Biological Molecules

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

Group name	Elements	Monomers	Polymers	% dry mass of a cell
Carbohydrates	CHO	monosaccharides	polysaccharides	15
Lipids	CHOP	fatty acids + glycerol*	triglycerides	10
Proteins	CHONS	amino acids	polypeptides	50
Nucleic acids	CHONP	nucleotides	polynucleotides	18

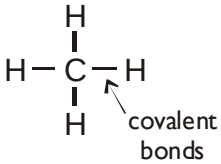
* These are not monomers, but rather the components of triglycerides.

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

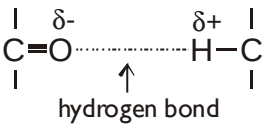
Chemical Bonds

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

Covalent bonds are strong. They hold together all the organic molecules in living organisms. Because they are strong, covalent bonds cannot be broken or made at the temperatures found in living cells. So in biology covalent bonds are always made or broken by the action of enzymes. Covalent bonds are represented by solid lines in chemical structures.



Hydrogen bonds are much weaker. They are formed between an atom (usually hydrogen) with a slight positive charge (denoted δ+) and an atom (usually oxygen or nitrogen) with a slight negative charge (denoted δ–). Because hydrogen bonds are weak they can be easily made and broken inside cells without needing enzymes. Hydrogen bonds are represented by dotted lines in chemical structures.



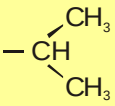
The Twenty Amino Acid R-Groups

Simple R groups

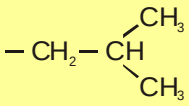
Glycine
Gly G

Alanine
Ala A

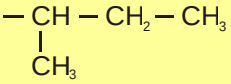
Valine
Val V



Leucine
Leu L

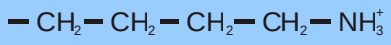


Isoleucine
Ile I

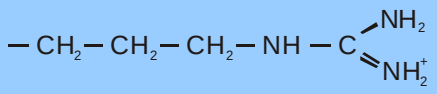


Basic R groups

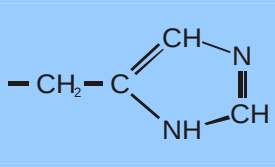
Lysine
Lys K



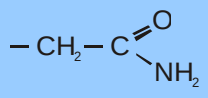
Arginine
Arg R



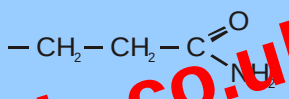
Histidine
His H



Asparagine
Asn N

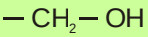


Glutamine
Gln Q

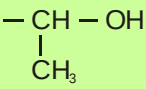


Hydroxyl R groups

Serine
Ser S

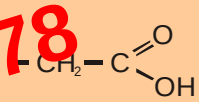


Threonine
Thr T

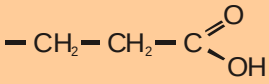


Acidic R groups

Aspartate
Asp D



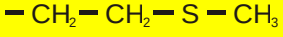
Glutamate
Glu E



Sulphur R groups

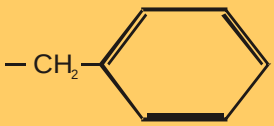
Cysteine
Cys C

Methionine
Met M

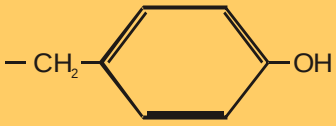


Ringed R groups

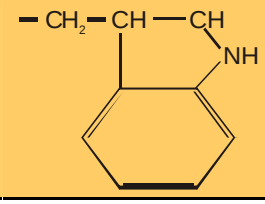
Phenylalanine
Phe F



Tyrosine
Tyr Y

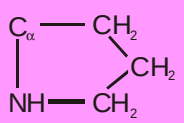


Tryptophan
Trp W



Cyclic R group

Proline
Pro P

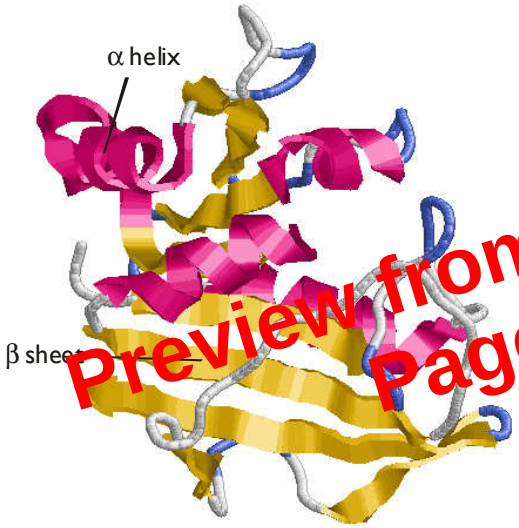


These four structures are not real stages in the formation of a protein, but are simply a convenient classification that scientists invented to help them to understand proteins. In fact proteins fold into all these structures at the same time, as they are synthesised.

