

Effect of adding an enzyme on activation energy



The specificity of enzymes depends on their tertiary and quaternary structure. The part of an enzyme that reacts with the substrate is known as the active site. This is a groove or pocket in the enzyme where the substrate will bind. The site is not necessarily rigid but can alter its shape to allow for a better fit – known as the induced fit theory.

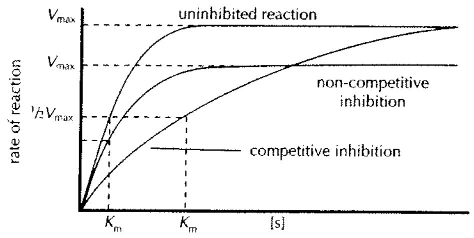


Induced fit theory of enzyme catalysis

COMPETITIVE AND NON-COMPETITIVE INHIBITION

Inhibitors are substances that slow down the rate of enzyme-catalyzed reactions. Competitive inhibitors resemble the substrate in shape, but cannot react. They slow down the reaction because they can occupy the active site on the enzyme thus making it less accessible to the substrate. Non-competitive inhibitors also bind to the enzyme, but not on the active site. This causes the enzyme to change its shape so that the substrate cannot bind. As the substrate concentration is increased the effect of competitive inhibitors lessens, as there is increased competition for the active sites by the substrate. With non-competitive inhibitors increasing the substrate concentration has no effect, as the enzyme's shape still remains altered.

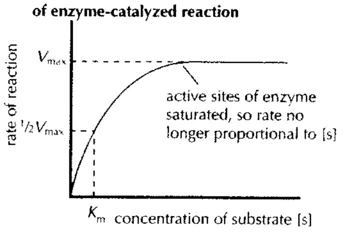
Effect of substrate concentration on inhibitors



For non-competitive inhibitors, V_{max} is lower but K_m is the same.
For competitive inhibitors, V_{max} is the same but K_m is increased.

ENZYME KINETICS

Effect of concentration of substrate on rate of enzyme-catalyzed reaction



At low substrate concentrations the rate of reaction is proportional to the concentration of the substrate. However, at higher concentrations the rate reaches a maximum known as V_{max}. This can be explained in terms of enzyme saturation. At low substrate concentrations there are enough active sites present for the substrate to bind to and react. Once all the sites are used up the enzyme cannot work any faster.

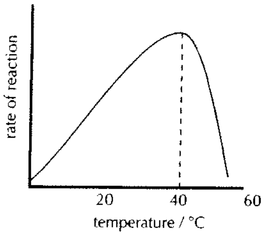
The Michaelis–Menten constant K_m is the substrate concentration when the rate of the reaction is $\frac{1}{2} V_{max}$. K_m for a particular enzyme with a particular substrate will always be the same. It indicates whether the enzyme functions efficiently at low substrate concentrations, or whether high substrate concentrations are necessary for efficient catalysis.

EFFECT OF TEMPERATURE, pH, AND HEAVY METAL IONS ON ENZYME ACTIVITY

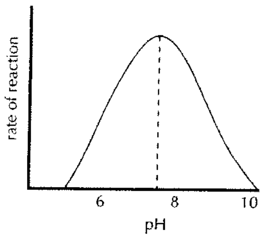
The action of an enzyme depends on its specific shape. Increasing the temperature will initially increase the rate of enzyme-catalyzed reactions, as more of the reactants will possess the minimum activation energy. The optimum temperature for most enzymes is about 40 °C. Above this temperature enzymes rapidly become denatured as the weak bonds holding the tertiary structure together break.

At different pH values the charges on the amino acid residues change affecting the bonds between them, and so altering the tertiary structure and making the enzyme ineffective. Heavy metals can poison enzymes by reacting with –SH groups replacing the hydrogen atom with a heavy metal atom, or ion so that the tertiary structure is altered.

