10. Blood

10.1 Composition and Functions of Blood

Blood circulates through the body bringing O_2 and nutrients to the tissues and removing CO_2 and other waste products. As it moves around the body it aids interchange between the fluid compartments, dissipates heat and distributes hormones, thus helping to maintain homeostasis and to coordinate the activities of the various organs. In addition blood contains haemostatic components that control bleeding. Finally, it performs a role in defending the body against foreign invaders as it carries cells and antibodies that seek out and destroy microorganisms and foreign proteins.

Blood can be separated into two components - a yellowish fluid, *plasma*, and *cells* which are suspended in it. Plasma is that part of the extracellular fluid which is restricted to the blood vessels. The cells are of three kinds - red cells (erythrocytes), white cells (leucocytes) and platelets (thrombocytes).

Plasma

Of thee 5-6 L of blood in the adult body, approximately 3 L is plasma. Approximately 90% of the plasma is water, 8% is protein and 2% is organic compounds of low molecular weight and electrolytes.

The plasma proteins, because they do not cross capillary walls readily, are largely restricted to the blood vessels. The major plasma proteins are albumin, globulins and fibrinogen.

Albumin (MW 69000), the most abundant protein (about 40 g/L plasma), is synthesized in the liver at the rate of about 15 g per day. It is the major contributor to the colloid osmotic pressure of the blood which determines the return of fluid to capillaries at the venous end. It can carry in loose combination certain hormones (i.e., corticosteroids), bilirubin, fatty acids, salts of bile, heavy metals (i.e., mercury and copper) and some drugs. It also plays a role as a buffer for plasma H+ ions and carries a small amount of CO_2 as a carbamino complex.

Globulins were originally separated from albumins by salt fractionation and are differentiated from them by lesser solubility, greater molecular weight and slower electrophoretic mobilities. They can be further fractionated by electrophoresis into alpha, beta and gamma globulins. These proteins vary widely in molecular weight and some contain carbohydrate (glycoproteins). Most of the alpha and beta globulins are synthesized in the liver but the gamma globulins are produced by plasma cells. Specific alpha and beta globulins are important in the transport of various substances i.e., thyroxine-binding globulin for thyroxine, transcortin for corticosteroids, transcobalamin for vitamin B_{12}, transferrin for iron, ceruloplasmin for copper and various lipoproteins for lipids. Kininogens and angiotensinogen are alpha globulins which are precursors of kinins and angiotensin I respectively. The gamma globulins and some of the beta globulins form a very important group called *immunoglobulins* which are *antibodies.*

Fibrinogen, synthesized in liver, is a dimer, each subunit (MW 340000) being composed of three peptide chains. It is converted to the insoluble form, fibrin, in the coagulation process, in the course of which several coagulation factors are consumed. The remaining fluid is called *serum.*

Red Blood Cells (Erythrocytes)

These cells, which comprise more than 99% of the blood cells, have as their primary function the carriage of respiratory gases. In man the red blood cells are biconcave discs, of diameter about 7-8 microm and of thickness about 1 microm in the centre and 2 microm at the edge. This curious shape has several advantages: (i) It produces a larger surface area/volume ratio than would be the case in a spherical cell of the same volume. There is thus more surface available for gas diffusion into and out of the cell. (ii) It enhances cell flexibility, permitting distortion as the cell passes through small capillaries with subsequent rapid restoration of the normal shape. (iii) It results in minimal tension on the membrane when volume changes occur between oxygenated and venous blood.

The red cells contain *haemoglobin* (35% of their net weight). There is no turnover of haemoglobin in the mature cell. Each haemoglobin molecule is made up of the protein *globin* (MW 65000) to which are attached four *haem* groups, containing iron in the ferrous form. The ferrous ion combines quickly and reversibly with O_2, one molecule of haemoglobin combining with up to four molecules of O_2. In this way haemoglobin carries out the primary function of the red cell which is *oxygen transport.* In addition, the globin part of haemoglobin carries some of the CO_2. It acts too as a buffer for H+ ions. Red cells also contain the enzyme *carbonic anhydrase,* which plays a crucial role in the rapid hydration of CO_2. This process is important for *carbon dioxide transport* in the blood.