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

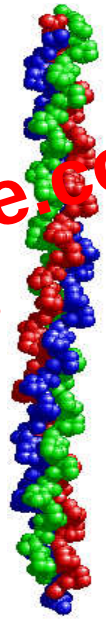
Globular Proteins

The vast majority of proteins are globular, i.e. they have a compact, ball-shaped structure. This group includes enzymes, membrane proteins, receptors and storage proteins. The diagram below shows a typical globular enzyme molecule. It has been drawn to highlight the different secondary structures.



Fibrous (or Filamentous) Proteins

Fibrous proteins are long and thin, like ropes. They tend to have structural roles, such as collagen (bone), keratin (hair), tubulin (cytoskeleton) and actin (muscle). They are always composed of many polypeptide chains. This diagram shows part of a molecule of collagen, which is found in bone and cartilage.



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

Protein Denaturing

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

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

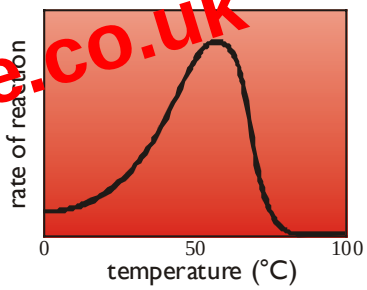
For example for the breakdown of hydrogen peroxide ($2H_2O_2 \rightleftharpoons 2H_2O + O_2$):

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

Factors that Affect the Rate of Enzyme Reactions

1. Temperature

All chemical reactions get faster as the temperature increases, but with enzyme reactions this is only true up to a certain temperature, above which the rate slows down again. This optimum temperature is about 40°C for mammalian enzymes but there are enzymes that work best at very different temperatures, e.g. enzymes from the arctic snow flea work at 4°C, and enzymes from thermophilic bacteria work at 90°C.



Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line). The rate increases because the enzyme and substrate molecules both have more kinetic energy so collide more often, and also because more molecules have sufficient energy to overcome the (greatly reduced) activation energy. The rate is not zero at 0°C, so enzymes still work in the fridge (and food still goes off), but they work slowly. Enzymes can even work in ice, though the rate is extremely slow due to the very slow diffusion of enzyme and substrate molecules through the ice lattice.

This increase in rate with temperature would continue indefinitely except that the enzyme molecule itself is affected by temperature. Above about 40°C there is enough thermal energy to break the weak hydrogen bonds holding the secondary, tertiary and quaternary structures of

