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BIOCHEMICAL BASIS OF DISEASE

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Biochemical Basis of Disease

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The Biochemistry Across the School Curriculum Group (BASC) was set up by the Biochemical Society in 1985. Its membership includes education professionals as well as Society members with an interest in school science education. Its first task has been to produce this series of booklets, designed to help teachers of syllabuses which have a high biochemical content.

Other topics covered by this series include: *Essential Chemistry for Biochemistry; The Structure and Function of Nucleic Acids; Enzymes and their Role in Biotechnology; Metabolism; Immunology; Photosynthesis; Recombinant DNA Technology;* and *Biological Membranes.*

More information on the work of BASC and these booklets is available from the Education Officer at the Biochemical Society, 59 Portland Place, London W1N 3AJ.

Comments on the content of this booklet will be welcomed by the Series Editor Mrs D. Gull at the above address.

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Preface

These days, students of Biology and Human Biology at A' level are expected to have a good appreciation of various disease processes. This can provide an area of considerable interest, as advances in medical knowledge regularly make the headlines, and illness and the requirements for good health are a familiar and everyday topic of debate. Yet the complexities that surround many disease states are not always fully addressed from solely a physiological or pathological account. A more complete understanding can often be achieved once the details of associated biochemical events become apparent. Thus the detection of a molecular defect, such as a malfunctioning or damaged protein, will begin to explain not just the clinical manifestations of a particular disease, as seen in affected individuals, but may also help with the design of a rational and effective treatment.

The aim of this booklet is, therefore, to assist A' level teachers by providing a biochemical perspective on human health and disease. Its content has been chosen to reflect that of modules designed by the various examination boards to cover human health, nutrition, disease and physiological processes. The present account draws on concepts which have been the subject of previous BASC booklets: *Essential Chemistry*, *Metabolism* and *Immunology*. Unfortunately, it has not been possible to cover all aspects of disease, and it is therefore anticipated that topics such as the Genetic Basis of Disease will be the subject of a future volume. Nonetheless, it is hoped that the text will provide a useful resource to assist with the teaching of human health and disease to sixth form and first year college students.
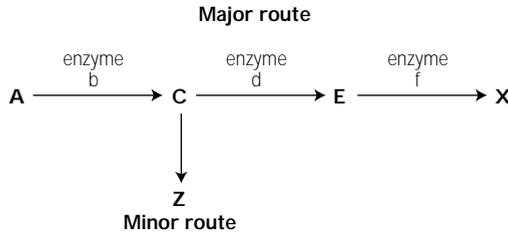
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The Metabolic Basis of Disease

Enzymes: a role in defining disease

The elucidation of basic metabolic pathways in living organisms has been a prerequisite to understanding the nature of many diseases. Details of the way in which metabolic fuels derived from carbohydrates, fats and proteins, are oxidized and linked to the synthesis and subsequent use of ATP, may at first glance seem theoretical and somewhat remote. However, studies on intermediary metabolism have opened up new ways to analyse and characterize many diseases, as well as providing an insight into the mechanisms involved when cells function abnormally. It is now possible to explain a variety of clinical and pathological situations in terms of problems associated either with specific enzymes, or with the factors that control and integrate enzyme activity in human tissues and organs.

An important link between metabolism and disease was made in 1908 by Archibald Garrod. He correctly appreciated that diseases can be caused by the genetically determined absence or modification of specific enzymes. This condition was described as an **inborn error of metabolism**. While Garrod himself reported on only four cases, there are now known to be many hundreds of such conditions. Although individually these hereditary defects of metabolism are rare, they are very important and can have disastrous consequences for the young children who are affected. Examples of such disorders include **phenylketonuria**, which can affect one in every 10 000 human beings, **cystic fibrosis**, which has an incidence of one in 2 500 live births, and **galactosaemia**, occurring in about one in 40 000 live births.

Major route

$$A \xrightarrow[b]{enzyme} C \xrightarrow[d]{enzyme} E \xrightarrow[f]{enzyme} X$$

Scheme **1**.

(with C branching down to Z via the **Minor route**)

Diagnosis of an inherited metabolic disorder can involve measuring the activity of a given enzyme in a sample taken from an accessible tissue, such as blood cells. When enzyme defects are associated with internal organs, such as the liver, the presence of the disease may more easily be inferred, by measuring levels of accumulated substrates of the enzyme or other metabolites, in various body fluids.

In the metabolic route shown in Scheme 1, product X is synthesized from substrate A by a series of enzyme-catalysed reactions. In addition, A can be metabolized to Z by a minor route, so that normally only a small amount of Z is made. If a patient lacks enzyme d, the following consequences may be observed.

- Reduced formation of E and X. If either or both are essential cell products, with no alternative route for their synthesis, a deficiency condition may result.
- Accumulation of C and possibly A. If these substances are toxic, cell damage may result.
- Increased levels of Z. The production of this molecule is now enhanced and it may be toxic.

The increased amounts of C and Z may accumulate in the blood and, subsequently, can be excreted in the urine.

An example of a metabolic disorder that involves a situation like this is **phenylketonuria**, which results from the inability to convert the amino acid phenylalanine (C) into tyrosine (E). This is due to the lack of **phenylalanine hydroxylase** (enzyme d) activity, which is normally present in the liver. Diagnosis can be achieved by carrying out simple clinical tests. High levels of phenylalanine are detected in the bloodstream (0.6 mM; compared with normal values in the order of 0.06 mM), and metabolites of phenylalanine, such as phenylpyruvic acid (Z), are excreted in the urine. Tyrosine, which is not normally an essential amino acid for growth, now becomes required in the diet. It is thought that the high circulating levels of phenylpyruvate and other metabolites inhibit

brain development by interfering with myelin synthesis. This results in mental retardation unless the condition is treated. Phenylketonuria has become a public health matter in most countries, and in the UK all babies are screened for the disease at birth.

The defective protein associated with an inherited disease may not always be an enzyme. The gene product could, for example, function as a **receptor molecule** or a **transport channel** in the cell membrane. An example of this is seen in cystic fibrosis, a common genetic disease among Caucasians in Northern America and Europe. It is characterized by chronic pulmonary difficulties, and patients can die as a result of the airways becoming progressively blocked by a thick mucus secretion. Mucus can also form plugs in the pancreatic ducts preventing enzymes from being secreted in adequate amounts to digest food and facilitate absorption. Cystic fibrosis is diagnosed by excessively salty sweat. Recent evidence suggests that the defective gene is associated with a **chloride ion channel** in epithelial cells, which results in abnormal electrolyte reabsorption.

Knowledge of the enzymes of intermediary metabolism has also provided techniques for the clinical diagnosis of many diseases. Most enzymes within cells are components of metabolic routes and are often associated with a particular subcellular compartment, such as mitochondria or lysosomes. Thus the normal concentration of most enzymes within the blood is very low. However, when cells are damaged or disintegrate, enzymes can be released, and their appearance in serum can be a sign of cell injury and death.

During a **heart attack** (**myocardial infarction**), part of the cardiac muscle is damaged when its blood supply is obstructed by a clot. Specific forms of tissue enzymes, such as **creatine kinase**, **aspartate aminotransferase** and **lactate dehydrogenase**, appear in the blood. A similar situation occurs when liver cells are damaged by infection or toxic agents. The presence in the plasma of **aminotransferase** and **phosphatase** enzymes are indicative of various types of liver disease. The appearance in the plasma of certain enzymes of the **ornithine cycle** (normally associated with mitochondria) can indicate that damage to the mitochondrion has taken place.

Clinical science has derived many other important benefits from studies on intermediary metabolism. One of these is an understanding of the mechanisms by which drugs and other chemotherapeutic agents achieve their effects, and are subsequently removed from the body. The

process of **drug detoxification** can be seen as a protective mechanism. It involves a number of enzymes that transform pharmacologically active compounds into more water-soluble and readily excretable substances. A prime example of this is **cytochrome *P*-450**. This is the name given to a group of structurally related, haem-containing enzymes principally located in the endoplasmic reticulum membranes of the liver. These enzymes can introduce a hydroxyl group into a large number of drugs, steroid hormones, carcinogens and environmental chemicals. This means that there are thousands of potential substrates for cytochrome *P*-450. It is now recognized that many poisons do not act directly but undergo metabolism first to cause cell damage and death. This enzyme-mediated process is known as **lethal synthesis** or **bioactivation**. Thus **benzo(a)pyrene**, a pollutant of air, water and soil that results from the incomplete combustion of organic material, is metabolized by cytochrome *P*-450 to give reactive chemicals that can attack DNA and RNA, and induce cancer.

These ideas will be developed further by considering the effect of a number of different types of disease on cell and tissue metabolism. As metabolism is the subject of a previous review, readers are recommended to consult *BASC Guidance Notes for Advanced Biology No. 4* to familiarize themselves with the basic principles of the subject.

Cholera: a usurper of intestinal metabolism

Cholera is an infectious disease, contracted by drinking water containing the bacterium *Vibrio cholerae*. The vibrios grow in the gut, but do not spread to other body parts. Within hours, patients become ill with diarrhoea and there is a profuse outpouring of thin, watery fluid at rates of up to 20 litres per day. This massive flow of water from the body into the gut drains the tissues and dehydrates them, the blood becomes thick and viscous and the victim can die within days.

This frightening disease was originally confined to Asia until the 19th century, when it spread to Europe, the USA and parts of South America. Although today the disease can easily be treated and is normally unlikely to spread, cholera will develop rapidly where sanitation is poor and drinking water is contaminated. Although cholera is only one of a number of diarrhoeal diseases, it does cause the most severe loss of fluid, and is responsible for much illness and death in areas where it is endemic, such as India and Pakistan.

At one time it was thought the bacterium caused considerable anatomical damage to intestinal epithelium. This is apparently not the case. The vibrios colonize and adhere to the small intestine, and then secrete a protein exotoxin called **choleragen**. This alters the metabolism of the mucosal cells. Choleragen's action is similar to that of a hormone, and the toxin will affect the metabolism of other tissues if it can gain access to them. In fat cells it will stimulate lipolysis, and in liver cells glycogenolysis; however, in clinical cholera only the intestine is affected.

The toxin molecule is made in two parts, known as subunits A and B. Subunit B binds strongly to a specific, complex lipid receptor in the cell membrane. Subunit A, which is responsible for cell toxicity, then gains entry to the cell. Once inside, it can work without subunit B and activates an enzyme called **adenyl cyclase**, which is attached to the inner face of the plasma membrane. Adenyl cyclase influences the regulation of cell metabolism and hormones such as **glucagon** and **adrenaline** have their actions mediated by it. When activated, the enzyme catalyses the production of **cyclic AMP (cAMP)** from ATP. cAMP is a small molecule whose concentration within the cell has a marked effect on the rate of many metabolic processes. In the intestinal epithelium, stimulation of adenyl cyclase results in the active secretion of chloride ions from the mucosa and a passive efflux of water, causing diarrhoea. This is outlined in Figure 1.
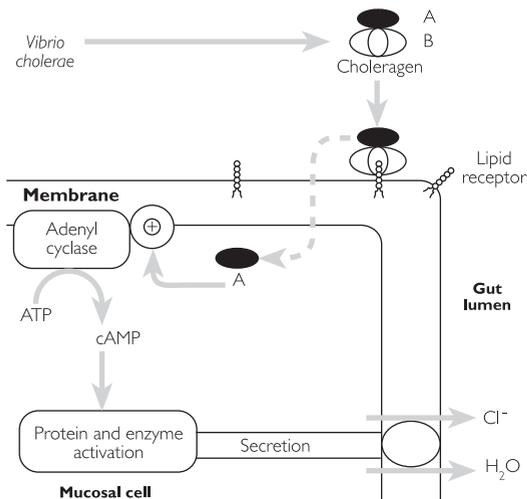
**Figure 1**. The effect of cholera toxin on the intestinal mucosa

Treatment for cholera is relatively simple. The fluid loss can be overcome by an intravenous infusion of water and salts, while the administration of an antibiotic such as tetracycline removes the infection. Alternative approaches to improve the prevention and treatment of the disease are being considered, including an oral cholera vaccine based on subunit B of the toxin molecule.

Diabetes mellitus: a catabolic disease

A major characteristic of diabetes mellitus is the inability to control blood sugar levels. High blood glucose concentrations (**hyper-glycaemia**) are associated with the disease, and result from an increased production of glucose by the liver and a reduced uptake by muscle and adipose tissue. In the kidneys, filtered glucose is normally completely reabsorbed, but when blood glucose levels of 10 mM are exceeded, reabsorption becomes saturated and glucose appears in the urine. This osmotically enhances water excretion and stimulates thirst, giving the classical symptoms of the disease.

A number of tests can be used to diagnose diabetes, the most common being the measurement of blood sugar two hours after a carbohydrate meal. In non-diabetics, the blood glucose will have returned to normal levels, whereas in the diabetic it will remain high (above 11 mM. In healthy people, blood glucose concentrations are regulated over a range of 2.5–8 mM, regardless of the nutritive state. This is achieved through the action of hormones, the most important being **insulin** and glucagon. Both are polypeptide hormones made in the pancreatic **islets of Langerhans**. However while insulin is produced by β-**cells** in response to a rise in blood sugar, glucagon secretion by α-**cells** is reduced. The role of these two hormones in glucostasis is illustrated in Figure 2.