The mature mammalian red cell is unusual in that it has no nucleus or mitochondria. It derives its energy in the form of ATP from anaerobic glycolysis. Compared with other cells its energy requirements are very small, but some energy is required to operate the sodium pump, maintain cellular volume and preserve the integrity of the cell. In addition, because of the high ambient oxygen tension to which the red cell is exposed, it has a reducing system to counteract the effects of oxidation. A small percentage of haemoglobin continually undergoes oxidation to *methaemoglobin* which contains iron in the ferric form, in which state it cannot combine with O_2. NADH generated in the glycolytic pathway is utilized to reduce methaemoglobin back to haemoglobin. There is also an oxidative pentose-phosphate pathway in the red cell which accounts for some 5% of glucose utilization. It results in the generation of NADPH which, together with glutathione, keeps intact the sulphydryl groups of haemoglobin and other proteins. The red cell also possesses an enzyme which can convert 1,3-diphosphoglycerate (DPG), generated within the glycolytic pathway, into 2,3-DPG. This combines reversibly with the globin chain of the haemoglobin molecule to reduce the affinity of haemoglobin for oxygen.

The *haematocrit* or *packed cell volume (PCV)* is the fraction of a volume of blood occupied by the erythrocytes. The *erythrocyte sedimentation rate (ESR)* is obtained by allowing anticoagulated blood to stand in a vertical tube of narrow bore. The red cells, being more dense than the plasma, will gradually sink under the action of gravity leaving a clear

layer of plasma at the upper end of the tube. The ESR (Westergren method) is the length in mm of the column of plasma above the red cells after 1 h at room temperature. Various factors influence the ESR but of particular importance is the extent to which red cells form rouleaux (in which the cells are arranged like piles of coins) because rouleaux sediment more rapidly than single cells. Rouleaux formation is enhanced by increases in the concentration of various plasma proteins, particularly fibrinogen. Its magnitude is often an indicator of the severity of a variety of diseases so that the test is useful but non-specific.

In disease abnormal red cells may appear in the blood. They can be detected by microscopic examination of a blood film stained, commonly, by the Romanovsky stain in which eosin stains cytoplasm red and methylene blue stains nuclear material blue. Red cells may vary in size (*anisocytosis*). They may be larger than normal (*macrocytes*) or smaller than normal (*microcytes*). They mal also vary in shape (*poikilocytosis*). Some may lose their biconcave shape and become spherical (*spherocytes*). Others may be distorted as a result of abnormal haemoglobin formation (i.e., *sickle cells*).

Normal blood contains a small number of *reticulocytes* (0.5-2.0%) which are immature red cells containing ribosomal remnants in their cytoplasm. After 1 to 2 days in the peripheral blood these remnants are completely lost and mature erythrocytes result. Reticulocytes can be identified in a blood film by staining, before fixation, with cresyl blue which reveals the ribosomal material. The reticulocyte count gives an indication of the erythropoietic activity of the bone marrow.

White Blood Cells (Leucocytes)

Unlike red cells, white cells are nucleated. They are usually larger (diameter 8-20 microm) than red cells, but are greatly outnumbered by them. Three groups of white cells can be recognized in blood films stained with a Romanovsky stain: granulocytes, lymphocytes and monocytes.

1. *Granulocytes* contain obvious granules in the cytoplasm. Depending on the staining reaction of the granules, they can be further subdivided into neutrophils, eosinophils and basophils.

Neutrophils (diameter 10-12 microm) in the adult comprise 40 to 75% of the total white count. Their cytoplasm contains fine pink-purple granules. Their nucleus usually has two to five lobes, the number increasing with cell age. Neutrophils act as phagocytes in the acute inflammatory response.