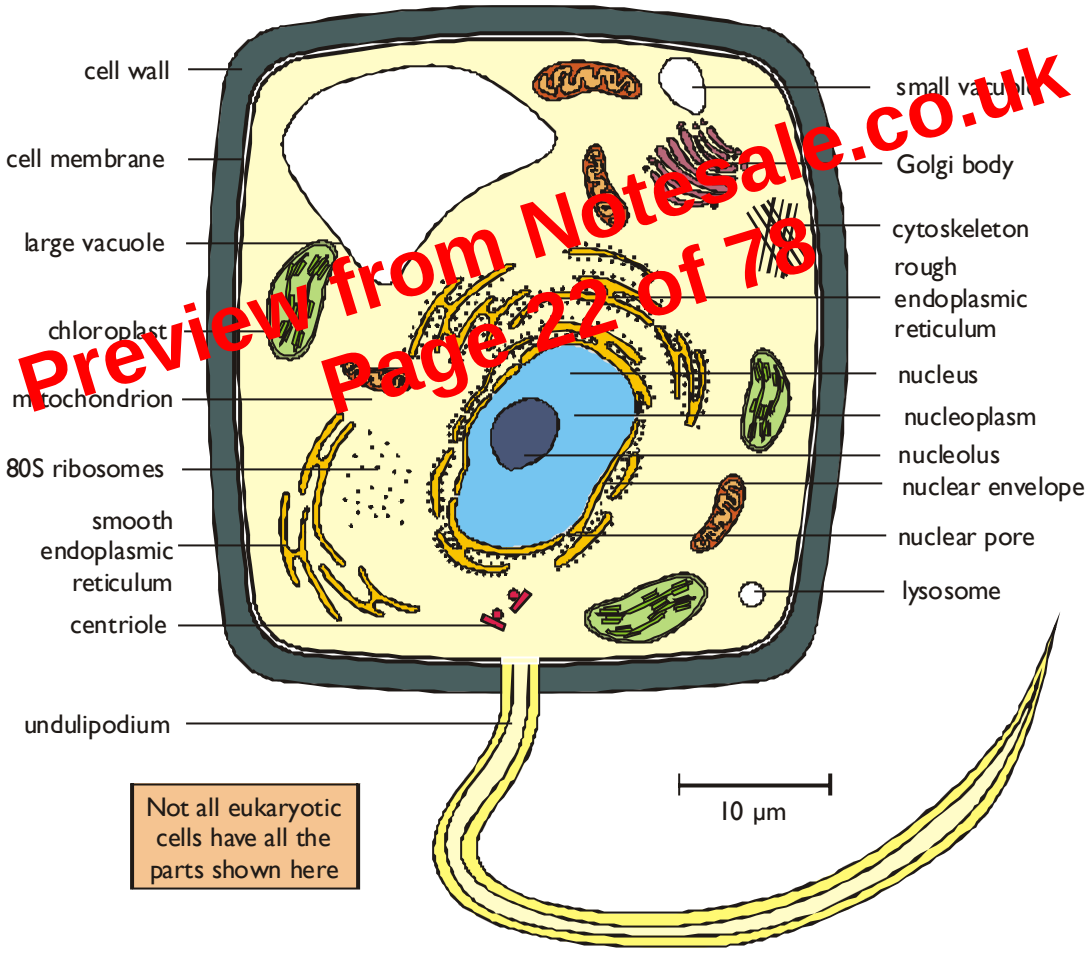
Cells

All living things are made of cells, and cells are the smallest units that can be alive. There are thousands of different kinds of cell, but the biggest division is between the cells of the prokaryote kingdom (the bacteria) and those of the other four kingdoms (animals, plants, fungi and protoctista), which are all eukaryotic cells. Prokaryotic cells are smaller and simpler than eukaryotic cells, and do not have a nucleus.

- Prokaryote = without a nucleus (think "before carrier bag")
- Eukaryote = with a nucleus (think "good carrier bag")

We'll examine these two kinds of cell in detail, based on structures seen in electron micrographs. These show the individual organelles inside a cell.