Glucagon causes a rapid increase in blood glucose concentrations by its action on the liver, stimulating glycogen breakdown and enhancing gluconeogenesis from protein. Glucose promotes the secretion of insulin by the β-cell provided that glucagon is present. Insulin, in turn, promotes the removal of glucose from the blood and its storage as glycogen. It will also inhibit the effects of glucagon on hepatic glucose production and, in the presence of glucose, insulin inhibits the secretion of glucagon by the cell. Thus, through a balance of these two hormones,
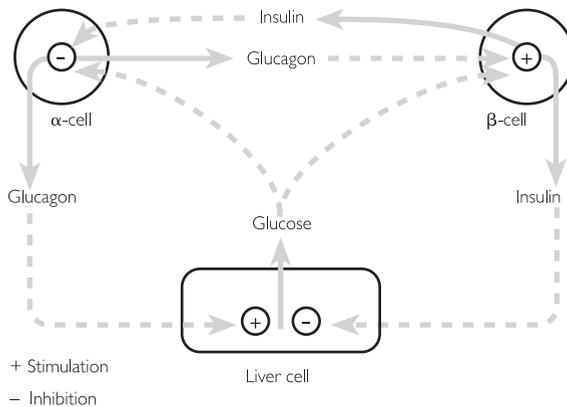
Figure 2. Control of blood glucose levels by insulin and glucagon

whose actions generally oppose each other, blood glucose concentrations can be kept within a narrow range.

Although insulin's action in the control of blood sugar levels has been an important focus in understanding diabetes, disorders of carbohydrate, fat and protein metabolism are characteristic of the disease, with later vascular complications. Diabetes is primarily the result of a relative or absolute deficiency of insulin. Insulin should, however, be considered to be an **anabolic hormone** that functions to coordinate the metabolic processes in a number of body tissues. A lack of insulin will, therefore, lead to enhanced catabolism, a view reported on many centuries ago by the Roman physician Arataeus (81–138 AD) when he wrote:

> *"Diabetes is a wonderful affection not very frequent among men, being a melting down of the flesh and limbs into urine".*

This "rapid melting" reflects the high plasma glucagon levels in untreated diabetes and the loss of coordinated antagonism between the two hormones. The effect of insulin on various metabolic processes is summarized in Table 1.

One of the most prominent features of insulin deficiency is the rapid mobilization of fatty acids from adipose tissue. These are taken up by the liver and converted into ketone bodies. **Pathological ketosis** develops, with large quantities of ketone bodies being produced in excess of the body's requirements. The most severe ketosis occurs in the terminal stages of diabetes, when gluconeogenesis is also greatly

Table 1

Metabolic process	Insulin excess	Insulin deficiency
Anabolic processes		
Lipid and fatty acid synthesis		
Protein synthesis	↑	
Glycogen synthesis		
Glucose uptake into muscle and		↓
adipose tissue		
Catabolic processes		
Lipolysis		↑
Fatty acid levels in plasma		
Ketogenesis from fatty acid oxidation		
Gluconeogenesis from protein	↓	
Glycogenolysis		

increased. This process synthesizes glucose from amino acids to make good the loss through urinary excretion, and to maintain high blood sugar levels so that some glucose is utilized in the absence of insulin.

The excretion by the kidney of large amounts of ketone bodies into the urine (**ketonuria**) not only represents a waste of energy, but can cause a loss of cations, a decrease in blood pH (**acidosis**) and eventually leads to diabetic coma and death.

Diabetes occurs as one of two clinically recognizable forms. One type appears in children and young people and, although the symptoms can develop suddenly and dramatically, it is likely the disease has been progressing slowly over several years. This is **juvenile-onset diabetes (insulin-dependent diabetes mellitus; IDDM)**. The β-cells of the pancreas are largely destroyed so no insulin is produced. It is probable that immunological attack causes progressive β-cell damage, and treatment of the disease involves insulin administration. The other type of diabetes develops symptoms in middle age and is referred to as **maturity-onset diabetes (non-insulin-dependent diabetes mellitus; NIDDM)**. Here, insulin levels are normal, but the cells of the body are less sensitive to the hormone, owing perhaps to receptor malfunction. NIDDM is generally less serious — but more common — than IDDM, and most patients can be treated with diet and drugs, such as sulphony-lureas.

Long-term diabetes brings with it risks of other complications, par-ticularly associated with the vascular system. Thus diabetics are more

prone to suffer from coronary heart disease or a stroke, as well as develop damage to the retina, kidney and nerves. Some of these problems may be a result of the hyperglycaemia; for example, the increased metabolism of glucose to the sugar alcohol sorbitol may be a contributory factor to cataract formation in the lens of the eye. At high concentrations, glucose can also link covalently to many proteins by non-enzymic reactions. Such glycosylation — and subsequent thickening of the basement membrane of capillaries in the kidney glomerulus and retina — gives rise to renal and ocular problems in diabetics.

Alcoholic liver disease: damage by metabolism

Alcohol (ethanol) consumption by man has long been recognized to be harmful. It is a common cause of **cirrhosis** of the liver, where increased formation of fibrous tissue destroys the normal liver lobule structure and seriously disturbs hepatic functions. As a result, patients with cirrhosis have a short life expectancy. While alcohol abuse over a period of 10–20 years can result in cirrhosis, other agents, such as hepatitis B virus infection will also cause the disease. Alcohol not only affects the liver, but can also have serious consequences for the nervous, cardiovascular and endocrine systems, and can damage the developing fetus.

Ethanol is metabolized by the liver in two steps, leading via ethanal (acetaldehyde) to the formation of acetate. The principal enzymes involved are **alcohol dehydrogenase (ADH)**, located in the cytoplasm and **aldehyde dehydrogenase (ALDH)**, predominantly associated with mitochondria. There is a cytochrome P-450 enzyme in the endoplasmic reticulum that also oxidizes ethanol. This is a minor route normally, but during chronic alcohol abuse the membranes of the endoplasmic reticulum proliferate and metabolism by this system is induced. Chronic alcohol abusers develop a metabolic tolerance to alcohol, reflected in an increased ability to oxidize ethanol, and the response of the cytochrome P-450 enzyme may contribute to this. The metabolism of alcohol is summarized in Figure 3.

As lactate dehydrogenase (LDH) is also a cytoplasmic enzyme, its role in catalysing the interconversion of lactate and pyruvate is linked to that of ADH through common NAD^+ coenzymes. Alcohol metabolism is known to inhibit liver gluconeogenesis from lactate and amino acids. This can be explained as a result of alcohol oxidation by the ADH reaction increasing the amount of NADH relative to NAD^+ in the
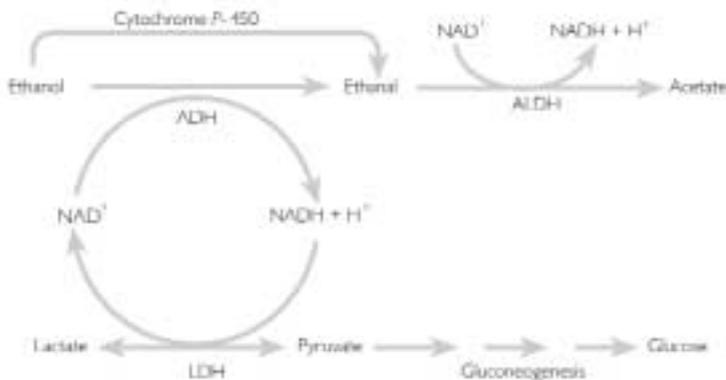
Figure 3. Alcohol metabolism in the liver

cytoplasm. Through the reaction catalysed by LDH, this will reduce the availability of pyruvate, thereby restricting its involvement in gluconeo-genesis.

Hans Krebs estimated that an intake of 200 cm^3 of sherry, equivalent to three small glasses, caused a sufficient increase in blood alcohol levels to inhibit gluconeogenesis. If gluconeogenesis is not effective, hypogly-caemia will result, and this is a major abnormality of carbohydrate metabolism associated with alcoholism.

A further important consequence of ethanol metabolism is the pro-duction of **ethanal**. This is considerably more toxic than alcohol, and its accumulation in the body produces a number of unpleasant and harmful physiological reactions. It is chemically very reactive, forming covalent bonds with a range of cellular molecules, particularly proteins. Ethanal will bind to the protein **tubulin**, which is associated with the micro-tubules of the cytoskeleton. This reduces the liver's ability to secrete proteins such as albumin, and the subsequent retention can result in hepatocyte swelling. Ethanal is also known to impair the activity of certain enzymes and promote membrane damage, so that alterations to the normal structure and functioning of organelles such as mitochondria are observed.

Another conspicuous feature produced by alcohol metabolism is the **deposition of fat** within the liver. This is believed to be, in part, a metabolic consequence of the increased NADH/NAD$^+$ ratio that follows ethanol oxidation. The NADH, through the electron-transport chain, supplies the liver with the means of obtaining plenty of energy (ATP), hence other fuels are not so essential. Thus catabolism of fatty

acids is reduced and lipid biosynthesis promoted, with fat droplets appearing in hepatocytes.

Alcoholic fatty liver is an early complication of alcohol abuse, and occurs quickly after a bout of heavy drinking. The condition is considered benign, since once drinking ceases, it is normally reversible. Continued alcohol consumption leads from fatty liver to **alcoholic hepatitis**, where a number of cells die, and this necrosis causes inflammation. Perhaps only one in 12 alcoholics will develop cirrhosis; however, once established, life expectation is short and 50% will die within two years if drinking if not curtailed. The disease is irreversible and characterized by increased **collagen** synthesis. Instead of a fine meshwork of fibrils that normally contain the liver, broad bands of fibrous tissue form septa (walls) that destroy the hepatic architecture. Nodules of liver tissue develop with hepatocytes, blood vessels and bile ducts not in their normal spatial arrangement. Consequently, blood from the gut is no longer processed by the liver before entering the general circulation.

Treatment of alcoholism is complex, as alcohol consumption can be addictive and the body becomes dependent upon it. Drugs such as **Disulfiram** (Antabuse) have been used as a form of aversion therapy. Disulfiram inhibits ALDH so that many unpleasant symptoms occur if alcohol is ingested. These include nausea and vomiting, and are presumably a consequence of ethanal accumulation. Interestingly, a natural condition occurs among groups of Japanese and Chinese who lack mitochondrial ALDH and for whom alcohol consumption is unpleasant and is accompanied by facial flushing.

Jaundice: a metabolic indicator of disease

Occasionally, patients with a disease such as cirrhosis develop jaundice, where the skin and whites of the eyes become yellowish in colour. This is due to excessive amounts of an insoluble yellow pigment called bilirubin being present in the blood and depositing in the tissues. Jaundice is not a disease, but is an important symptom of a number of underlying diseases — often, but not exclusively, associated with the liver.

Bilirubin is a waste product, derived from the haem group of haemoglobin, when damaged or old red cells are destroyed. In humans about 7 g of haemoglobin are broken down daily, and 250 mg of bilirubin produced. The cells that metabolize haemoglobin and form

bilirubin are called phagocytic macrophages and they occur in most tissues, but are particularly prevalent in the spleen, bone marrow and liver, where they are jointly referred to as the **reticulo-endothelial system (RES)**.

Although the liver is not essential for bilirubin formation, it is responsible for the excretion of the bile pigment. A major function of normal adult liver is the production of bile, a complex secretion containing cholesterol, bile salts and **bilirubin glucuronide**. This is excreted via the bile ducts into the intestine. The fundamental distinction between bilirubin and bilirubin glucuronide is that the former is very insoluble, and must be transported from the RES to the liver in the plasma bound to albumin. Bilirubin glucuronide however is fully water-soluble and can be excreted into the bile. The conversion of insoluble bilirubin into soluble bile pigment is an important metabolic reaction catalysed by a specific **transferase** enzyme in the liver. The process is essential for bilirubin secretion and is known as **glucuronide conjugation**. An outline of bilirubin excretion by the liver is given in Figure 4.

Intestinal bacteria metabolize bilirubin glucuronide into a colourless product called **urobilinogen**. Some of this is reabsorbed by the portal blood, but the majority is converted into the brown faecal pigment urobilin.

Figure 4.   An outline of bilirubin metabolism
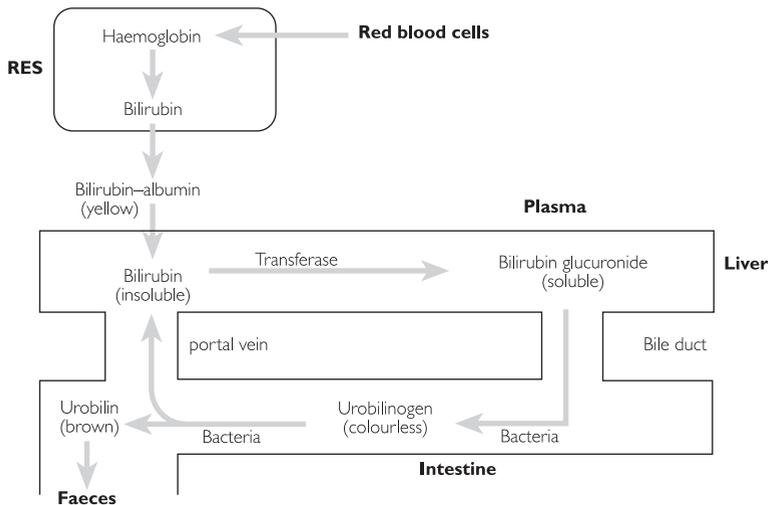
The concentration of bilirubin in the plasma of adults is normally below 0.025 mM, and depends on the individual's balance between production of the pigment and its removal by the liver. If this rises to 0.05 mM or more, jaundice can be detected. A newborn infant may show symptoms of jaundice during the first days of life because the transferase enzyme does not develop until after birth. This will normally correct itself as the activity of the enzyme rises; however, the condition must be monitored, as high levels of bilirubin cause brain damage in the young. There are several inborn errors of metabolism where bilirubin glucuronide synthesis is impaired and this can have dangerous consequences. Other conditions that can result in jaundice include increased red cell destruction associated with **haemolytic anaemia**, liver damage such as cirrhosis, and blockage of the bile ducts by gall stones or a tumour which prevents bilirubin glucuronide from passing into the intestine.