Eosinophils (diameter 10-12 microm) have a cytoplasm packed with coarse red of orange granules. Their nucleus is typically bilobed. Their functions include (i) inactivation of histamine released by mast cells and basophils in inflammatory and immunological responses; (ii) phagocytosis of antigen-antibody complexes; and (iii) release of fibrinolysin which dissolves fibrin clots. An eosinophilia, i.e., an increase in eosinophil numbers beyond the normal range, occurs in allergic disorders and parasitic infestations.

Basophils (diameter 7-12 microm) have a cytoplasm containing coarse dark blue granules which obscure the nucleus. Their granules contain histamine and heparin which are released by local antigen-antibody reactions. Basophils are closely related to if not identical with mast cells of connective tissue.

2. *Lymphocytes* in the circulation are of two sizes - 'small' (diameter 7-9 microm) and 'large' (diameter 8-16 microm). They vary in appearance but characteristically are round cells with a large densely-staining nucleus surrounded by a thinner or thicker rim of cytoplasm. They play a key role in both humoral (production of antibodies) and cell-mediated immunity.

3. *Monocytes* (diameter 16-20 microm) are the largest blood cells. Their nucleus is variable in shape and their cytoplasm pale-staining, often showing, with special stain, small lilac-coloured granules. They act as tissue macrophages in the acute inflammatory response.

Platelets (Thrombocytes)

These (diameter 2-3 microm) are small pieces of cytoplasm lacking nuclei. The total platelet count in blood normally lies within the range 150-400 x 10^9 per L.

10.2 Blood Cell Production

In the developing fetus primitive red cells first appear in the mesoderm of the yolk sac. From the second to the seventh month of fetal life, all blood cell types are formed in the liver and spleen. Meanwhile, during the fifth month, production of blood cells begins in the red bone marrow which gradually takes over this function from the liver and spleen. After birth the red marrow is the only source of blood cells apart from lymphoid tissue in which lymphocytes are formed. In childhood red marrow occupies the cavities of all bones. In adults it is confined to the bones of the trunk, the skull and thee upper ends of the humerus and femur.

Ageing blood cells are continually being destroyed. In addition leukocytes are destroyed as a consequence of their activities. Blood cells are continually being replaced and their rate of production is regulated so that the numbers of each cell type remain remarkably constant. On average red cells have a life-span of 120 days, platelets 10 days, monocytes 24-48 hours (in blood) and neutrophils 6-8 hours (in blood). Note that monocytes and neutrophils spend a relatively short time in the blood. They use it only for transport to the interstitial space where they carry out their functions.

Dying blood cells are phagocytosed by *macrophages* of the *reticuloendothelial system (mononuclear phagocytic system).* This is a general name for those areas in the red bone marrow, liver, spleen, lymph nodes and loose connective tissue which contain these macrophages. Platelets may also be destroyed by macrophages in the lung. When red blood cells are destroyed by the macrophages, the globin part is degraded into amino acids and the haem is converted into *bilirubin* and *iron.* Bilirubin is bound to the plasma albumin and transported to the liver to be excreted through the biliary system. The iron is bound to the plasma protein transferrin and transported to the erythroblasts of the red bone marrow to be re-used.

Development of Blood Cells

Blood cells are derived from primitive undifferentiated marrow cells known s *stem cells.* These cells can replace their own number by mitosis and, because they can develop along various pathways, they are called multipotent stem cells. From this reservoir of multipotent cells *committed* or *unipotent* stem cells develop which give rise to erythrocytes, granulocytes, lymphocytes, monocytes or platelets. Note that platelets are formed from the cytoplasm of *megakaryocytes.* Committed stem cells in the bone marrow undergo *proliferation* and *maturation* through a series of stages to form adult cells. The stem cells and, initially, the developing blood cells lie outside the blood vessels of the bone marrow. When still immature the blood cells enter the sinusoids eventually to enter the bloodstream.