Effect of temperature on enzyme activity



Effect of pH on enzyme activity



HL Nucleic acids

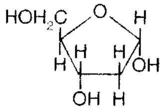
STRUCTURE OF NUCLEOTIDES AND NUCLEIC ACIDS

Almost all cells in the human body contain DNA (deoxyribonucleic acid). DNA and a related material RNA (ribonucleic acid) are macromolecules with relative molar masses of up to several million. Both nucleic acids are made up of repeating base-sugar-phosphate units called **nucleotides**. A nucleotide of DNA contains deoxyribose (a pentose sugar), a phosphate group, and one of four nitrogen-containing bases, adenine, guanine, cytosine, or thymine. RNA contains a different sugar, ribose, but also contains a phosphate group and four nitrogen-containing bases. Three of the bases are the same as those in DNA but the fourth, uracil, replaces thymine.

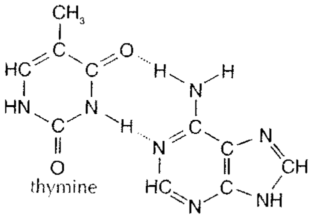
In DNA the polynucleotide units are wound into a helical shape with about 10 nucleotide units per complete turn. Two helices are then held together by hydrogen bonds between the bases to give the characteristic double helix structure. The hydrogen bonds are very specific. Cytosine can only hydrogen bond with guanine and adenine can only hydrogen bond with thymine (uracil in RNA).

sugars

bases (showing complementary hydrogen bonding)

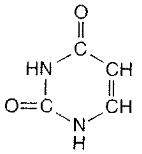


deoxyribose (used in DNA)

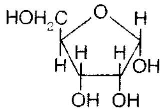


thymine

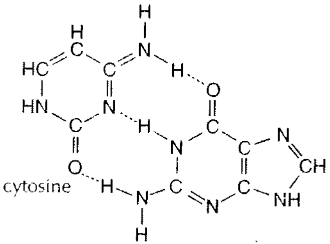
adenine



uracil
(replaces thymine
in RNA)

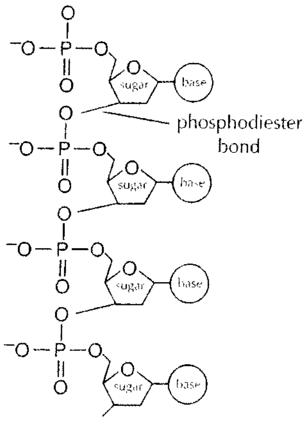


ribose (used in RNA)

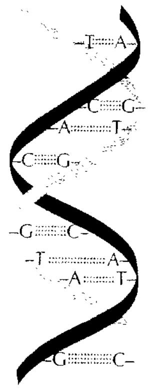


cytosine

guanine



Nucleotides condense to form a polynucleotide. Each nucleotide is joined by a phosphodiester bond between C_3 of the sugar and the neighbouring phosphate group.



The double helix structure of DNA is shown here. Note the hydrogen bonds between the two different strands of polynucleotides.

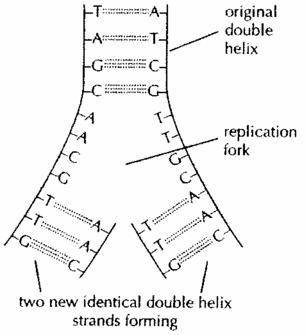
HL The genetic code

THE GENETIC CODE

When cells divide the genetic information has to be replicated intact. The genetic information is stored in chromosomes found inside the nucleus. In humans there are 23 pairs of chromosomes. Chromosomes are effectively a very long DNA sequence. The DNA in the cell starts to partly unzip as hydrogen bonds between the bases break. Sugar base units will be picked up from the aqueous solution to form a complementary new strand. Because adenosine can only hydrogen bond with thymine (A–T) and cytosine can only hydrogen bond with guanine (C–G) the new strand formed will be identical to the original.

DNA resides wholly in the nucleus whilst protein synthesis takes places in the cytoplasm. The information required to make complex proteins is passed from the DNA to messenger RNA by a similar unzipping process, known as transcription, except that the new strand of mRNA contains a different sugar and uracil in place of thymine.