Atherosclerosis: an aberration of lipid metabolism

Atherosclerosis is a disease that leads to the hardening and narrowing of the arteries. If this occurs in the coronary arteries that supply heart muscle or within the brain, a heart attack or stroke may result. It is one of the most widespread causes of death in Western societies, and the condition is exacerbated by stress, high blood pressure, high plasma cholesterol, a poor diet and smoking. Hardening of the arteries develops gradually throughout life, and is characterized by the accumulation of **cholesterol-rich deposits** in the artery wall. White fatty streaks called **atherosclerotic plaques** are seen, which progressively extend into the lumen of the vessel so that blood flow is impaired.

Over recent years cholesterol has attracted a bad press, yet it is a normal component of the human body. It is a lipid molecule that is an essential element of plasma membranes, and it is also the starting point for the synthesis of bile salts by the liver and steroid hormones by tissues such as the adrenal glands, testes and ovaries. Like other lipids, because of its insolubility it has to be transported in the blood as a complex with a protein, called a **lipoprotein**. Lipoproteins can be distinguished according to the type of protein they contain and their density. As their fat content increases, their density decreases. One type of lipoprotein, known as **low-density lipoprotein (LDL)**, carries most of the cholesterol in the human plasma.

The LDL particle is a sphere with a single hydrophobic protein called **apoprotein B** embedded in a non-polar core of cholesterol, which is linked to long-chain fatty acids to form cholesterol esters. For most cells, LDL particles are their major source of cholesterol. They possess special **receptor molecules** on their surface to recognize and bind the apoprotein, allowing the LDL to be internalized by **endocytosis** within the cell and subsequently used. These receptors are displayed on the surface of cells of the liver, where bile salts are made, or of the adrenal cortex, which synthesizes many important hormones.

The thickening of artery walls is associated with deposits of cholesterol which appear to originate from LDL particles that circulate in the blood. Current research suggests that damage to the endothelial cell inner lining of the vessel allows LDL particles and blood platelets to enter the artery wall. The LDL contains fatty acids that have more than one double bond, i.e. **polyunsaturated**, which can be attacked by reactive forms of oxygen. These are known as **free radicals**, and can be generated as part of normal metabolic processes. The LDL becomes oxidized and may give rise to products that are toxic to the cells of the artery wall.

Macrophages attempt to remove the oxidized LDL but are unable to degrade the cholesterol, which accumulates as droplets in the cells, thereby giving them a foamy appearance. Cholesterol-laden **foam cells** give a characteristic appearance to the fatty streaks observed with atherosclerosis. The toxic products can kill the foam cells leaving cholesterol deposits, and the accumulated cholesterol, cells and debris constitute an **atheroma** which can narrow the channel of the artery. LDL involvement in atherosclerosis is summarized in Figure 5.

The rate at which atherosclerosis develops depends in part on the amount of LDL in the blood; this in turn is controlled by the numbers and activity of specific LDL receptors displayed on the surface of liver cells. Should these receptors be defective or absent, hepatocytes cannot remove LDL from the plasma, and affected individuals have a predisposition to early atherosclerosis.

Hypercholesterolaemia is an inborn error of lipid metabolism associated with a defective gene for the LDL receptor. Blood cholesterol levels are raised above normal and heart attacks occur prematurely. The **heterozygous** form, where a person inherits a copy of the mutant gene from one parent only is quite common. It occurs in one in 500 people in most ethnic groups and leads to a two-fold increase in plasma LDL par-
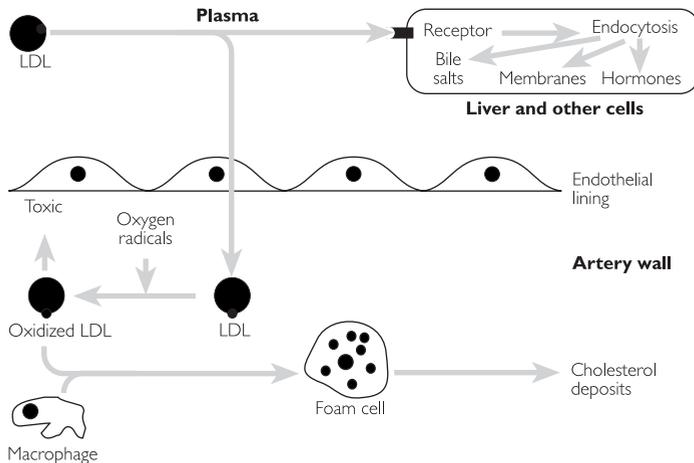
Figure 5. LDL uptake and its role in atherosclerosis

ticles. The more severe, **homozygous** form is rare (affecting one in 10^6 of the population), but the consequence of inheriting two copies of the mutant gene is that blood cholesterol levels are elevated from six to ten times above normal, and heart attacks can start in childhood.

None of these people have any of the risk factors normally associated with the disease. However, there is concern that atherosclerosis is developing at an early age, with young people with no apparent genetic susceptibility showing artery damage as a result of LDL oxidation. Also, there is considerable interest in the recent evidence that phenolic substances in red wine can inhibit LDL oxidation. This has raised the possibility that modest red wine consumption could protect against heart disease and may help explain the observation that in Southern France and other Mediterranean countries, the incidence of coronaries is low.

Free radicals and disease: metabolic attack and defence

Atherosclerosis is not the only human disease in which free radicals are thought to cause cell damage. There are at least 50 conditions in which free radicals have been implicated, either as the causative agent or as a component generated by other factors in the disease process. Some examples are listed below.

- Ageing
- Cancer
- Inflammatory bowel disease

- Kwashiorkor
- Myocardial infarction
- Parkinson's disease
- Rheumatoid arthritis

Free radicals are molecular entities that contain unpaired electrons, and to emphasize this a dot is included in their formula, e.g. A$^{\cdot}$. The unpaired electron makes the molecule a reactive species that can be stabilized by donating or removing electrons from other molecules. As a result of this process, new radicals are generated and a **chain reaction** can be propagated. This can be very destructive and can damage many biologically important molecules, including DNA, membrane lipids, proteins and carbohydrates. The chain reaction will only cease when two radicals meet and form a covalent bond.

Free radicals are continually produced by the human body, and some are made for normal physiological reasons. **Nitric oxide (NO$^{\cdot}$)** is made by the vascular system and promotes the relaxation of smooth muscle, helping to control blood pressure. **Phagocytic cells** involved in inflammation and infection, such as macrophages, neutrophils and monocytes, use free radicals as part of the body's defence mechanism. Large amounts of radicals are generated to break down pathogens and kill foreign organisms. However, in certain diseases, such as **rheumatoid arthritis**, these processes can be activated excessively. High levels of phagocyte activity cause free radicals to be released extracellularly and result in tissue injury.

Not all the free radicals produced in cells are equally harmful. The **hydroxyl radical (OH$^{\cdot}$)** is very reactive. It will attack virtually all biological molecules, initiating chain reactions and damaging cell membranes and DNA, thereby enhancing the risk of cancer developing. Hydroxyl radicals can be formed as a result of exposure of cell water to high energy radiation (e.g. from X-rays and radioactive isotopes), or produced within cells as intermediates of normal biochemical processes. **Superoxide radicals (O$_2^{\cdot}$)**, are less toxic, and are generated by metabolic reactions that involve the controlled movement of electrons. Thus in aerobic cells, mitochondria normally reduce oxygen via the electron-transport chain to water. However, a small amount of this oxygen can be released as superoxide radicals after having accepted only one electron.

$$O_2^{\cdot} \xleftarrow{\;+e^-\;} O_2 \xrightarrow{\;4e^- + 4H^+\;} 2H_2O$$

$$\text{Superoxide} \qquad\qquad \text{Electron-transport chain}$$
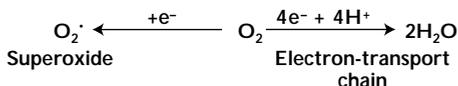
Superoxide is also made through the action of an enzyme present in the plasma membrane of phagocytes. Once bacteria have been engulfed, oxygen is taken up and the superoxide generated is responsible for microbial killing. These processes are summarized in Figure 6.

It is essential that levels of free radicals in the body are controlled because overproduction can be dangerous. For example, excess superoxide may be converted into the more damaging hydroxyl radicals. Consequently, cells and tissues have a number of protective mechanisms available to combat free radicals and eliminate them. This is known as the **antioxidant defence system**.

The range of antioxidant molecules in the body is diverse and includes enzymes, peptides, vitamins and other nutrient substances. Within the cell, the enzyme **superoxide dismutase** removes superoxide by catalysing its conversion into hydrogen peroxide (H_2O_2), which is disposed of by enzymes such as **catalase**.

$$2H^+ + 2O_2^{\cdot} \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$
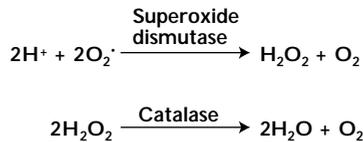
Figure 6. Free radical production and the role of antioxidants
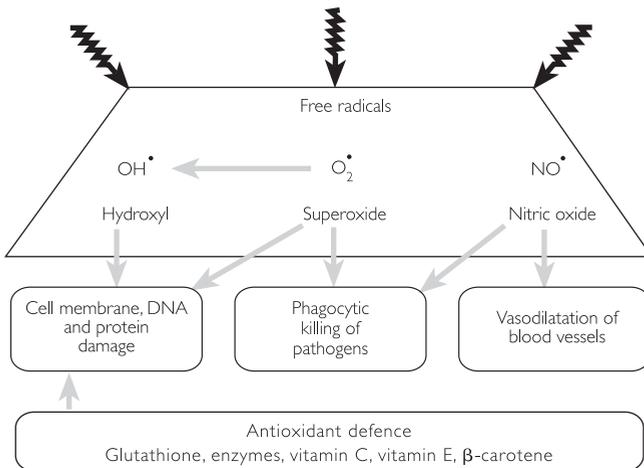
Another important antioxidant is **glutathione** (**GSH**), a tripeptide present in substantial amounts in most cells. This molecule has a **thiol** (-**SH**) group that can be oxidized easily, and various enzyme systems within the cells are responsible for maintaining it in a reduced state. In this form, GSH protects proteins and membranes against free radical attack and promotes the destruction of reactive species. Glutathione is an important coenzyme in a number of metabolic reactions, including that of a peroxidase enzyme which is involved in removing hydrogen peroxide.

Very recently the role of various dietary molecules as cellular antioxidants has been recognized. These molecules act as scavengers, trapping free radicals before they can damage tissues. **Vitamin E (tocopherol)** is a lipid-soluble antioxidant which is able to prevent chain reactions occurring with polyunsaturated fatty acids, thereby preventing irreparable damage to membranes. Vitamin E may also have a role in retarding atherosclerosis by protecting against lipoprotein oxidation. β-**Carotene**, found in the pigments of many fruits and vegetables, is not only the essential precursor of **vitamin A (retinol)**. It also plays a role in disease prevention by reacting with radicals to form more stable entities. There is considerable evidence to suggest that dietary β-carotene both protects against heart disease and reduces the risk of several types of cancer occurring.

Vitamin C (ascorbic acid) is an essential dietary component for humans. It prevents the deficiency disease **scurvy** by acting as a necessary cofactor in hydroxylation reactions associated with collagen biosynthesis. Being a highly water-soluble molecule, it also has a general role in scavenging free radicals in the aqueous environment. Vitamin C is considered to be the most important antioxidant in extracellular fluids, and it complements the action of vitamin E in the membranes.

In recent years there has been enhanced public awareness of the significance of antioxidants in promoting good health and preventing life-threatening diseases. This has been matched by a growing nutritional supplements industry which has marketed the importance to individuals of increasing significantly their intake of β-carotene and vitamins C and E. There is considerable evidence to suggest that dietary antioxidants can restrict free radical damage to cells, However beneficial, this is a controversial area and caution must be exercised when claims are made that antioxidants may cure such diseases.

2

Nutrition and Disease

Diet: a source of energy and essential nutrients

To survive in good health, humans require a daily diet containing a balanced quantity of nutrients. The principal components of the diet are **carbohydrates**, **lipids** and **proteins**. These molecules provide the major respiratory fuels such as glucose, fatty acids, ketone bodies and amino acids. They are metabolized by the body to produce carbon dioxide, water and urea. During this process a considerable amount of energy is conserved in the form of ATP, which can then be used to drive energy-requiring processes.

The proportions of these various fuels that are available to the body at any given time depends on the nutritional state. In the **fed situation**, an adult receiving an average UK diet will obtain approximately 47% of the energy requirement from carbohydrate, 38% from fat and 15% from protein. During **starvation**, however, the contribution that carbohydrate makes to the energy requirement is considerably reduced. Stored fat now becomes an important source of fuel, while tissue protein is broken down to provide amino acids that can either be oxidized or used as precursors for glucose synthesis.

The human body has an enormous capacity for the metabolic interconversion of various types of dietary molecule. Thus, provided that energy needs are being adequately met, it is not too important which of the principal dietary components are utilized to produce the necessary fuels. Food sources can, therefore, vary considerably as to the proportion and nature of their carbohydrate, fat and protein content as these are interchangeable energy sources. In theory, there is no need for carbohydrate to be present in the diet, since all the necessary carbohydrate compounds required by the body can be made from protein. In practice, however, if there is an insufficient supply of carbohydrate, the

extensive catabolism of lipid and protein that must occur to supply cells with energy results in elevated levels of ketone bodies. This **ketosis** — and subsequent **acidosis** — can produce illness; consequently, to avoid this situation, at least 20% of the total energy required by the body should be obtained from carbohydrate sources.

Birth is a special event in mammalian development. It represents a drastic environmental change for the newborn infant who has to adapt rapidly to a highly independent existence from its mother. At birth there is a sudden shift in nutrition from carbohydrate to lipid metabolism. The fetus normally receives a continuous supply of maternal glucose, and its abrupt cessation at birth is a hazardous event. During the suckling period the newborn baby receives an intermittent supply of milk, which represents a diet rich in fat. As a result, high levels of ketone bodies are produced in human infants. During this neonatal period **galactose** is also an important nutrient because it is a component of the milk sugar **lactose**. **Galactosaemia** is an inborn error of metabolism in children that reflects a reduced ability to convert galactose into glucose. The subsequent accumulation of phosphorylated sugar derivatives can lead to a very severe disease that causes liver damage and mental retardation.