As the red cells develop (*erythropoiesis*) in the bone marrow from *erythroblasts* they undergo characteristic changes. They become smaller, hemoglobin accumulates and the nucleus decreases in size and is finally extruded. Certain substances are necessary for normal red cell development. *Iron* is essential for haemoglobin synthesis. A deficiency of *vitamin* B_{12} or *folic acid* results in abnormal maturation of red cells in the marrow, a decrease in the number of cells in the blood, and the presence of both immature and abnormally large, fragile cells. *Pyridoxine* is required in the course of haem production. Traces of *cobalt,* which forms part of the vitamin B_{12} molecule, and *copper* are required, and so too is the hormone *thyroxine.*

Control of Blood Cell Production

There is good evidence that oxygen tension in the kidney is the major factor regulating erythropoiesis. When oxygen tension falls, renal erythropoietic factor, released by the kidney, combines with a plasma protein from the liver to form *erythropoietin* which increases thee number of stem cells committed to erythropoiesis and accelerates the rate of the process. The underlying cause of the anaemia associated with chronic kidney disease is erythropoietin deficiency. Although erythropoietin plays the main role in controlling erythropoiesis, the endocrine glands - gonads, pituitary, thyroid and adrenals - appear to have a secondary modifying influence.

Neutrophils in blood may produce a substance which circulates to the bone marrow and inhibits production and release of new cells. Thrombopoietin is a humoral agent which controls production of platelets.

By *anaemia* is meant a reduction in the concentration of haemoglobin in the blood below the lower level of the normal range for the age and sex of the patient. This may be due to (i) reduced production of red cells or to a decrease in their Hb content or to both, (ii) bleeding or (iii) a decrease in the life-span of the circulating cells. *Deficiency of iron* resulting in a reduced concentration of Hb in the red cell is the commonest cause of anaemia. The deficiency may arise from insufficient iron in the diet, from malabsorption in the gut or from excessive blood loss. Deficiencies of vitamin B_{12} or folic acid cause *megaloblastic anaemias* characterized by larger than normal red cells in the marrow (megaloblasts) and blood (macrocytes). *Pernicious anaemia* is a form of megaloblastic anaemia resulting from an inability to produce intrinsic factor in the stomach and hence to absorb vitamin B_{12} from the

ileum. *Sickle-cell anaemia* is due to the production of an abnormal haemoglobin. *Aplastic anaemia* is caused by bone marrow damage (most commonly drug-induced) resulting in a failure of production of all blood cell elements.

An increase in the total number of circulating white cells beyond the normal range (*leucocytosis*) occurs in bacterial infection. A decrease in the number of circulating white cells (*leucopenia*) can arise, for example, through the action of drugs which suppress white blood cell production in the bone marrow. The *leukaemias* are a group of diseases which are characterized by uncontrolled proliferation of white cells.

10.3 Defence Systems of the Body

The body's first line of defence against foreign invaders is the tough outer layer of the skin. Mucous membranes lining the mouth, gut and respiratory passages also provide protection. Cilia in the upper respiratory tract can sweep particles into the pharynx whence they are swallowed. Sweat, sebaceous secretions and tears contain bactericidal and fungicidal substances and acid secreted by the stomach destroys many ingested microorganisms. Finally, the normal microflora of the skin and gut helps to suppress the growth of more virulent microorganisms.