The coded information held in the mRNA is then used to direct protein synthesis using a triplet code by a process known as translation. Each sequence of three bases represents one amino acid and is known as the **triplet code**. The triplet code allows for up to 64 permutations known as **codons**. This is more than sufficient to represent the 20 amino acids and several different codons may represent the same amino acid. Consecutive DNA codons of AAA, TAA, AGA, GTG, and CTT will transcribe to RNA codons of UUU, AUU, UCU, CAC, and GAA which will cause part of a strand of a protein to be formed that contains the amino acid residues -Phe-Ile-Ser-His-Glu-. In 2000 the human genome – the complete sequence of bases in human DNA – was finally determined and published on the Internet.



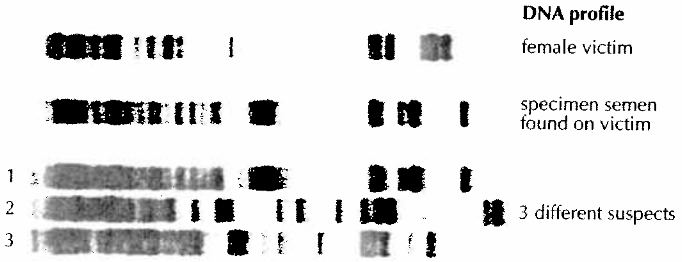
Replication of DNA

UUU UUC UUA UUG	Phe Phe Leu Leu	UCU UCC UCA UCG	Ser Ser Ser Ser	UAU UAC UAA UAA	Tyr Tyr Terminator Terminator	UGU UGC UGA UGG	Cys Cys Terminator Trp
CUU CUC CUA CUG	Leu Leu Leu Leu	CCU CCC CCA CCG	Pro Pro Pro Pro	CAU CAC CAA CAG	His His Gln Gln	CGU CGC CGA CGG	Arg Arg Arg Arg
AUU AUC AUA AUG	Ile Ile Ile Met	ACU ACC ACA ACG	Thr Thr Thr Thr	AAU AAC AAA AAG	Asn Asn Lys Lys	AGU AGC AGA AGG	Ser Ser Arg Arg
GUU GUC GUA GUG	Val Val Val Val	GCU GCC GCA GCG	Ala Ala Ala Ala	GAU GAC GAA GAG	Asp Asp Glu Glu	GGU GGC GGA GGG	Gly Gly Gly Gly

The genetic code carried by RNA

DNA PROFILING

DNA profiling (also known as DNA fingerprinting) was developed in 1985. In this process a small amount of cellular material is required. This may be obtained from blood, semen, hair, or saliva. The DNA is extracted and broken down into smaller fragments known as minisatellites using restriction enzymes. DNA not only contains coded triplets, but also has regions where there is no coded message in the base sequence. It is these regions where the splits occur to form the minisatellites which are unique to the person giving the sample (except in the case of identical twins). The fragments are separated into bands by gel electrophoresis. The resulting pattern is transferred to a nylon membrane and then labelled with radioactive ^{32}P which binds to particular bands of the DNA. X-ray film is then exposed to the radiation produced by the ^{32}P. The film is developed to give the characteristic 'fingerprint' of all the fragments. Use is made of this in court cases to positively identify murderers and rapists and to prove paternity. It also allows palaeontologists to map the evolutionary tree of extinct species.



DNA profiles of a rape victim, a specimen of semen found on her, and three different suspects. Which one was the rapist?

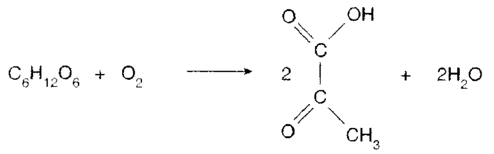
Respiration

AEROBIC AND ANAEROBIC RESPIRATION

When glucose burns in a plentiful supply of oxygen it forms carbon dioxide and water and releases energy.

$$C_6H_{12}O_6(s) + 6O_2(g) \longrightarrow 6CO_2(g) + 6H_2O\,(l) \quad \Delta H^{\ominus} = -2816\ \text{kJ mol}^{-1}$$