While certain components such as carbohydrates — and to a lesser extent fats — are not always required in the diet, other substances have great nutritional importance and have to be provided. For example, animals are incapable of synthesizing **benzene rings** and, therefore, must obtain molecules containing such structures from their diet. Microorganisms and plants can make these aromatic substances from carbohydrates by several special metabolic routes. They thus provide an important source for humans of **aromatic amino acids**, such as phenylalanine, tyrosine and tryptophan, as well as certain **vitamins** such as folate, vitamin K and coenzyme Q.

Essential amino and fatty acids: indispensable for health

The majority of amino acids required by the body are derived from the digestion of dietary protein in the gut. However, some amino acids can be made by human cells, such as those of muscle, liver, kidney and brain tissue. These are known as **non-essential amino acids** and are listed in the box that follows.

Non-essential amino acids		
Alanine	Aspartate	Asparagine
Glycine	Glutamate	Glutamine
Proline	Serine	

Non-essential amino acids are synthesized in the body from carbohydrate intermediates and can be omitted from the diet, provided that total amount of protein is adequate. Amino acids are continually required to make new tissue proteins. As they are the building blocks of these macromolecules, all 20 amino acids associated with protein structure must be available for protein synthesis to occur. In addition, some amino acids may be used by certain cells to synthesize **neurotransmitters**, whereas others can provide a source of special chemical structures such as methyl ($-CH_3$) groups, or simply be metabolized to give ketone bodies and glucose.

Of these 20 amino acids, 10 either cannot be synthesized by human beings at all, or cannot be synthesized at a rate sufficient to meet the body's requirements. These are known as the **essential amino acids**, and they must be provided in the diet. Of these, eight are indispensable at all times during life, the other two are required in the diet during periods of rapid growth (for example, during childhood the demand for amino acids exceeds the normal synthetic capacity of the body). The essential amino acids are listed in the box below.

Essential amino acids	
Indispensable for adults and children	**Additional indispensable amino acids for infants**
Isoleucine	Arginine
Leucine	Histidine
Lysine	
Methionine (Cysteine)	
Phenylalanine (Tyrosine)	
Threonine	
Tryptophan	
Valine	

Although **arginine** and **histidine** are made by humans, they have to be provided in the diet of young children to ensure they gain in body weight. **Tyrosine** is an amino acid that can be made in the body from

phenylalanine, and only becomes essential when phenylalanine is omitted from the diet. The treatment of **phenylketonuria** is an interesting example of **nutritional therapy** in children who have an inborn error of metabolism. Phenylalanine is an essential amino acid, and despite the block in its conversion to tyrosine that occurs with this disease, it cannot simply be excluded from the diet. This would immediately affect the growth of the child as the amino acid is required for the maintenance and development of body protein. Thus although a diet low in phenylalanine is prescribed in cases of phenylketonuria, the amino acid has to be present at levels that allow normal growth and development, but minimizes its accumulation and the potential for forming harmful metabolites. Similarly, **cysteine** only becomes essential if insufficient **methionine** is present in the diet.

The measurement of **nitrogen balance** can be used to determine the protein requirement of an individual. An adult is said to be in nitrogen balance when the amount of nitrogen consumed equals that excreted in the urine, faeces and sweat. When nitrogen intake exceeds its output, a **positive nitrogen balance** is recorded. This is commonly associated with growth. A **negative nitrogen balance** occurs when more nitrogen is lost from the body than is retained. If an essential amino acid is not adequately catered for in the diet, negative nitrogen balance is observed. This can also be seen during starvation and malnutrition and, temporarily, during a variety of circumstances — these include surgery, trauma, sepsis and burns, when an increased rate of skeletal muscle protein breakdown occurs, presumably to provide amino acids to assist with the repair processes and to support the immune system.

Not all dietary proteins provide a good balance of amino acids. The milk protein **casein** is considered a biologically complete protein as it can supply sufficient quantities of all the required amino acids. **Gelatin**, however, lacks **tryptophan** and many vegetable proteins can have a low level of one or more essential amino acids, for example, sweetcorn is deficient in tryptophan and **lysine**. This does not mean that a vegetarian diet poses a problem, provided that a mixture of plant foods are used to supply the necessary protein. The seeds of legumes are high in protein and low in starch, and their essential amino acid content resembles that of animal proteins. Supplemented with a small amount of **methionine** from cereals or animal sources, they adequately support human growth and development.

Occasionally some foods contain proteins that are toxic or able to produce an allergic reaction. For example, in **coeliac disease**, one specific protein called **gliadin**, which is associated with the **gluten** fraction of wheat, causes the intestinal wall to become inflamed. Damage to the villi of the mucosa results, they become flattened, and poor absorption of nutrients leads to diarrhoea and malnutrition. This process can be reversed if the patient avoids wheat products and maintains a gliadin-free or 'gluten-free' diet.

In contrast with proteins, there is no requirement for **triacyl-glycerols**, the common oils and fats of the diet. Most fatty acids and lipids in animals can be made either from carbohydrate or from other fatty acids. However, there are two groups of polyunsaturated fatty acids which are of great nutritional importance. Although humans can synthesize saturated and mono-unsaturated fatty acids, they cannot make linoleic acid ($C_{18:2}$) which has two *cis* double bonds, or linolenic acid ($C_{18:3}$) which has three *cis* double bonds. These are representatives of two classes of polyunsaturated fatty acids known as the **essential fatty acids** and must be obtained from plants, which can synthesize them. They are vital for health: polyunsaturated fatty acids are not just important components of membrane lipids and lipoproteins, they are also the starting point for the synthesis of a group of **local hormones** that include prostaglandins, **thromboxanes** and **leukotrienes**. These substances are involved in the regulation of blood pressure, vasodi-latation, blood clotting and the immune response.

Fish oils, particularly those of sardines, mackerel and herring are rich in polyunsaturated fatty acids derived from linolenic acid. Taken by humans in reasonable amounts, these fatty acids can have beneficial effects. They have anti-inflammatory properties, and dietary sup-plements of marine oils are used in the management of rheumatoid arthritis. The triacylglycerol level of blood is also lowered by these fatty acids, and it is proposed that they could reduce the risk of **athero-sclerosis**. Certainly Eskimos, who have a high intake of fish oil in their diet, have a low incidence of coronary heart disease.

Vitamins: not just vital amines for health

The term vitamin was introduced after studies on the disease **beriberi**, which is prevalent in south-east Asia and causes damage to the peripheral nervous system. The active principle that cured the disorder

was extracted from rice polishings. Since it appeared to be an amine that was 'essential for life', the term *vitamine* was proposed. The 'e' was later dropped when it was realized that many other small organic molecules, required by animals for normal metabolic activity, do not necessarily contain basic nitrogen. Most vitamins either cannot be synthesized by the body or cannot be made in sufficient amounts to meet its needs. They are therefore required in the diet, albeit in much smaller amounts (mg) than the quantities associated with essential amino acids and fatty acids (g).

There are 14 vitamins currently recognized as being important for health. These are normally classified into two large groups: **fat-soluble** and **water-soluble** (B-group) vitamins and are listed in the box below.

Fat-soluble vitamins	Water-soluble vitamins
Vitamin A (retinol)	Vitamin B_1 (thiamin)
Vitamin D (calciferol)	Vitamin B_2 (riboflavin)
Vitamin E (tocopherol)	Vitamin B_6 (pyridoxal)
Vitamin K (phylloquinone)	Vitamin B_{12} (cobalamin)
	Nicotinic acid
	Lipoic acid
	Folic acid
	Pantothenic acid
	Biotin

Mammalian tissues are able to metabolize many of the B-group vitamins, and incorporate them into the structure of important **coenzymes** or **prosthetic groups** that are bound to protein apoenzymes. These can then take an active part in the mechanism of enzyme catalysis, often acting as donors or acceptors of specific chemical groups. The role of some B-group vitamins as coenzymes in metabolism is summarized in Table 1.

As these vitamins are not normally stored in the body in significant amounts, a daily supply is needed. A dietary deficiency can occur in those vitamins that are involved in energy provision by providing coenzymes for metabolic steps associated with glycolysis and the tricarboxylic acid cycle. Examples include thiamin, riboflavin, nicotinic acid, and pantothenic acid. Their absence can lead to nervous disorders because neurons have a high metabolic rate that depends on glucose being effectively metabolized.

Table 1. A review of some B-group vitamins and the deficiency diseases associated with them

Vitamin	Coenzyme	Metabolic role	Deficiency disease
Thiamin B$_1$	Thiamine pyrophosphate (TPP)	Oxidative decarboxylation in the tricarboxylic acid cycle (TCA)	Beriberi — peripheral nerve damage
Riboflavin B$_2$	Flavin–adenine dinucleotide (FAD) Flavin mononucleotide (FMN)	Hydrogen atom transfer in some dehydrogenase enzymes	Skin lesions in the mouth and visual problems
Nicotinic acid	Nicotinamide–adenine dinucleotide (NAD) Nicotinamide–adenine dinucleotide phosphate (NADP)	Hydrogen atom transfer in many dehydrogenase enzymes	Pellagra — muscular weakness; dermatitis; dementia
Pyridoxal B$_6$	Pyridoxal phosphate	Aminotransferase and decarboxylase reactions in amino acid metabolism	Impaired amino acid and neuro-transmitter metabolism; convulsions
Pantothenic acid	Coenzyme A	Activation in fatty acid, carbohydrate and TCA cycle metabolism	Widely available; deficiency uncommon
Cobalamin B$_{12}$	B$_{12}$ coenzyme	Methyl group transfer in methionine and fatty acid metabolism	Pernicious/megaloblastic anaemia and spinal cord damage
Folic acid	Tetrahydrofolate	One-carbon transfer; important in DNA synthesis	Poor growth, megaloblastic anaemia and other blood disorders
Biotin	Protein-bound lysine	CO_2 fixation in gluconeogenesis and fatty acid metabolism	Dermatitis; nausea; vomiting, anorexia

Sometimes the inability to absorb a vitamin from ingested food can cause a problem. **Pernicious anaemia** is a disease that results in a deficiency of **vitamin B_{12}** — owing to the absence of a specific protein in the gastric juice. This **intrinsic factor** is normally synthesized by the gastric mucosa. Micro-organisms produce vitamin B_{12}, but it is normally obtained from food derived from animal sources. Hydrochloric acid in the stomach releases the vitamin from the proteins that contain it. Intrinsic factor then binds to vitamin B_{12} and facilitates its transport across the intestinal mucosal cell surface. Vitamin B_{12}, folic acid and methionine are essential for the metabolism of all rapidly dividing cells, particularly those of the bone marrow and nervous system.

A deficiency in these dietary molecules will impair DNA synthesis so that large, abnormal, immature erythrocytes with low levels of haemoglobin (**megaloblastic cells**) are produced. Therefore, a lack of either vitamin B_{12} or folic acid results in a similar anaemia. At the same time, neurological changes can occur, including degeneration of the spinal cord. Dietary folic acid deficiency may occur during pregnancy, in the elderly, or when intestinal malabsorption — such as that associated with coeliac disease — develops.

The fat-soluble vitamins are found associated with natural lipid foods and tend to be absorbed with them from the intestine. They are transported as a component of **lipoproteins** to the liver, before being stored in various body tissues. Each fat-soluble vitamin has a distinctive role, and these are summarized in Table 2.

Table 2. Nature of the fat-soluble vitamins

Vitamin	Active form	Metabolic role	Deficiency disease
Vitamin A	11-*cis*-retinal retinoic acid	Visual cycle in the retina; cell growth and development	Night blindness and xerophthalmia
Vitamin D	Dihydroxy-cholecalciferol	Hormone involved in Ca^{2+} and phosphate metabolism	Rickets
Vitamin E	α-Tocopherol	Antioxidant	Rare (membrane damage)
Vitamin K	Phylloquinone	Modifies blood-clotting enzymes	Impaired blood clotting

Sometimes a nutritional imbalance can result in vitamin deficiencies that may enhance the development of a particular disease. For example people with a high alcohol intake obtain considerable energy from the metabolism of ethanol and, consequently, do not eat sufficient quantities

of other foods to provide the necessary amounts of vitamins. Alcohol can also interfere with vitamin uptake from the gut. Thus, in undernourished drinkers, deficiencies of vitamins B_1, B_6, B_{12}, folic acid and the fat-soluble vitamins can occur. This may exacerbate the health problems, such as liver disease, that these individuals develop.

Fibre and minerals: valuable dietary components

One aspect of diet which has received considerable media interest is **fibre** — the components of plant cell walls that remain in the alimentary tract after digestion. Fibre is made up of **non-starch polysaccharides**, which include the soluble pectins and gums as well as insoluble cellulose and some hemicelluloses. While they have limited direct nutritional value, these materials play an important role in the metabolic activity occurring in the colon. This organ is rich in bacteria that are capable of carrying out **anaerobic fermentation** of complex carbohydrates. **Short-chain fatty acids** (acetate, propionate and butyrate) are produced that can be used as an important source of energy by the colon and other body tissues. Gases such as carbon dioxide, hydrogen and methane are also released.