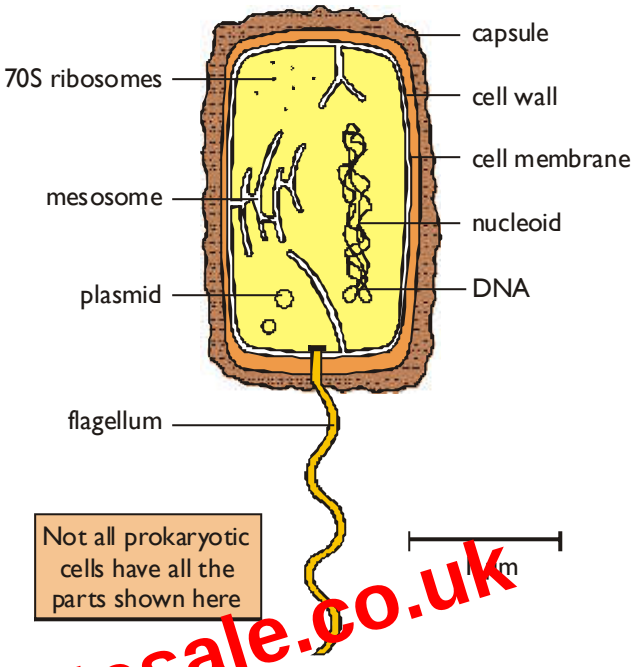
Euakryotic Cells



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

Prokaryotic Cells

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



- **Cytoplasm**. Contains all the enzymes needed for all metabolic reactions, since there are no organelles
- **Ribosomes**. The smaller (70S) type, all free in the cytoplasm and never attached to membranes. Used for protein synthesis.
- **Nuclear Zone** (or **Nucleoid**). The region of the cytoplasm that contains DNA. It is not surrounded by a nuclear membrane.
- **DNA**. Always circular (i.e. a closed loop), and not associated with any proteins to form chromatin. Sometimes confusingly referred to as the bacterial chromosome.
- **Plasmid**. Small circles of DNA separate from the main DNA loop. Used to exchange DNA between bacterial cells, and also very useful for genetic engineering (see unit 4).
- **Plasma membrane**. Made of phospholipids and proteins, like eukaryotic membranes.
- **Cell Wall**. Made of murein (not cellulose), which is a glycoprotein (i.e. a protein/carbohydrate complex, also called peptidoglycan).
- **Capsule**. A thick polysaccharide layer outside of the cell wall. Used for sticking cells together, as a food reserve, as protection against desiccation and chemicals, and as protection against phagocytosis. In some species the capsules of many cells fuse together forming a mass of sticky cells called a biofilm. Dental plaque is an example of a biofilm.
- **Flagellum**. A rigid rotating helical-shaped tail used for propulsion. The motor is embedded in the cell membrane and is driven by a H^+ gradient across the membrane. Anticlockwise rotation drives the cell forwards, while clockwise rotation causes a chaotic spin.

Exchange

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

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

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

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

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

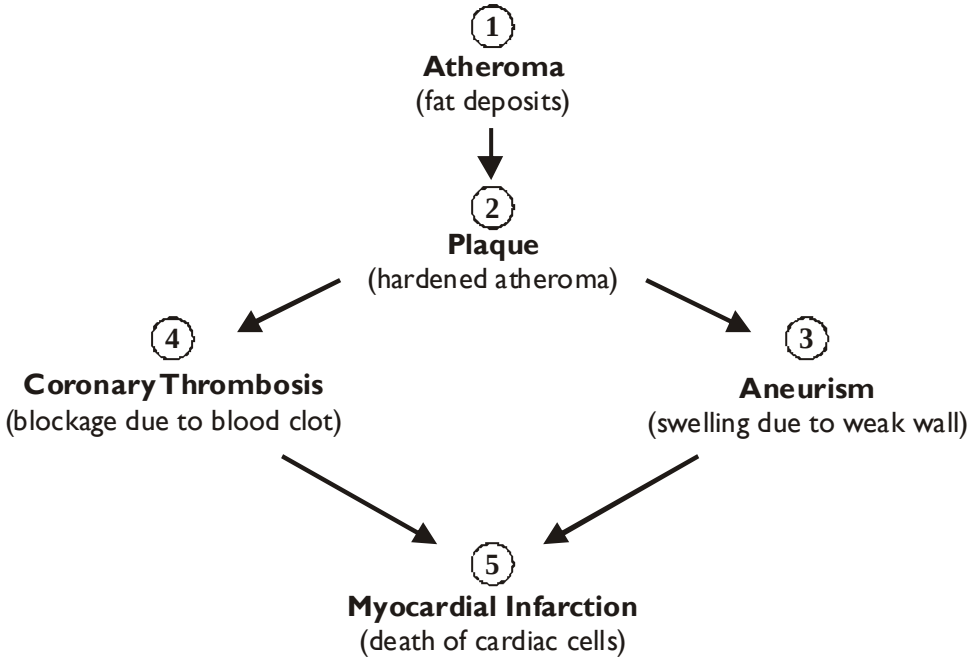
system	large surface area	small distance	high concentration gradient
Human small intestine	7m long, folds, villi and microvilli give surface area of 2000m²	blood capillaries close to surface of villus	stirred by peristalsis and by microvilli
Human circulatory system	100m of capillaries with a surface area of 1000m²	capillary walls are only one cell thick	constant blood flow replenishes the blood
Human lungs	600 million alveoli with a total area of 100m²	each alveolus is only one cell thick	constant ventilation replaces the air

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

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

Some pathogens are more harmful than others; in other words they are have a greater pathogenicity or virulence. For a pathogen to cause a disease these steps must take place:

1. The pathogen must be transmitted to the human host. Pathogens can be transmitted through drinking water, eating food, breathing aerosol droplets, animal bites, or direct contact.
2. The pathogen must gain entry inside the human body. The human body is protected by a tough layer of skin, but pathogens can enter via cuts in the skin (e.g. rabies, malaria); or the thinner interfaces, such as the digestive system (e.g. cholera, typhoid); gas exchange system (e.g. influenza, tuberculosis) or reproductive system (e.g. AIDS).
3. The pathogen must evade the defences of the host. Humans have a range of defences, such as stomach acid, lysozyme enzymes and the immune system, and these defences are usually very effective at preventing disease. But it only takes a few pathogen cells resisting the defences to multiply and cause a disease.
4. The pathogen must harm the host. Pathogens harm their hosts in two ways. First, by reproducing inside host cells, using up cellular resources and preventing the cell from carrying out its normal reactions. The microbes then usually burst out of the host cell, rupturing the cell membrane and killing the cell in the process. Second, by producing toxins – chemicals that interfere with the body's reactions. These chemicals may inhibit enzymes, bind to receptors, bind to DNA causing mutations, interfere with synapses and so on.



Risk Factors for Coronary Heart Disease

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

- **Blood Cholesterol**. Cholesterol in the blood comes from the diet and from the liver, where it is synthesised. Cholesterol is carried in large complexes with proteins, called lipoproteins. High-density lipoproteins (HDLs) remove cholesterol from tissues, so decrease the risk of atheromas, while Low-density lipoproteins (LDLs) deliver cholesterol to tissues, so increase the risk of atheroma.
- **Blood Pressure**. High blood pressure increases the risk of an aneurism and stimulates thickening of artery wall, increasing the risk of thrombosis. Stress, diet and lack of exercise can all increase blood pressure.
- **Genetics**. Both blood pressure and fat metabolism are affected by genes, so genes undoubtedly affect the chance of a coronary thrombosis. This doesn't mean that, for some people, a heart attack is inevitable; it just means some people have to be even more careful about their lifestyle risk factors.
- **Diet**. High levels of saturated fat increase the amount of cholesterol carried in the blood and so increase the risk of atherosclerosis. High levels of salt increase blood pressure and so increase the risk of aneurism. However, fibre and vitamin C reduce the risk of heart disease.
- **Smoking**. Smokers are between two and six times more likely to suffer from coronary heart disease than non-smokers. The carbon monoxide and nicotine in cigarette smoke both cause an increase in blood pressure.

The Three Lines of Defence

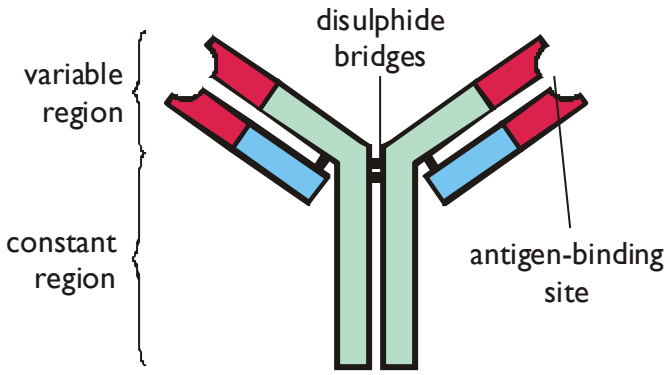
Humans have three lines of defence against invading pathogens:

1. Barriers – the skin and associated chemicals stop microbes entering the body
2. The non-specific immune system – phagocytes quickly destroy microbes that pass the first line of defence
3. The specific immune system – lymphocytes kill any microbes that pass the second line of defence, and remain on guard for future attacks.

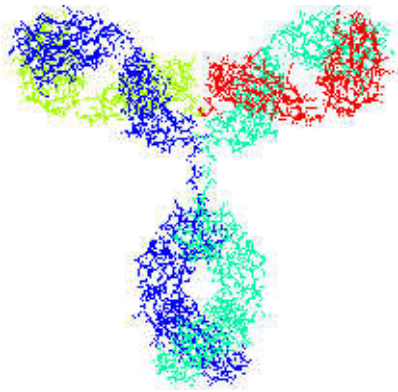
The First Line of Defence – Barriers

The body has many mechanism to try to stop microbes entering the body, particularly the bloodstream.