Acute Inflammatory Response

When an area of the body becomes invaded by bacteria, *acute inflammation* develops in response to the toxins and enzymes released by the bacteria. There is local dilatation of blood vessels, mediated by the activation of tissue plasma *kinins* and by the release of *histamine* from mast cells and basophils. The dilatation increases blood flow causing local *redness* and *heat,* and bringing more white cells to the site of infection. Kinins and histamine also increase the *permeability* of the capillary walls resulting in a protein-rich fluid entering the interstitium with local *swelling.* Kinins also stimulate nerves in the infected area producing the sensation of *pain. Neutrophils* adhere to the vessel walls and then pass through into the interstitial spaces by an amoeboid movement called *diapedesis.* They are attracted into the infected area by kinins and other chemical compounds (*chemotaxis*) and phagocytose the bacteria, this process being enhanced by the *complement system of proteins.* The ingested bacteria are then destroyed by lysosomes contained in the neutrophil granules. *Monocytes* follow neutrophils into the infected area and are transformed into tissue *macrophages* which also phagocytose bacteria. In addition chemicals (*pyrogens*) produced by white cells and bacteria may induce *fever.*

A substance which plays an important, albeit non-specific, role in defending the body against viral infection is *interferon.* It is a protein produced in the body in response to viral infection and it inhibits viral replication.

Specific Immune Response

The body has a second defence system directed against specific invaders or foreign tissue. This system is characterized by its ability to produce a *specific immune response,* i.e., a specific reaction to the entry into the body of a 'foreign' or 'non-self' substance (i.e.,

bacteria, viruses, proteins of various kinds). A foreign substance which evokes such a response is called an *antigen.*

There two kinds of specific immune mechanisms - humoral and cell-mediated. In both cases *lymhocytes* are involved. In *humoral immunity* B lymphocytes proliferate and differentiate into *plasma cells* which produce *antibodies* (immunoglobulins) that can combine chemically with the specific antigen that stimulated their production. By so doing antibodies may coat bacteria, enhancing the likelihood of their phagocytosis, or they may combine with and neutralize bacterial toxins. In *cell-mediated immunity* T lymphocytes react directly with the foreign cells, for example tissue transplants. They may also act on cells which are harbouring organisms such as the tubercle bacillus and viruses.

Lymphocytes are found in the lymphatic tissue in lymph nodes, spleen and the lining of the GIT (i.e., tonsils, Peyer's patches). There are always some circulating lymphocytes which pass from the blood into the lymphatic system and back again. The *B lymphocyte,* so called because in birds it undergoes the final stage of maturation in the bursa of Fabricius, is in adult mammals derived directly from the bone marrow. On contact with a particular antigen for which it has the appropriate receptor, a B lymphocyte undergoes proliferation to form a colony or 'clone' of plasma cells. The plasma cells secrete antibodies which combine with the specific antigens. In initiating antibody production B lymphocytes depend on cooperation from T lymphocytes and macrophages. The latter somehow process the antigen into a form suitable for interaction with the B cells. The B lymphocytes is distinguished from the T lymphocyte in that the former has immunoglobulins on its surface which act as receptors for specific antigens. The T lymphocyte can be recognized by the presence of T-specific antigens.

The *T lymphocyte* arises in the bone marrow and matures under the influence of hormones produced by the thymus. Hence the 'T' refers to the influence of the thymus. In infancy the thymus is large and active but after puberty it undergoes atrophy and in the adult it is much reduced in size. On contact with an antigen the T lymphocyte becomes 'sensitized' and on subsequent exposure to that antigen it may proliferate into helper cells, suppressor cells, or other effector cells involved in cell-mediated immunity. *Helper cells* assist the B lymphocytes to make antibodies. *Suppressor cells* inhibit this production. In cell-mediated immunity T cells may release *lymphokines* which recruit (by chemotaxis) and activate other cells, particularly monocytes and macrophages, or they may be directly cytotoxic and so are called *killer cells.* These may attack host cells infected with a virus or graft cells of foreign tissue.

The immunological system responsible for the specific immune response has several important properties. It possesses *memory* inasmuch as the system reacts more vigorously and more quickly to an antigen which it has met previously. The first contact with the antigen leads to a small rise in serum antibody titre (*primary response*) but subsequent exposure leads to a much larger increase in titre (*secondary response*). The basis of these response is believed to be the formation of *memory cells* from both B and T cells. The immunological system shows *tolerance* in that it does not normally attack 'self' material, although in some diseases (*autoimmune diseases*) this is not the case. It may also produce other ill-effects. For

example acute *allergic reactions* are caused by the release of histamine, triggered by antigen-antibody reactions.