A consequence of this metabolism is that **plasma cholesterol** is lowered. This may be a result of the non-starch polysaccharides binding to cholesterol and bile acids which are released into the intestine, thereby preventing their reabsorption and promoting their excretion. Such an effect may offer protection against coronary disease and strokes. Chemicals liable to enhance the risk of diseases, such as colorectal cancer, are also produced in the intestinal tract, sometimes as a result of bacterial activity. Non-starch polysaccharides bind these chemicals, thus preventing them from damaging the epithelial lining and enabling them to be excreted in the faeces. This has led to manufacturers' claims that foods which are high in fibre, such as oat bran, promote health. However, it does appear that fibre, by increasing the bulk of the faeces, facilitates defecation and therefore reduces the incidence of constipation, haemorrhoids and varicose veins. A high stool mass is also associated with a reduced risk of colon cancer, and the fermentation product **butyrate** is believed to protect against the disease. Therefore, an increased fibre content in the diet is thought to be a significant factor in reducing the risks of both coronary heart disease and colon cancer.

The inorganic elements that comprise the human body also have to be provided in the diet. The principal mineral components are sodium,

potassium, calcium, magnesium, iron, sulphur, chloride and phosphate. They tend to reflect the inorganic nature of either the skeletal system or the body's buffers.

In addition, copper, zinc, manganese, iodine, cobalt, nickel and selenium are required in trace amounts.

Although **mineral deficiencies** are unlikely for most people on a balanced diet, problems can arise in certain situations. For example, although **iron** is efficiently conserved by the body, a heavy loss of blood after an injury or haemorrhage can place excessive demands on the body's reserves. Iron is principally stored in association with the protein **ferritin** in the liver, spleen and bone marrow. After absorption, it is transported from the intestinal mucosa to these tissues by the plasma protein **transferrin**. The human body contains about 4 g of iron, the majority of which is bound to the haem-containing proteins, **haemoglobin** and **myoglobin**. These are found in red cells and muscle tissues, respectively, and are concerned with the transport and storage of oxygen. The **cytochromes** that function in both mitochondria and in the endoplasmic reticulum, and the enzyme **catalase**, are also important iron-containing molecules.

A lack of iron results in the reduced synthesis of these molecules, particularly haemoglobin, so that **iron-deficiency anaemia** can develop. Women are particularly susceptible to iron depletion and anaemia, as a result of menstrual blood loss, pregnancy and lactation. Deficiencies in **manganese** and **selenium** can have important consequences for the body's **antioxidant defence**. These elements are active components of the enzymes **superoxide dismutase** (manganese) and **glutathione peroxidase** (selenium) that are involved in removing reactive oxygen species to protect cells against free radical damage.

Vitamin A: more than just a visionary molecule

Vitamin A plays a fundamental role in the visual process, but it is also essential for normal cell growth and development, and a severe lack can lead to blindness, a predisposition to infectious disease and death.
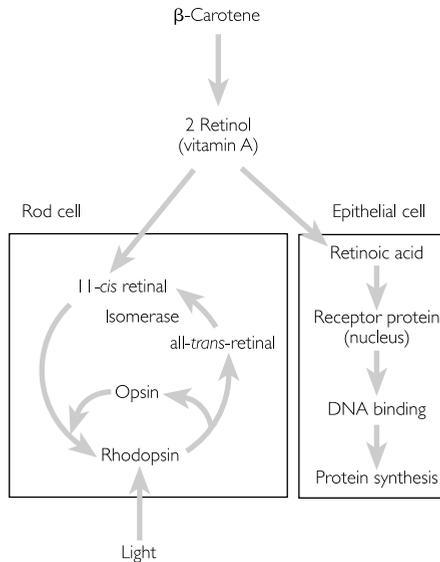
The human retina contains two types of light-sensitive cell. These are **cone cells**, which mediate colour vision and are adapted for high light intensities, and **rod cells** which cannot determine colour; however, they detect low levels of illumination and provide nocturnal vision which is essentially monochromatic in nature. This is due to the characteristics of

rhodopsin (purple visual pigment), the photoreceptor molecule present in rod cells. Rhodopsin is made up of a protein called **opsin**, which has a pigment known as **11-*cis*-retinal** tightly bound to it. Retinal is the aldehyde form of vitamin A (retinol) and it is vision at low light intensities that is impaired when vitamin A deficiency occurs (night blindness).

Vitamin A is made from dietary β-**carotene**, an orange pigment found in plant material such as green leaves and carrots. β-Carotene is split by enzymes in the intestinal mucosa and liver, to give two molecules of retinol that can be readily oxidized to 11-*cis*-retinal. The presence of the *cis* double bond in the molecule twists retinal into a bent shape so it can bind with opsin to give rhodopsin. When rhodopsin is exposed to light, the 11-*cis*-retinal absorbs the radiation and its *cis* double bond is **isomerized** to the *trans* form. This causes the molecule to undergo a profound change in shape, and the **all-*trans*-retinal** that results can no longer bind to the opsin. Rhodopsin is therefore 'bleached' when the all-*trans*-retinal dissociates from the opsin. Such a change in shape alters sodium ion channels in the rod cell membrane, thereby generating a nerve impulse which is sent to the brain. Resynthesis of rhodopsin only occurs after the all-*trans*-retinal is converted back to the 11-*cis* form. This reaction is catalysed by an **isomerase** enzyme, and the 11-*cis*-retinal is then able to bind to the opsin. The visual cycle in rod cells is outlined in Figure 1.

Vitamin A has another important role in maintaining the epithelial lining that covers most external and internal surfaces of the human body. A lack of the vitamin causes soft mucous membranes to become dry and thickened. The eyes are conspicuously affected, and in young children the condition known as **xerophthalmia** (dry eye) can develop. This results from epithelial cells of the conjunctival membrane, which covers the outer aspect of the eyeball, becoming keratinized and hard. In certain tropical countries it is a common cause of permanent blindness, because eventually the cornea can become irreversibly damaged. Modification of epithelial cells in this way can also enhance infectious disease, as access to the body by bacteria is made easier and the immune system is impaired.

To promote healthy mucous membranes, vitamin A has a hormone-like function. It is metabolized to **retinoic acid**, which then binds to a receptor protein in the nucleus of **basal cells** that differentiate into epithelia. The receptor–retinoic acid complex binds to DNA, and

β-Carotene

2 Retinol
(vitamin A)

Rod cell

Epithelial cell

11-*cis* retinal

Isomerase

all-*trans*-retinal

Opsin

Rhodopsin

Light

Retinoic acid

Receptor protein
(nucleus)

DNA binding

Protein synthesis

Figure 1. Vitamin A metabolism and function

influences the synthesis of specific proteins so that normal development of mucous epithelia is achieved. Vitamin A also plays a similar role in embryonic development, where it is thought to act as a signal molecule in the fetus to ensure the normal development of specific body parts. As the ultimate source of all vitamin A is plant carotenoids, the inclusion of green vegetables in the diet is essential for health, especially for young children who are most susceptible to a lack of this vitamin.

Vitamin D: the sunshine vitamin with hormone action

Vitamin D is a lipid molecule that occurs in fatty foods and is associated with the proper mineralization of bone by calcium. It is linked to the disease **rickets**, which is prevalent either in industrialized urban areas or in regions with long winters; in both situations there can be limited exposure to sunshine. Children show deformities in the long bones of the legs, so they walk with a bow-legged gait. Adults develop **osteo**-**malacia**, which manifests itself as an increasing tendency for the skeletal system to fracture. This condition is commonly seen in elderly people who may have restricted opportunities to go outside; however, it should

not be confused with **osteoporosis**, which results from a change in the balance of sex hormones with age.

Precursors of vitamin D are found in plants (ergosterol) and animals (dehydrocholesterol) and both are transformed by UV radiation into substances with vitamin D activity. In animals, **dehydrocholesterol** is found in the epidermis of the skin, and exposure to sunlight causes the molecule to be converted into vitamin D (cholecalciferol) which is then released into the bloodstream, attached to a carrier protein.

Vitamin D has to be metabolized to make it biologically active. This occurs first in the liver, where it is hydroxylated to give 25-hydroxyvitamin D, the principal form of the vitamin in the blood. This is largely inactive, and full activity is not achieved until the blood has passed through the kidney which further hydroxylates the vitamin to give 1,25-hydroxyvitamin D, or '**active vitamin D**'. This is outlined in Figure 2.

Figure 2. Metabolism and action of vitamin D
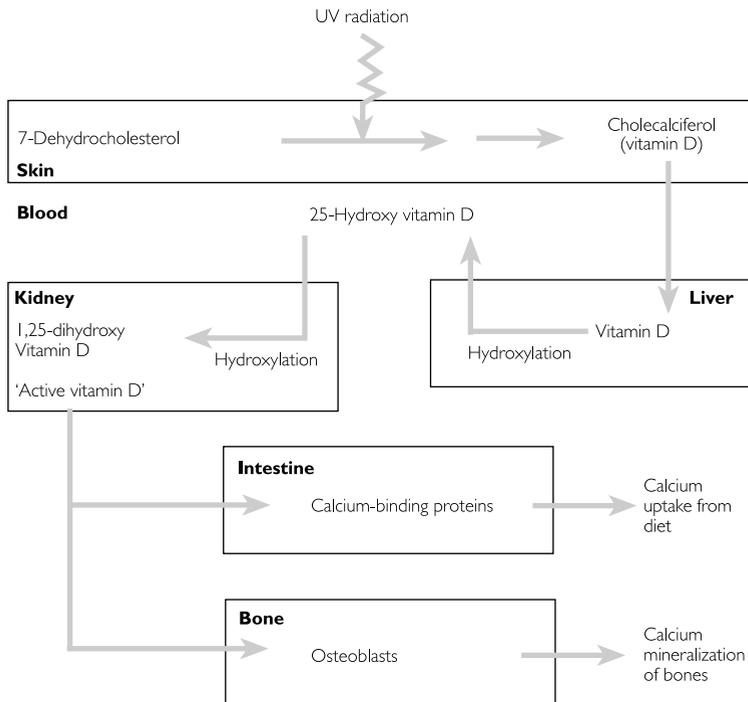
Active vitamin D now acts like a steroid hormone and enters the cells of the **intestinal mucosa** where it binds to a receptor protein in the nucleus. The gene that makes calcium-binding proteins (calbindins) is enhanced so that the intestine absorbs more calcium from the diet and raises the plasma concentration accordingly. 1,25-dihydroxyvitamin D also interacts with bone-forming cells called **osteoblasts**, to increase their ability to take up calcium and promote bone mineralization, thereby preventing rickets.

Although vitamin D is metabolized by the liver, it is not stored there. Cholecalciferol is deposited in adipose tissue and skeletal muscle, and these tissues act as important reservoirs of the vitamin. The UV radiation required to make the vitamin is not always readily accessible and, therefore, vitamin D made during the summer months can be stored and made available for winter use. When levels of active vitamin D are not maintained in the blood plasma, the intestinal mucosa will not be stimulated appropriately to absorb calcium from the diet. In growing children this means that bones are **under**-**mineralized** and rickets can result.

Calcium intake and the availability of vitamin D are also thought to be important dietary factors that influence osteoporosis. This degenerative disease is associated with a loss of bone substance with advancing age, owing to a reduction in the levels of oestrogens in women and androgens in men. It is most often seen in post-menopausal women, although the condition can occasionally develop in young men and women.

Undernutrition: diseases of dietary deprivation

The problems of malnutrition result from an inadequate intake of food and may arise in a variety of ways. Often these reflect more complex social issues that include economic considerations and the cultural attitude of various groups.

An example of the latter is **anorexia nervosa**, an eating disorder common in western countries that primarily affects young women during adolescence. The problem is complex and often has a psychological basis. Patients lose weight apparently to avoid obesity, although the reasons behind this are not fully understood and are not the simple consequences of a slimming programme. If dietary restriction continues, and is carried to excess, severe weight loss and emaciation occur, which
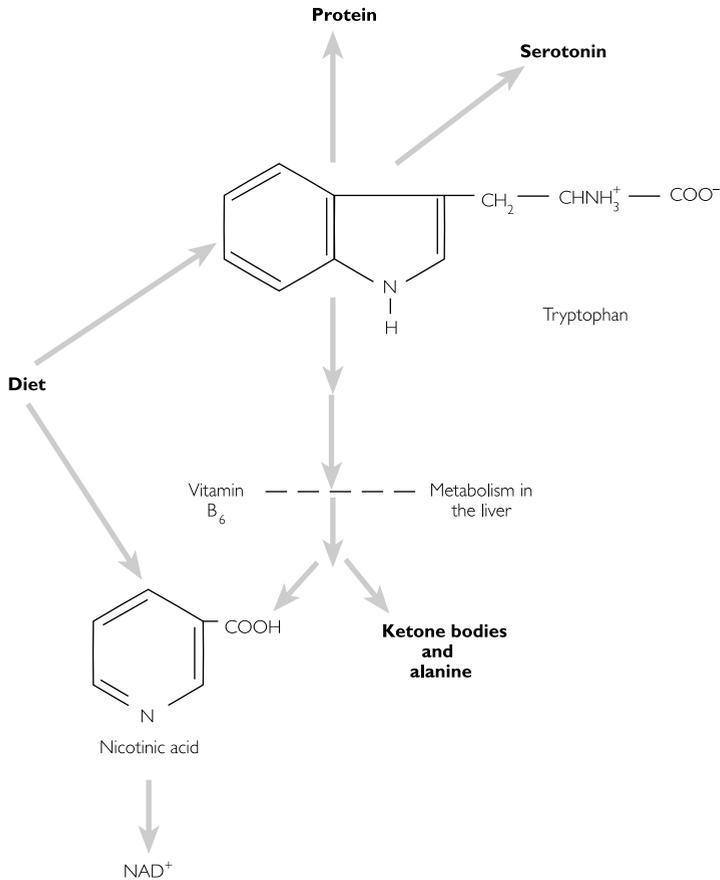
can prove fatal. In many instances multiple vitamin and mineral deficiencies result, menstruation ceases, the heart rate slows and hair growth is reduced. Treatment involves obtaining the consent of the patient to increase their food intake and providing assistance to overcome any depression, compulsive disorders, phobias or other psychological problems.