- The skin is a tough, impenetrable barrier (which is why we use it to make leather shoes). The outer layer, the epidermis, is 20-30 cells thick (about as thick as a sheet of paper) and its cells are toughened by the protein keratin.
- Sweat and tears, secreted by glands in the skin, contain lysozyme enzymes, which destroy (lyse) bacteria growing on the surface of the skin by digesting their peptidoglycan cell walls.
- The digestive tract is a potential entry route for pathogens, but it is protected by concentrated acid in the stomach, which denatures microbial enzymes and cell surface proteins, as well as protease enzymes. Saliva also contains lysozymes.
- The respiratory tract is another potential entry route, but it is protected by sticky mucus secreted by glands in the bronchi and bronchioles, which traps microbes and other particles in inhaled air before they can reach the delicate alveoli. Mucus contains lysozymes, and cilia constantly sweep the mucus upwards to the throat, where it is swallowed so that the microbes are killed by the stomach acid.
- The human body is home to billions of bacterial cells called variously the natural microbiota, the normal flora, the commensal flora (because they have a non-harmful or commensal relationship with their host) or even the "friendly bacteria". There are in fact twenty times more bacteria cells in a human than there are human cells! These commensal bacteria colonise the skin, mouth, lower digestive tract, respiratory tract and vagina, and they help prevent infection by out-competing pathogenic microbes for food and space.

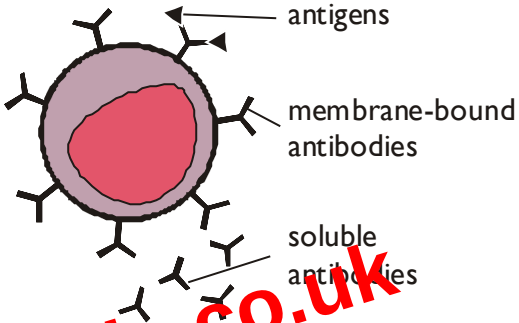


stylised diagram

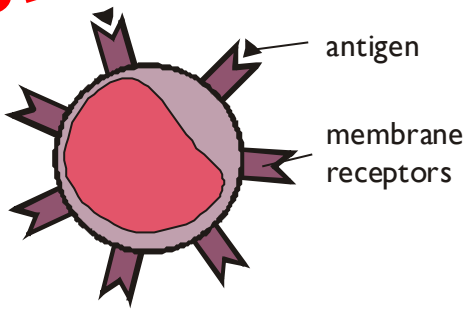


Rasmol molecular model

Each B-cell has around 10^5 membrane-bound antibody molecules on its surface and can also secrete soluble antibodies into its surroundings. Every human has around 10^8 different types of B cell, each making antibodies with slightly different variable regions. Between them, these antibodies can therefore bind specifically to 10^8 different antigens, so there will be an antibody to match almost every conceivable antigen that might enter the body.