Complement System

This is a group of eleven plasma proteins, consisting of nine components (C1 to C9) with three main subfractions of the first, which participate in the immune response leading to the destruction of foreign invaders. The system can be activated directly by the surface proteins of some bacteria (*alternative pathway*) but usually it is activated by the formation of antibody-antigen complexes. When antibodies are produced as a result of infection, they combine with antigens on the surface of the invading microorganisms and this leads to the activation of the first protein in the complement system. The other proteins in the group are activated sequentially. The activated complement enhances the inflammatory response by causing vasodilatation and increased vascular permeability, by attracting neutrophils and mast cells to the site of invasion and by coating (*opsinizing*) the surface of the microorganisms so as to enhance the possibility of their phagocytosis. They may also kill microbes directly by causing their *lysis.*

Antibodies and Their Functions

Antibodies are synthesized and secreted by plasma cells. They are found in the gamma and to a lesser extent the beta globulin fractions of serum and are referred to as immunoglobulins. They share the same light chains which may be of two types, kappa or lambda, but differ in the structure of their heavy chains. The variable regions of the light and heavy chains are the sites of interaction with antigens. Heavy chains are capable of binding complement.

IgG (MW 150000) is the most abundant immunoglobulin in serum. It also occurs in extravascular fluids and can cross the placenta. It is bivalent, i.e., it can bind two antigen molecules, and several of its subclasses also bind complement. It appears to be the main immunoglobulin synthesized during the secondary response to an antigen and is of major importance in defence against microorganisms and their toxins.

IgM (MW 900000) is a much larger pentameric form. Little is found outside the vascular system. It is produced particularly during the primary response. It is polyvalent for antigens and fixes complement avidly.

IgA exists mainly as a monomer (MW 160000) in serum and as a dimer in saliva, tears, colostrum and gastrointestinal and bronchial secretions. It is therefore primarily concerned in defending the body's surfaces and is especially effective against viral infections.

IgE (MW 200000) occurs in serum at very low concentrations. It does not bind complement but sensitizes mast cells and basophils causing them to release histamine. It is responsible for allergic symptoms in the respiratory and gastrointestinal tracts.

IgD (MW 185000) is present mainly on the surface of lymphocytes. Its function is not well understood.

By binding to bacterial antigens they immobilize bacteria or cause them to agglutinate. By interacting with bacterial toxins they neutralize their toxicity. By coating viruses they reduce their pathogenicity. Most importantly, antibodies bind complement and, when microorganisms are coated with antibody, complement-fixation may lead to (i) lysis of microorganisms, (ii) attraction (chemotaxis) of phagocytic cells toward the invasion site and (iii) facilitation of phagocytosis of the invading microorganisms.

Tissue Transplantation

The cell-mediated immune response causes the rejection of organ grafts from genetically dissimilar animals of the same species (*allograft*). For successful transplantation the tissues of the donor must be matched as closely as possible to those of the recipient. This is best achieved with two people who are closely related genetically (ideally identical twins). In transplant operations where tissue matching is imperfect, the immune system can be suppressed with drugs such as the glucocorticoids (i.e., prednisone), cyclosporin A and azathioprine. Obviously this will also lower the patient's resistance to infections.

10.4 Blood Groups

ABO System

Failures in transfusion were often due to *agglutination* of the donor's red cells in the recipient's blood. Plasma contains factors which agglutinate the red cells of some but not of other individuals. These factors are *antibodies.* Agglutination of red cells may be followed by intravascular haemolysis with liberation of haemoglobin into the bloodstream, kidney damage and death.