Another disease that can result from a poor or unusual diet is **pellagra**. In the early 1900s, this was responsible for many deaths in the southern states of the USA, where cornmeal was an important dietary component. It also occurs in parts of India where jowar (*Sorghum vulgare*) is regularly eaten. Pellagra is a result of a lack of the B-group vitamin, **nicotinic acid**. In humans this can be synthesized from the essential amino acid tryptophan, but normally at a rate insufficient to maintain good health. Thus a dietary source of the vitamin is also required. Because tryptophan is a precursor of nicotinic acid, tryptophan deficiency is associated with pellagra. Corn has a very low tryptophan content, and the composition of jowar is such that tryptophan degradation is enhanced. Thus a diet that comprises chiefly these foods, or of cereals in which nicotinic acid is not readily accessible, will result in pellagra.

Nicotinic acid is a component of the coenzymes NAD^+ and $NADP^+$. These are ultimately linked with oxidative reactions that supply the energy for tissues such as the brain. Mental disturbances, like depression, are amongst the first signs of pellagra and this can develop into dementia, while other symptoms include dermatitis, diarrhoea and muscular weakness.

Tryptophan has a complex metabolism and, apart from being incorporated into proteins, it also gives rise to the neurotransmitter **serotonin** (5-hydroxytryptamine), which is used by the brain. The body's supply of the tryptophan is usually quite small because it is the least-abundant amino acid in dietary protein. However, as shown in Figure 3, tryptophan will spare the dietary requirement for nicotinic acid and the symptoms of pellagra can be treated successfully with this amino acid.

Providing a diet with plenty of protein rich in tryptophan will cure the disease. Most foods rich in animal protein are an excellent source of tryptophan, including lean meat, poultry, fish, eggs and milk. In a normal person, 60 mg of tryptophan is equivalent to 1 mg of nicotinic acid. The conversion of tryptophan to nicotinic acid occurs in the liver

Protein

Serotonin

CH_2 —— $CHNH_3^+$ —— COO^-

N
|
H

Tryptophan

Diet

Vitamin — — — — — — Metabolism in
B_6 the liver

COOH

**Ketone bodies
and
alanine**

N

Nicotinic acid

NAD^+

Figure 3. Tryptophan and nicotinic acid in NAD^+ synthesis

and a key reaction in this process is dependent on vitamin B_6. Vitamin-deficient diets often lack more than one vitamin and a deficiency of vitamin B_6 will impair the metabolism of tryptophan, thereby preventing this route from sparing the dietary need for nicotinic acid.

There are several extreme forms of protein and energy malnutrition. These are particularly prevalent in underdeveloped countries where famine is rife and whole populations live in poverty. **Marasmus** afflicts both adults and children. It is a condition of semi-starvation, with muscular wasting and a loss of body fat and protein that affects the heart, liver, kidneys and gastrointestinal tract as well as depressing the immune

system. Restriction of liver function, particularly in synthesis of transport proteins for mobilizing stored vitamin A, results in an apparent deficiency of this vitamin. As retinol is essential to maintain general health, this deficiency contributes to the reduced growth seen in children with the condition

Kwashiorkor appears in young children and is a lethal disease. It was first described in 1933 by the paediatrician Cicely Williams who worked with children in Ghana. Kwashiorkor is a native word and means "the disease of the deposed baby when the next one is born". At first glance, afflicted infants may not appear to be malnourished, for sub-cutaneous fat is present and their bodies seem swollen and bloated. This is a cruel deception: muscle wasting is masked by an excessive accumulation of tissue fluid, which results from reduced synthesis of plasma proteins. The presence of plasma proteins normally ensures that an osmotic pressure develops across the capillary wall, which opposes the hydrostatic pressure of the blood. This facilitates fluid retention and consequently a reduction in plasma protein concentration can lead to severe oedema. The liver is fatty and enlarged, giving the children a 'pot-bellied' appearance that is made even more pathetic by sparsely growing hair and poorly pigmented skin. Kwashiorkor is a disease which suddenly precipitates after infants have been on a monotonous, starchy and inadequate diet for several months. Although often considered to be a result of extreme protein deficiency, it is much more complex, as the children have an impaired ability to synthesize a range of proteins.

It is currently thought that the antioxidant defence system becomes thoroughly undermined as a result of dietary neglect. In Kwashiorkor, plasma levels of tocopherol, retinol and β-carotene are low, and the essential elements zinc, manganese and selenium that assist various antioxidant enzymes are much reduced. **Free radical attack** can therefore disrupt protein synthesis, damage membranes and destabilize tissues so that fatty liver, oedema and skin lesions result. Treatment requires the progressive introduction of a series of easily assimilated foods — because the gastrointestinal tract is in a poor state; without such treatment most of these children die.
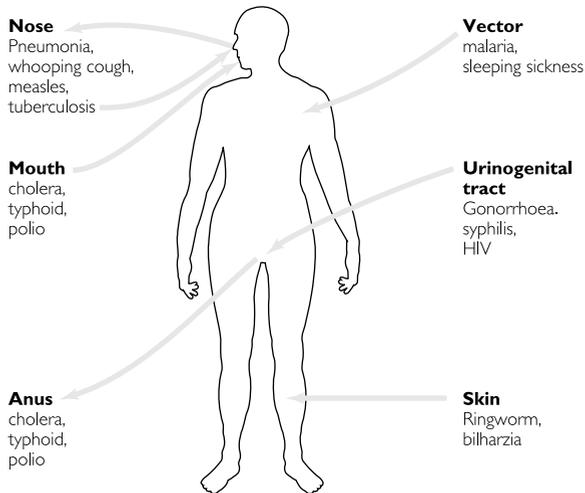
3

Micro-organisms and Disease

Infectious disease

Disease can be defined as any condition where the normal functioning of part of the body — or a bodily function — is impaired, leading to a change in the normal state of health of an individual. One important group of diseases is known collectively as the **infectious diseases**. They are those caused by a large variety of **pathogenic micro-organisms** within the **bacteria**, **fungi**, **protozoa** and **viruses**, which cause pathological damage to their host and, if untreated, may eventually cause death. During the last century, the study of infectious diseases lead Pasteur and Koch to advance the **germ theory** of disease, which proposed that minute organisms in the environment called **microbes** were responsible for these diseases. It was proposed that different diseases were caused by different microbes, each with their own characteristics. The scientific study of the different populations of microbes has led to the development of modern bacteriology. Every year, infectious diseases are still responsible for millions of deaths in the world.

To cause disease, an infectious micro-organism must either colonize the host surface or invade the sterile tissues of a susceptible individual and produce an injury to which the host will respond (Figure 1). A combination of the effects of this damage to the host and the response to the injury will give the **symptoms** of the disease — such as inflammation, fever and pain. The precise symptoms that are observed will depend on the species of infecting micro-organism, the physiological processes within the host that are affected and the host response. Disease is, therefore, caused by a complex series of interactions between the infecting micro-organism and its host.

Nose
Pneumonia,
whooping cough,
measles,
tuberculosis

Vector
malaria,
sleeping sickness

Mouth
cholera,
typhoid,
polio

**Urinogenital
tract**
Gonorrhoea.
syphilis,
HIV

Anus
cholera,
typhoid,
polio

Skin
Ringworm,
bilharzia

Figure 1. Routes by which pathogens enter and leave the body

Acute infections, which appear rapidly and are of short duration, are often the result of violent interactions between the host and the infecting micro-organisms, as in **lobar pneumonia** which is a severe lung infection caused by the bacterium *Streptococcus pneumoniae.* **The slow onset of disease often results in a chronic infection** (of long duration), as is caused by *Mycobacterium tuberculosis* which infects the lungs and causes **tuberculosis**. This used to be a common cause of death in the world and its occurrence is increasing again in the UK and USA.

Categories of pathogenic micro-organisms

Micro-organisms that cause disease are called **parasites**, since they grow on or within the body at the expense of the host. The parasite initially gains all the advantages of the association, such as a ready source of food and a constant environment. This can deprive the host of essential nutrients, which can often lead to pathological damage of body systems. Eventually this can also be detrimental to the parasite as it may cause the host to eliminate the parasite or, in extreme cases, lead to death of the host.

Some micro-organisms have developed a milder association with the host known as **commensalism**, whereby the parasite benefits but the host is not harmed. Good examples of this type of relationship can be

found in the **natural microbial flora** of the skin and mucous membranes. **Micrococci** are bacteria that normally grow on the surface of the skin and cause no harm under normal circumstances.

An understanding of the interactions between an infecting micro-organism and its host is of vital importance for the design of protective therapies such as **vaccination**.

Vibrio cholera, the causative agent of cholera, colonizes the surface of the intestine and causes disease by releasing an **exotoxin** that is adsorbed by the surface epithelial cells (see Chapter 1). It is, therefore, pointless to induce immunological protection within the tissues and body fluids of the host, since the organism and its products remain outside these protected areas. Vaccines must therefore be designed to induce specific protection at the intestinal surface if protection against cholera is to be achieved.

Micro-organisms are normally divided into four categories according to their behaviour in the host. These are categorized as follows.

Facultative parasites

These organisms either live and multiply outside the host as saprophytes (which live on decaying organic matter) or, under certain conditions, can survive as parasites which feed in the host at the host's expense. Facultative parasites normally invade a host after it has been damaged by a previous infection of another micro-organism, physical trauma or by the use of immunosuppressive therapy. As these organisms form a permanent source of infection in the environment, it is essential that precautions are taken to prevent infection by nursing patients with severe burns or those that have recently undergone organ transplantation in a **sterile** environment. Organisms in this category infect the **body cavities** and **tissue spaces** as they do not have the abilities either to survive in or infect living cells. Examples of this type of parasite include members of the bacterial genus *Clostridium* which cause tetanus and gangrene, the bacterial genera *Escherichia* and *Pseudomonas* which can infect the blood, and the fungus *Aspergillus* which can infect the tissue spaces of patients who are on immunosuppressive therapy.

Obligate extracellular parasites

Organisms of this type can, under normal conditions only, multiply in the body cavities and tissue spaces in the host. They will also persist for

some time on inanimate objects in the environment where they form a reservoir of infection. If these organisms fail to infect another host they will die out in the environment as they cannot grow. Infections caused by obligate extracellular parasites are contracted by direct or indirect contact with an infected individual.

Bacteria of this type include *Corynebacterium diphtheriae*, **which causes diphtheria** — a common cause of infant death until the 1940s in the UK; *Staphylococcus aureus*, which infects blocks sweat and sebaceous glands producing boils and carbuncles; and *Streptococcus pyogenes*, a cause of sore throats and scarlet fever.

Facultative intracellular parasites

These can multiply either in the tissue spaces or within phagocytic cells but they are incapable of actively invading host cells and, therefore, must be taken up by phagocytosis. Facultative intracellular parasites have evolved mechanisms which allow them to be ingested and to survive and grow in phagocytes such as macrophages. Like the obligate extracellular parasites, they can survive for only a limited time in the external environment and so have to be passed from host to host if they are to survive. Diseases caused by these organisms are often severe, long lasting and difficult to treat. Examples include **brucellosis** caused by *Brucella abortus* and **tuberculosis** caused by *Mycobacterium tuberculosis*.

Obligate intracellular parasites.

These parasites multiply only in living cells and are capable of invading most if not all body cells without the requirement for phagocytosis. Organisms of this type include all animal viruses and the malarial parasite *Plasmodium*.

In addition to the four main groups of pathogenic micro-organisms there are some species which colonize and multiply on the external surfaces of the body and cause disease by the release of poisonous toxins which are then adsorbed by the host. An example of this type of organism *Vibrio cholera* has been mentioned earlier in this chapter.

Factors that determine infection

The likelihood that an individual will become infected by a specific micro-organism depends on a number of factors.

Virulence is a measure of the ability of a **given strain** or **pure culture** of a micro-organism to produce disease, and is determined by the genetic make-up of the organism. Pathogenic micro-organisms have a number of genes which are known as **virulence genes**, if one or more of these genes is lost or damaged by mutation the micro-organism becomes **avirulent** and is no longer capable of causing disease. Microbiologists often deliberately mutate virulent organisms to produce stable avirulent strains which can be used as live vaccines. For example, the **Aro A**- mutants of *Salmonella typhi* have lost their ability to cause **typhoid** as a consequence of a defect in aromatic metabolism. These bacteria are now being used to develop more effective vaccines.

A loss in virulence can often be seen when a micro-organism is repeatedly grown on artificial media. This selection of variants that survive better in successive sub-culture is known as **attenuation**. If the attenuated organism is stable and does not revert back to the virulent form when it is passaged through a live animal, it is a stable genetic variant and can be used as a vaccine.

Another important factor that determines the risk of infection is the **number of organisms** to which a susceptible host is exposed. The greater the number of organisms, the more chance there is that an infection will occur. The infective dose of an organism is not just determined by the number of organisms, but is influenced by the virulence of the organism and the resistance of the host to the infection.

Host resistance to the first exposure of a specific infectious micro-organism is determined by a multitude of factors, which include the host's genetic make-up, age, nutritional status and emotional state. These factors will influence the functioning of the antimicrobial defence systems of the host, including the **non-specific** and **specific immune systems**. Any weakening in the host's defences will make that individual more susceptible to infection.

The process of infection

A virulent micro-organism must be able to go through a complex sequence of interactions with a susceptible host to produce disease. The micro-organism has first to attach to and survive on one of the outer surfaces of the host. Once this has been accomplished, the infecting organism normally has to penetrate the outer surface and then multiply in the host's body fluids or tissues. The growth of the organism will

induce a defensive response by the host which the pathogen has to overcome so that it can cause the damage that results in disease. If any of these interactions fails to happen, the disease will not develop.

Outer defences of the body

As previously mentioned, when a pathogenic micro-organism encounters its potential host it has to survive on and penetrate one of the **external mechanical barriers** of its target. The mechanical barriers consist of the **skin** and the **mucous membranes** of the **gut**, the **uro-genital tract** and the **respiratory tract**, all of which are environments where micro-organisms find it difficult to survive.