In the body one mole of glucose is broken down in a process known as glycolysis to two moles of pyruvate (2-oxopropanoate). Glycolysis produces energy by storing it in molecules of adenosine triphosphate, ATP. The pyruvate may then break down aerobically or anaerobically. In the aerobic decomposition glucose is oxidised by molecular oxygen (which is itself reduced) to form carbon dioxide and water and release energy. It can be summarized by the following two reactions:



pyruvic acid
(pyruvate is the anion of the acid)

then

$$2\,C_3H_4O_3 + 5O_2 \longrightarrow 6\,CO_2 + 4H_2O$$

overall

$$2\,C_6H_{12}O_6 + 6O_2 \longrightarrow 6\,CO_2 + 6H_2O$$

Theoretically one mole of glucose can produce thirty six moles of ATP by aerobic decomposition. During anaerobic decomposition, which occurs in the absence of oxygen, only two moles of ATP are produced and the product formed in humans is lactic acid (2-hydroxypropanoic acid), $CH_3CH(OH)COOH$. However ATP is created more quickly during anaerobic decomposition so during short bursts of strenuous activity, muscle cells may also use anaerobic respiration which accounts for the build up of lactic acid in the muscles. In yeast the pyruvate is decomposed not to lactic acid but to ethanol and carbon dioxide. This is the basis of fermentation.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

ELECTRON TRANSPORT

Food does not burn in the body to produce energy, instead it is oxidized by a series of redox reactions involving the transport of electrons. The reactions take place in the mitochondria found inside cells. The oxidizing enzymes are called cytochromes. Cytochromes contain copper or iron. The metal ion in cytochrome oxidase is surrounded by a porphyrin ligand. This contains four nitrogen atoms each of which donates a pair of electrons, so that it occupies four of the sites around the metal ion and is said to be a tetradentate ligand. During each step of the the overall oxidation of glucose the Fe^{3+} ion is reduced to Fe^{2+} (or the Cu^{2+} ion is reduced to Cu^{+}). In the reduction stage when oxygen is reduced to water the Fe^{2+} ion is oxidized back to Fe^{3+} (or Cu^{+} is oxidized to Cu^{2+}).

Oxidation step:

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

$$Fe^{3+} + e \longrightarrow Fe^{2+} \text{ (or } Cu^{2+} + e \rightarrow Cu^+\text{)}$$

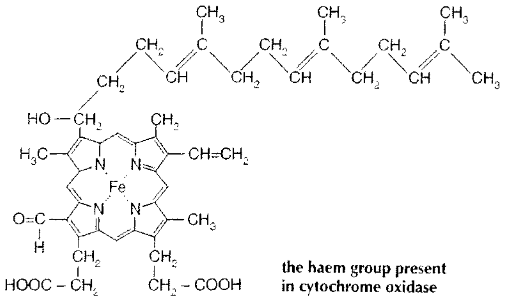
metal ion reduced

Reduction step:

$$O_2 + 4H^+ + 4e \longrightarrow 2H_2O$$

$$Fe^{2+} \longrightarrow Fe^{3+} + e \text{ (or } Cu^+ \rightarrow Cu^{2+} + e\text{)}$$

metal ion oxidized

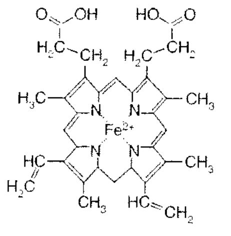


the haem group present in cytochrome oxidase

HAEMOGLOBIN AS AN OXYGEN CARRIER

The ability of iron to form complexes is also important in haemoglobin. Haemoglobin in the blood is responsible for carrying oxygen around the body during respiration. Haemoglobin contains four large polypeptide groups, and four iron ions each surrounded by flat porphyrin ligands known as haem groups. Haem is a prosthetic group, a group essential for the protein to be able to carry out its function.

At high oxygen concentrations haemoglobin binds to oxygen molecules. In the process the oxygen bonds onto the iron in the haem group as an extra ligand. At low concentrations the reverse process occurs. Carbon monoxide and cyanide ions are poisonous as they form irreversible complex ions with the iron preventing it from carrying oxygen.



one of four haem prosthetic groups present in haemoglobin