Human red cells have on their membranes *antigenic substances.* These antigens, which are genetically determined, permit the classification of blood into groups. They can be detected by means of appropriate antibodies which elicit agglutination.

Landsteiner (1868-1943) showed that all human blood could be divided into four groups, depending on the presence or absence of the antigenic substances, A and B. In addition antibodies corresponding to the absent antigens occur in the plasma. The ABO blood group of any person can be determined either by adding known antisera to unknown red cells (*cell testing*) or adding unknown plasma or serum to known red cells (*serum testing*). In practice, before giving a transfusion the patient's blood is tested directly with the donor's blood. This is referred to as *cross-matching.*

Frequency of ABO Blood Groups

These groups are distributed differently in different races. In most European populations groups A (about 40%) and O (about 45%) predominate with groups B (about 10%) and AB (about 5%) being rare. Group B, rare in Europeans and Polynesians, is more common in Negroes (about 20%) and even more common in the Chinese (about 35%). The stability of these and other distributions assists in tracing the ancestry of racial groups.

Inheritance of Blood Groups

The four main ABO groups are determined by three dominant allelic genes, A, B and O, which control the formation of antigenic substance on the red cell membrane. The O gene produces no recognizable antigen on the red cells. There are thus six possible combinations of genes (genotypes) but only phenotypes.

Subgroups of ABO System

Subgroups of the main groups can be identified. For example in Group A 80% of people belong to Group A_1 and 20% to Group A_2. Consequently, in Group AB there are two corresponding subgroups, A_1B and A_2B. The subgroups are important in blood grouping because A_1 antigen produces a stronger antigen-antibody reaction than the A_2 antigen. The weak agglutination of the A_2 antigen is easily missed, causing incorrect grouping of the blood.

Secretors and Non-Secretors

A and B antigens are not confined to red cell membranes. They appear in secretions such as tears, sweat, urine, breast milk and saliva in 80% of people, who are called *secretors.* The remaining 20% are *non-secretors.* It is possible with minute quantities of secretion to classify secretors into ABO groups. This finding has proved of great importance in medico-legal work.

Origin of Blood Group Antibodies

A and B antibodies are not present in the blood at birth. They appear during the first six month of life. It is possible that this is genetically determined. Alternatively, it could reflect a response to A and B antigens soon after birth because these antigens are found in the gut and are widely present in the environment, for example on foodstuffs and on bacteria.

Rhesus System

The ABO system is one of at least fifteen well-established blood group systems, but it is by far the most important clinically. The other system which is of great importance in transfusion is the *Rhesus system,* discovered by Landsteiner in 1941. In his search of blood-group antigens he injected red cells from rhesus monkeys into rabbits and guinea-pigs, and produced anti-rhesus antibodies which agglutinated the red cells of monkeys. These antibodies were found to agglutinate the blood from 85% of humans (*Rh-positive*). The 15% whose blood failed to agglutinate were termed *Rh-negative.* In the Rhesus system there are five main antigens (C, D, E, c, e) with corresponding genes (C, D, E, c and e). There is also a *d* gene but it has no known effect on the red cells. A child inherits a set of three Rhesus genes from each parent, i.e., CDE/cDE. Most of the problems arising from the Rhesus system are due to the D antigen. Rhesus grouping is performed with anti-D serum. Rhesus positive persons have the genotype *DD* or *Dd.*

There are no 'naturally occurring' antibodies as in the ABO system, but there are two ways in which a specific immune response with antibody production is generated:

1. *Transfusion of Rh-positive blood into a Rh-negative person.* This stimulates B lymphocytes to form plasma cells which in turn form antibodies against the Rhesus antigen. Should there be a second transfusion of Rh-positive blood, memory cells stimulate a faster and more vigorous response which results in haemolysis of the transfused red cells.