Intact skin is an excellent barrier to infection: it is unstable as the outer layer of the skin — the **stratum corneum** — is continually being shed, together with any attached bacteria. The food supply is poor as the **nutrients** that are available on the skin for bacterial growth are few and, because the skin's surface is normally colonized by a natural **microbial flora**, most of the food supply is consumed rapidly by the resident micro-organisms.

The natural flora also has another important role in preventing infection as it occupies the areas on the skin surface that are favourable for growth and survival so it crowds out the invading pathogens.

If a pathogenic micro-organism establishes a foothold on the skin, it then has to contend with the anti-microbial substances that are continuously being released onto the surface. The skin is covered with **lipids**, **sebaceous gland secretions** and **sweat**, which all contain components that will either kill micro-organisms or inhibit their growth. Sebaceous secretions are very important in preventing fungal infections such as **ringworm** (caused by *Trichophyton* sp.): as young children do not produce these secretions, they are more susceptible to this disease.

It is thus very difficult for a pathogenic micro-organism to colonize and penetrate the skin unless it is damaged by injury or the natural flora is disturbed by the long-term use of antibiotics or antiseptic products. The precautions taken by surgeons and operating-theatre staff to maintain a sterile environment during operations are of vital importance in the prevention of infections when the patient's skin is deliberately cut. *Staphylococcus epidermidis* is a very common invader of damaged skin, where it often causes minor skin abscesses or, in serious cases, can invade the blood and tissue spaces.

Some micro-organisms, such as the malarial parasite (*Plasmodium vivax*), overcome the skin barrier by first invading the salivary glands of certain *Anopheles* mosquitoes. The parasite then gains entry into the body when the mosquito feeds by injecting its saliva, which contains the parasite, through the skin.

The biting arthropods, such as fleas, lice, mites, mosquitoes, sandflies and ticks, are all important vectors in the transmission of infectious disease. For example, fleas can transmit the black plague bacillus *Yersinia pestis*, lice can transmit typhus (a rickettsial disease), and the Tsetse fly will transmit sleeping sickness (caused by *Trypanosoma* sp.) from infected animals to man. The diseases transmitted by these vectors can be reduced to some extent by controlling the vectors with insecticides. The use of the insecticide DDT to control the mosquito has largely eliminated malaria from Europe; however, as the mosquito is becoming resistant to modern insecticides, the disease is slowly moving back to these areas.

Infections through the skin can also occur as a result of a bite from a mammal. A good example of this is the transmission of the **rabies** virus from the bite of an infected animal (such as a dog or a vampire bat). The saliva from the infected animal will be transferred into the wound as the animal bites, and this will cause the individual who has been bitten to develop rabies.

Some infections are introduced into the body by accidental injection of contaminated blood. **Hepatitis B virus** and the **human immunodeficiency virus (HIV)** can be introduced into the body by a contaminated blood transfusion or by the use of shared hypodermic needles by drug addicts. This route of infection is entirely man-made and can be controlled by better screening of blood for viruses and by better health education.

A small number of parasites have developed mechanisms which enable them to penetrate intact skin. For example, the human blood flukes of the genus *Schistosoma* produce free-swimming, aquatic *Cercariae* which penetrate unprotected skin and invade the bloodstream causing the disease schistosomiasis (bilharzia). Humans can become infected by swimming or wading in water that contains the parasite, which is found in tropical countries such as Africa, the Far East and South America.

The mucous membranes that line the gut, respiratory and urinogenital tracts are constantly exposed to external micro-organisms, and

the majority of infections start on these membranes. The structure of a mucous membrane is much more delicate than that of the skin. The outer surface consists of a layer of **epithelial cells** and mucus-secreting **goblet cells** which are attached to a basal membrane. The mucus produced by the goblet cells bathes the surface of the membrane and is continually being swept to the outside of the body (Figure 2).

Moving mucus is a vital defence against infection which is shown in the disease **cystic fibrosis**. Sufferers produce a highly viscous mucus which is not cleared from the lung making these individuals very prone to respiratory tract infections.

Beneath the outer epithelial layer is found a region known as the **lamina propria**. This region consists of a loose web of **connective tissue fibres**, in which are found phagocytes and the cells of the immune system. Occasionally the lamina propria will penetrate into the outer epithelial layer of the mucous membrane forming nodules of lymphoid tissue which in the small intestine are known as **Peyer's patches**.

In addition to the physiological defence mechanisms, further protection is provided by the natural microbial flora of the mucous membrane. All mucous membranes are colonized by a distinctive natural flora which is attached to the outer surfaces of the epithelial cells. The bacterial species which are found in the natural flora vary at different sites along the mucosa. Therefore, any invading micro-organism faces a similar situation to that of a micro-organism attempting to colonize the skin; but in this case an additional problem of a moving layer of mucus has to be overcome.

Figure 2. Ciliated epithelium with goblet cells.
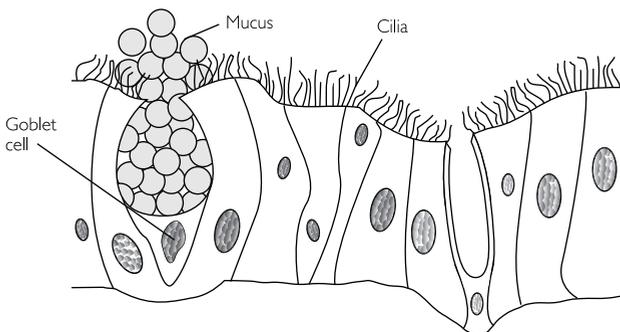
Most micro-organisms that infect mucous membranes have developed mechanisms that enable them to attach to the host's epithelial cells. The surface structures on the micro-organisms that are responsible for this adherence are called **adhesins**. Adhesins are often filamentous proteins, such as **pili**, or cell wall components.

Adhesin molecules bind to specific surface structures on the host's epithelial cells which allow the invading micro-organism to attach to specific areas of the mucosa and to resist being swept away by the stream of mucus. Bacterial adhesins are, therefore, important virulence factors in the early stages of infection.

Micro-organisms that infect the respiratory tract, such as the whooping cough bacillus *Bordatella pertussis*, will inhibit and then attach to the cilia of human ciliary epithelial cells — the disease is then caused by the release of an exotoxin that causes death of the local epithelia. A similar mechanism is used by *Vibrio cholera* when it infects the small intestine where the organism adheres to the intestinal epithelial cells and releases an exotoxin which disturbs fluid transport across the intestinal epithelium.

The above examples are all infections that are caused by Gram-negative micro-organisms, which colonize the surface of the mucosa and release exotoxins. Many micro-organisms, however, cause disease by first colonizing the mucosa and then invading the sterile body tissues underneath.

Bacteria such as *Salmonella typhi*, which causes the blood infection known as typhoid, attach to the epithelial cells of the intestinal mucosa and are taken up by specialized mucosal cells known as **microfold (M) cells**. The M cells carry the ingested bacteria through their cytoplasm and release them, unharmed, into the lamina propria (Figure 3).

The normal role of the M cells is to sample the environment external to the mucosa and to deliver it to the mucosal immune system so that the system is aware of the external environment. It is unfortunate for mammals that some pathogenic micro-organisms have also discovered this route for entry into the body.

Gram-positive bacteria, unlike Gram-negative bacteria, do not produce fimbriae and so can only attach to the mucosa by specialized cell wall structures. Virulent forms of *Streptococcus pyogenes* Group A attach to the extracellular matrix protein **fibronectin**, which is present on the surfaces of epithelial cells with a cell wall protein. *Streptococcus pneumoniae* adheres to the respiratory mucosa by a different mechanism
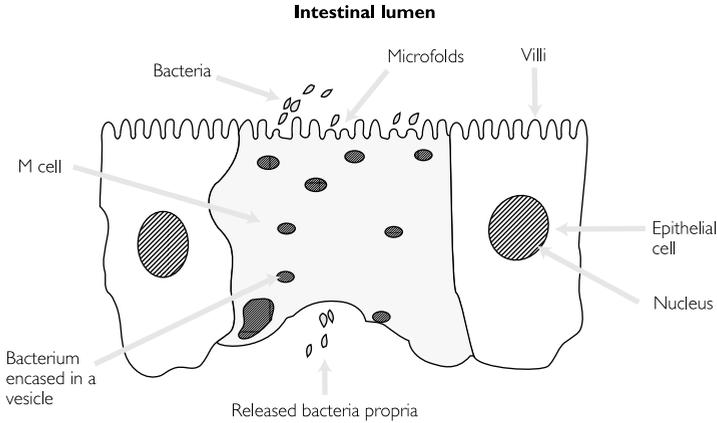
Intestinal lumen

Figure 3. The uptake and release of pathogenic bacteria by M–cells in the intestinal lymphoepithelium.

which uses a cell wall protein that recognizes specific sugars expressed on the epithelial cell's surface.

These proteins, which recognize specific sugars, are known as **lectin-like proteins** and are often used by both Gram-positive and -negative micro-organisms to attach to specific surfaces. A well-known example of a Gram-negative organism that uses this mechanism is the intestinal bacterium *Escherichia coli*, whose type-1 common fimbriae recognize α1-linked **mannose**, a common component of mammalian cell-surface **glycoproteins** and **glycolipids**.

Viruses can also infect mucosal cells by binding to specific cell-surface receptors with receptors that are expressed on the protein coat or membrane of the virus. Once a virus has gained entry to the body it will multiply and spread, often infecting specific tissues which it recognizes by its viral receptors. The **polio virus**, which normally causes an infection of the gut, can also spread and infect the central nervous system causing **paralytic poliomyelitis**.

Micro-organisms which infect the body at the mucosal surfaces use a variety of mechanisms to attach to the mucosal epithelial cells. Once these mechanisms are discovered it should be possible to develop vaccines that stimulate the immune system to block the attachment of these organisms.

Mechanisms for survival in the host

Pathogenic micro-organisms are unique in that they have mechanisms that enable them to survive in the host by interfering with and overcoming the host's defence mechanisms.

Bacteria can do this by producing compounds known as aggressins which act on the non-specific and the specific immune systems (Table 1). Aggressins are very important virulence factors in the later stages of a **systemic** microbial infection.

Table 1. The main components of the non-specific and specific immune systems

Non-specific immune system	Specific immune system
Soluble components	**Soluble components**
Acute phase proteins	Antibodies
Complement	Cytokines
Interferons	
Lysozyme	
Cells	**Cells**
Natural killer cells	B-lymphocytes
Phagocytes	T-lymphocytes

The non-specific immune system is a basic defence system consisting of anti-microbial serum proteins, such as the **complement system**, and the mobile and fixed phagocytic cells such as **neutrophils** and **macrophages** which will take up and destroy invading micro-organisms. (Figure 4).

The specific immune system will recognize specific molecules that are produced by an invading micro-organism. These are known as **antigens** and can be polysaccharides, proteins, glycoproteins and lipoproteins.

The immune system responds and produces **antibodies** and **killer small lymphocytes** which react with and destroy the antigen. If an individual becomes infected for the first time by a pathogenic micro-organism this will trigger an immune response which is known as a **primary response**. During the primary response the immune system develops what is known as **immunological memory**, which enables the immune system to remember its response to the infecting organism (Figure 5).

Once immunological memory has developed, this will cause the system to produce a rapid **secondary response** if it encounters the same micro-organism again. An individual who develops immunological
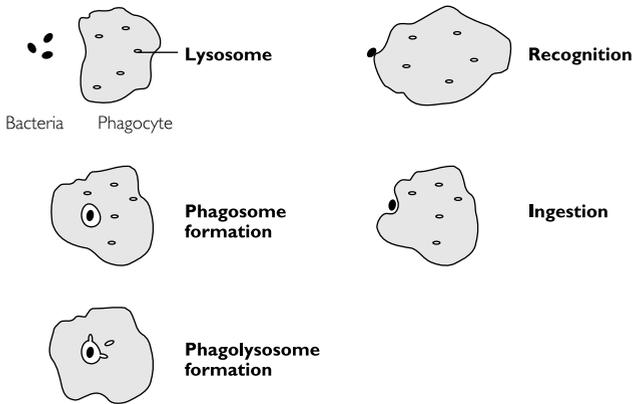
Figure 4. Uptake of bacteria by phagocytic cells (neutrophils and macrophages)
The process of phagocytosis starts with the recognition of the bacterial cell as foreign by
the phagocyte. The cytoplasmic membrane of the phagocyte then moves around the bac-
terium and it is taken into the cell contained in a phagosome. Once inside the phagocyte,
the phagosome fuses with a lysosome to form a phagolysosome, and the bacterium is
killed by antimicrobial substances.

memory to a pathogenic micro-organism will normally be **immune** to
that specific organism for the rest of his/her life. For example, if an indi-
vidual recovers from an infection caused by the mumps virus, it is very
unlikely that he/she will become reinfected with the virus.

It is, therefore, vitally important for a pathogenic micro-organism to
avoid these defences if it is to survive and produce an infection. The main
ways by which micro-organisms avoid the body defences are detailed in
the following sections.

Avoidance of the non-cellular (humoral) defence systems
One of the most effective ways of doing this is to hide from the soluble
defence mediators by invading and growing inside the host cells.
Organisms that do this can avoid the actions of both complement and
antibodies. The best exponents of this type of defence are the obligate
intracellular parasites (viruses) which only become susceptible to the
host humoral defence mechanisms if they leave an infected cell, or if
antigens from the virus are displayed on the infected cell surface.
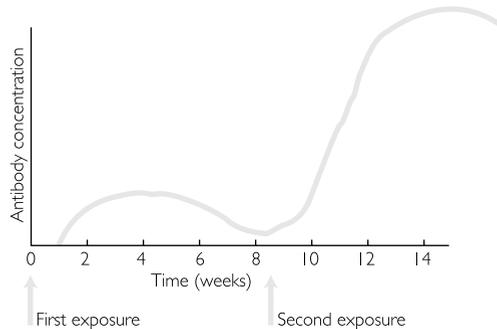
Figure 5. Primary and secondary immune responses after exposure to antigen
The first exposure of a non-immune individual to antigen results in a low-level primary immune response, which can be detected by a rise in antibody levels after 5–7 days. A secondary immune response will be produced if the individual is re-exposed to the same antigen as the primary response declines. The onset of the secondary response is rapid, and high levels of antibody are produced which persist for several months.