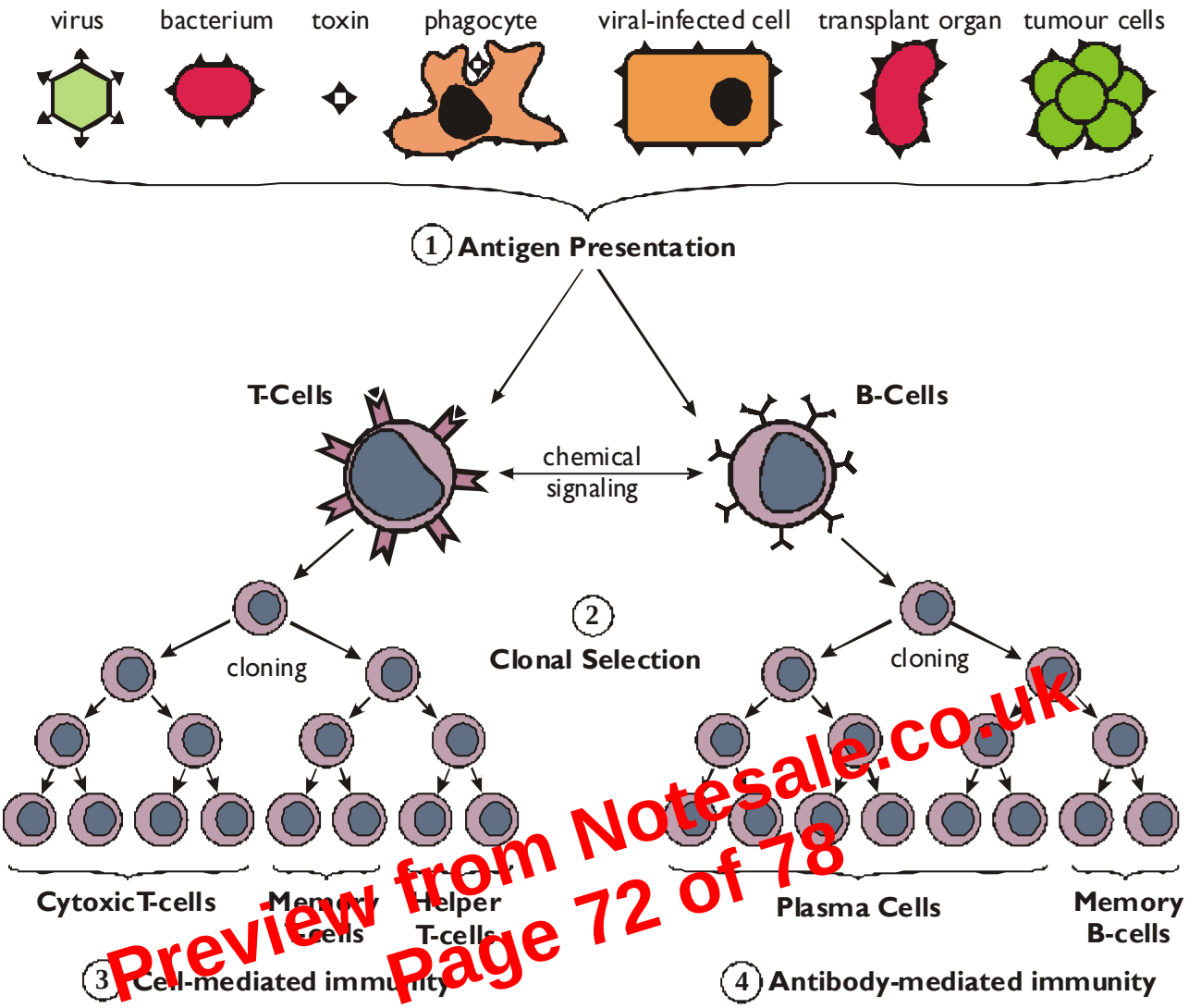


T-cells have receptor molecules on their surfaces which are very similar, but not identical, to antibodies. These receptors also bind specifically to antigens to form antigen-receptor complexes. Each T-cell has around 10^5 receptor proteins, and again there are about 10^8 different types of T-cell, each with slightly different receptor molecules, so they can also specifically bind to any conceivable antigen. T-cells do not secrete soluble proteins.



The B and T cells are exposed to so many "self" antigens on every normal cell they come across, that they quickly "learn" to recognise them very early in life. From then on self antigens are ignored, but any non-self antigens are recognised and stimulate an immune response as described below.

The actions of the specific immune system are summarised in this diagram:



1. Antigen Presentation

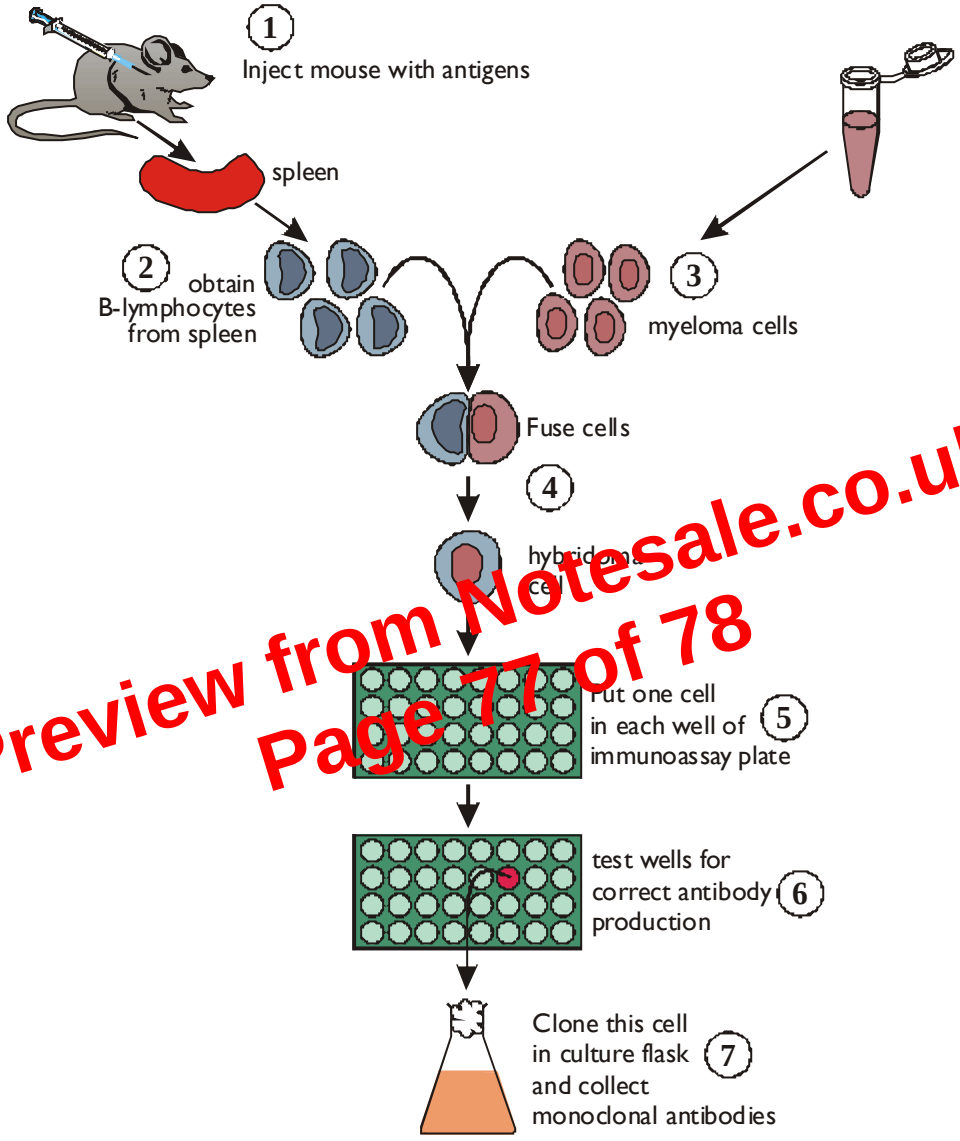
Infection is started when cells with non-self antigens enter the blood or tissue fluid. The antigens can be from a variety of sources:

- a virus capsid protein or envelope protein
- on the surface of a bacterial cell
- a toxin released from a bacterium
- on a phagocyte that has ingested a pathogen
- on a cell infected with a virus so that it has viral proteins on its surface
- on the surface of cells of a transplant
- on a cancerous cell

Macrophages are the most important antigen-presenting cells because they are the most numerous. They constantly inspect the surface of every cell they come into contact with in the blood, spleen, lymph nodes, tissue fluid and alveolar spaces. If the antigens are not recognised as self antigens, then the macrophage ingests the antigen and its cell by phagocytosis. Some of the antigens

Monoclonal Antibodies

Scientists quickly realised that the remarkable specific binding property of antibody proteins *in vivo* would make them very useful tools in medicine and research *in vitro*. [*In vivo* means "in life", i.e. in a living organism; and *in vitro* means "in glass", i.e. in a test tube.] For example antigens could be used as a "magic bullet" to target drugs at one specific cell type in the body, or antibodies could be used to detect the presence of specific proteins in very low concentrations. So in 1975 Kohler and Milstein found a way to make pure monoclonal antibody proteins in the lab. This is their technique:



1. Inject a mouse with the antigen proteins that you want antibodies for. These antigens could be from a human cancer cell or a particular protein. The mouse will show a primary immune response and make a clone army of B lymphocytes with antibodies specific for that antigen.
2. After a few days, kill the mouse and extract B lymphocyte cells from its spleen. Although these B cells will make antibodies, there are two problems: B lymphocyte cells won't grow *in vitro*; and the spleen extract contains a mixture of thousands of different B cells, each making their own specific antibodies.