Some bacteria have also developed this avoidance strategy: for example, *Neisseria gonorrhoeae*, which is susceptible to complement and avoids its action by invading the mucosal epithelial cells of the reproductive tract causing gonorrhoea.

Other organisms, such as *Yersinia*, can also invade epithelial cells by producing bacterial surface proteins known as **invasins** which bind the organism to the cell and then stimulate its uptake.

The facultative intracellular parasites have adopted a different approach to avoid humoral defences as they do not have the ability to invade cells. These organisms, if they are taken up by phagocytic cells, can avoid their killing mechanisms and multiply in the phagocyte.

The ways by which this is achieved are not clear at present but it is known that some organisms — such as *Brucella abortus*, which causes abortion in cattle — release soluble mediators which prevent the activation of the phagocytic killing mechanisms. Others, such as *Salmonella typhi*, have cell wall structures that resist phagocytic killing. Extracellular parasites can also resist the humoral defence systems; for example, Gram-positive bacteria are resistant to the lytic action of complement due to the thickness of their cell walls. Gram-negative bacteria, such as *Salmonella typhimurium*, have long chains of the cell wall component **lipopolysaccharide** extending from the cell which protects against complement damage.

The action of specific antibodies can be resisted by an organism if it mutates and its progeny express surface antigens that are different to those expressed by the parent cell to which the antibodies were raised. This type of resistance is shown in *Neisseria gonorrhoeae*, which has multiple genes coding for common fimbriae which enables the organism to alter its antigenic structure by genetic reassortment.

The protozoan parasites of the genus *Trypanosoma* have also adapted this strategy since they have multiple genes which code for cell-surface glycoproteins, thereby allowing the organism to alter its antigenic structure by gene switching.

The action of antibodies which normally bind to the antigens on the cell surface can also be resisted by binding them in an incorrect configuration. This is done by the cell wall protein, **Protein A**, expressed by *Staphylococcus aureus* which binds IgG by the Fc region (Figure 6) so that it cannot by recognized by the IgG receptors expressed on phagocytes. Some micro-organisms use destructive mechanisms to avoid the specific immune system. These include suppressing the immune response by destroying T-helper cells, as seen in HIV infections, and by releasing protease enzymes that destroy antibodies, e.g. *Neisseria gonorrhoeae* releases an IgA protease which destroys IgA class antibodies.

Avoidance of phagocytosis

When extracellular parasites are taken up by phagocytes they are invariably destroyed and, if the phagocytic cell involved is a macrophage, the specific immune system will also be triggered. The ability to avoid phagocytosis is, therefore, an important pathogenic mechanism for these organisms. As mentioned previously, facultative intracellular parasites use other mechanisms to survive phagocytosis which resist the phagocyte's killing mechanisms.

Many extracellular bacteria have surface structures, such as polysaccharide capsules, fimbriae or lipopolysaccharide, which enable them to avoid phagocytosis. Bacterial capsules are non-toxic structures that surround the bacterial cell wall and hinder contact with phagocytes, thus preventing effective phagocytosis.

Streptococcus pneumoniae, *Haemophilus influenzae* type b and *Neisseria meningitidis*, which cause the majority of cases of bacterial meningitis in children, produce capsules that are the main virulence factors for these organisms. Experiments on microbial pathogenicity have demonstrated clearly the importance of bacterial capsules, e.g. the

loss of a capsule by *Streptococcus pneumoniae* renders the organism avirulent.

The production of specific anti-capsule antibodies by an infected host is an important defence against these structures. Antibodies that bind to the capsular surface will neutralize its antiphagocytic effects, as phagocytes have cell-surface receptors which recognize and bind to IgG class antibodies, resulting in phagocytosis. Before effective antibiotic therapy was available, this effect was frequently seen in the disease lobar pneumonia, caused by *Streptococcus pneumoniae* — the disease would reach a crisis point after about 7 days which coincided with the production of anti-capsular antibodies; if the antibody response was effective the patient recovered but if the response was ineffective the patient died.

An alternative antiphagocytic strategy is shown by *Staphylococcus aureus*, which produces a lethal exotoxin known as **PV leucocidin.** The toxin kills both neutrophils and macrophages and is responsible for the accumulation of dead phagocytes, which form pus around the infection seen in the formation of a boil.
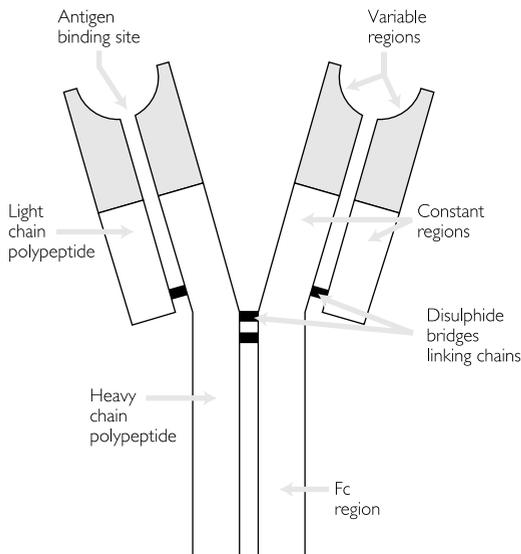
Figure 6.
Structure of an IgG class antibody molecule

Host damage resulting from a microbial infection

There are three main ways by which micro-organisms can cause damage to the host: direct damage, which is due to the growth or the release of enzymes and toxins by the invading organism; damage due to the activation of the immune system; and the induction of cancer.

Direct damage

Direct damage to the host tissues usually occurs soon after the establishment of an infection, for example many viral infections result in the damage or lysis of infected cells. Polio virus will destroy the nerve cells that it infects and the **influenza virus** damages the mucosal cells of the respiratory tract. The ability to damage body cells is not confined to the viruses — the malarial parasite *Plasmodium* damages red blood cells, and facultative intracellular parasites will damage phagocytes.

Bacterial exotoxins are heat-labile protein toxins which are secreted during growth. Exotoxins are highly active compounds. A single **neurotoxin** produced by *Clostridium tetani* is responsible for the symptoms of tetanus. The toxin acts by binding to the central nervous system where it blocks the normal inhibitory mechanisms that control the spinal motor nerves. The lack of inhibition causes the spinal nerves to fire continuously so that all the body's effector muscles contract, resulting in spastic paralysis.

Some exotoxins are regarded as chemical poisons since they are produced outside the body and are ingested with food. *Clostridium botulinum* neurotoxin is produced when the organism grows in food; if the food is eaten, the toxin is activated by protease enzymes in the gut and the active toxin is then adsorbed into the blood. The targets for the toxin are the neurones at neuromuscular junctions, where it blocks the release of the neurotransmitter acetylcholine thus causing flaccid paralysis.

Other bacterial toxins contribute to the spread and survival of the organism. Many of these toxins are enzymes that destroy body cells and tissues; for example, *Streptococcus pyogenes* excretes **hyaluronidase**, which breaks down the intracellular matrix that holds connective tissue together, allowing the organism to spread. **Streptokinase** is another enzyme produced by streptococci which aids the spread of these organisms by dissolving blood clots that form around the site of infection.

Haemolysins and **leucocidins** are bacterial toxins which help survival *in vivo* by destroying red blood cells and white cells. The release

of the essential growth nutrient iron from lysed red blood cells will aid the growth of the infecting organisms as the metal is highly conserved by the human body for which it is also an essential nutrient. The roles of leucocidins *in vivo* are difficult to evaluate but, because they destroy the white blood cells of the non-specific and specific immune systems, this must prolong the survival *in vivo* of the organisms that produce them.

An important group of bacterial exotoxins that have only recently been recognized are the **superantigens**, which include *Staphylococcus aureus* **enterotoxin** and **exfoliatin**, and the **pyogenic** toxins of *Streptococcus pyogenes*. These toxins trigger non-specific overactivation of the immune system, causing the release of soluble mediators known as **cytokines** which stimulate, suppress or alter cell function. Cytokines cause the symptoms associated with **toxic shock syndrome**, **bacterial food poisoning** and inflammation. Overstimulation of the body systems by cytokines results in shock and, in many cases, this leads to death, which cannot be prevented by prompt medical intervention.

A component which is only released from Gram-negative organisms that has a powerful stimulatory effect on the immune system is **endotoxin**. Bacterial endotoxin is composed of lipopolysaccharide, which forms part of the outer cell wall layer of these organisms. The presence of endotoxin in the blood due to a Gram-negative infection will cause fever, inflammation and **septic shock** which, like toxic shock syndrome, may be irreversible. Septic shock syndrome is a major threat to human health and is responsible for over 500 000 deaths per year worldwide.

The similarities between the symptoms of toxic shock syndrome and septic shock suggest that these diseases have a common underlying mechanism. The superantigens and bacterial endotoxin both cause the release of the cytokines **tumour necrosis factor (TNF)**, **interleukin 1**, **interleukin 6** and **interleukin 8,** but there are differences in the cell populations involved in cytokine release. Both macrophages and T-lymphocytes are activated by superantigens, whereas only macrophages are activated by endotoxin.

Activation of the immune system

Stimulation of the specific immune system by microbial antigens is beneficial in most cases but, with certain types of infection, can result in damage to the host. For example, infected body cells that display microbial antigens on their surfaces will be destroyed by the immune

system and, if the cells are vital to the body's function and are not replaced, this will cause permanent damage.

The immune system can be tricked to react with its own normal body cells by micro-organisms which produce antigens very similar to those expressed by the host. *Streptococci* have antigens which are similar to those expressed on human cells; consequently, an immune response against a streptococcus will cause the destruction of normal body cells.

The production of antibodies against a pathogenic micro-organism or its products normally results in the removal of the antibody/antigen complex by the phagocytic system, with little if any permanent damage to the host. In chronic infections caused by *Aspergillus fumigatus*, hepatitis B virus, malaria and *Streptococci* there can be a failure in the removal of the **immune complexes** which deposit in the small blood vessels of the kidney glomeruli, joints and lungs.

Neutrophil phagocytes will try to remove the immune complexes, which results in serious damage to the blood vessels and tissues. This can be seen in the disease **glomerulonephritis**, where immune complex deposition in the kidney results in severe damage to the glomeruli which can eventually lead to kidney failure.

Similar reactions in the lungs as a result an infection of *Aspergillus fumigatus* will cause the destruction of the alveoli and a decrease in lung function.

The immunological defence against facultative intracellular parasites can be very destructive as this involves **cell-mediated immunity** which is due to T-lymphocytes not antibodies. T-lymphocytes are activated by microbial antigens to produce cytokines, which attract macrophages into the infected area and activate the cells to produce **activated macrophages**. Macrophages at this level of activation are killer cells and will cause non-specific damage to surrounding tissues while trying to remove the organism.

The activated macrophages and T-lymphocytes also form a barrier around the infection known as a **granuloma**, which can eventually become calcified causing loss of normal tissue in the area. Granuloma formation and tissue damage are commonly found in chronic bacterial infections caused by *Mycobacterium tuberculosis* and *Mycobacterium leprae* where the immune response is responsible for many of the disease symptoms. The tissue damage that results from cell-mediated immunity is better than no response at all, as both these infections are lethal.

Induction of malignant disease

A group of viruses, known as the **oncoviruses**, are able to convert the cells that they infect into **malignant** tumour cells. The best-known examples of oncoviruses are the **human T-cell leukaemia viruses**, which are **retroviruses**, and **Epstein-Barr virus**, which normally causes glandular fever but can also cause nasopharyngeal carcinoma in China and Japan and tumours of B-lymphocytes in a disease known as **Burkitt's lymphoma** in East Africa.

Many other viruses are suspected of causing tumours, for example the **human papilloma viruses** are thought to cause **cervical cancer**; however, more evidence is require before this can be confirmed.

Transmission of infection

An organism that causes an infectious disease has to be transmitted to a new host if it is to survive since the infected host will either become immune to the organism or die. The main routes for the transmission of infectious diseases are via the gastrointestinal, respiratory and urogenital routes. Infections transmitted by infected blood being transferred by animal, arthropod and insect bites are less common. Direct contact is the route by which skin infections such as boils, impetigo, fungal ringworm and warts are spread.

Gastrointestinal transmission

Organisms that cause gastrointestinal infections are shed from the body in the faeces. If faecal contamination of food or water occurs as a result of poor hygiene, a potent source of infection is produced.

The contaminating organisms will then be transmitted by ingestion if the infected material is not properly treated before consumption to remove the organisms. Diseases such as cholera, poliomyelitis, typhoid and food poisoning are transmitted via this route.

Respiratory transmission

Respiratory tract secretions are the means by which respiratory infections such as the common cold virus, influenza virus, *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* are transmitted.

Aerosol droplets that are produced by coughing, sneezing and talking can contain infectious micro-organisms which, if inhaled by a non-immune individual, will colonize the respiratory tract and cause an

infection. Transmission is made more efficient by overcrowding in poorly ventilated rooms — which is why respiratory tract infections are more common in the winter months.

Urinogenital transmission

Sexually transmitted diseases such as AIDS, gonorrhoea and syphilis are caused by organisms that cannot survive for long periods of time outside the human body as they are susceptible to drying out. Infections can, therefore, only be transmitted by direct contact between the infecting organism and the mucous membranes of the genital tract. The urinary tract can also become infected during sexual intercourse, owing to its proximity to the genital tract.

4

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