2. *Pregnancy.* If a Rh-negative mother is carrying a Rh-positive (DD or Dd) fetus, the mother may develop antibodies, due to the passage of some fetal red cells across the placenta during pregnancy or, particularly, at the time of delivery. The mother is unaffected because her red cells do not carry the D antigen and the first baby is usually healthy. However the mother continues to carry lymphocytes which have been sensitized to the D antigen.

During subsequent pregnancies, further leakage of fetal red cells across the placenta produces a more vigorous antibody response in the mother. This antibody can cross the placenta to the fetus during the pregnancy and destroy the fetal red cells. The fetus may suffer from *haemolytic disease of the newborn* or *die in utero,* depending on the amount of antibody that it has received. The disease is now largely preventable by giving a Rh-negative mother an injection of anti-D antibody *immediately* after each birth. This prevents the cells of the baby from sensitizing the mother's lymphocytes. In rare cases the condition can be caused by antibodies to antigens other than D.

10.5 Haemostasis

Haemostasis is the control and arrest of bleeding. The normal haemostatic response to bleeding depends on three interrelated factors: (i) the reaction of the blood vessel wall; (ii) the reactions of platelets, and (iii) the activation of the blood coagulation mechanism.

Reaction of Blood Vessels

Injury to a blood vessel causes immediate local *vasoconstriction,* accompanied by reflex constriction of surrounding vessels. The consequent reduction in blood flow to the injured area lessens blood loss and assists the onset of platelet and coagulation activity. Minute holes in damaged capillaries may be sealed completely by lateral spreading of endothelial cells. Bleeding may or may not cease depending on the degree of injury, the size of the vessels and the pressure of blood within them. When large vessels are injured, local compression may be required to stop bleeding.

Reactions of Platelets

Platelets form a *plug* at the site of damage to the vessel. During injury collagen fibres in the subendothelial connective tissue are exposed and platelets adhere to them. This adhesion is dependent on the von Willebrand part of coagulation factor VIII in the plasma linking with a receptor glycoprotein on the membrane of the platelet. As a result of adhesion, platelets release various substances into the fluid around them. These include serotonin and adenosine diphosphate (ADP). *Serotonin* constricts the blood vessels, maintaining and reinforcing the initial vasoconstriction and thus reducing blood flow. *ADP* causes other platelets to aggregate on the already adhered platelets, forming a plug which slows or stops bleeding. Aggregated platelets become fused irreversibly. The process involves thrombasthenin

filaments lying beneath the platelet membrane. Fibrin formation, which is the end result of the blood coagulation process, increases the stability of the plug. In the process of plug formation *phsopholipids* which catalyse blood coagulation are exposed on the platelet membrane. Platelets are important therefore in helping to initiate the coagulation process.

The platelet membrane contains other varieties of phospholipid which on activation by collagen produce the prostaglandin derivative, *thromboxane* A_2. This substance is a potent aggregator of platelets and is also a powerful vasoconstrictor. Vascular endothelial cells synthesize another prostaglandin, *prostacyclin* (PGI_2). It is the most potent inhibitor of platelet aggregation yet discovered, and is also a vasodilator. The opposing effects of these two prostaglandins balance each other so that in intact vessels there is normally no platelet aggregation. Aspirin inhibits the synthesis of thromboxane A_2 by platelets and has an anti-clotting effect.

Blood Coagulation

This involves, as a final step, the conversion by thrombin of soluble *fibrinogen* to insoluble *fibrin. Thrombin* is a proteolytic enzyme which splits two pairs of polypeptides from fibrinogen so that the remaining molecules can polymerize to form a fibrin mesh around the platelet plug. Blood cells become entangled in this mesh. *Factor XII,* activated by thrombin in the presence of *calcium ions,* stabilizes the fibrin.

Thrombin is formed from an inactive precursor *prothrombin,* as the result of a linked series of enzymatic reactions involving other coagulation factors present in the blood or tissues. The coagulation factors are numbered I to XIII, omitting factor VI which is no longer recognized. Factors I to IV are usually known by their common names: fibrinogen (I), prothrombin (II), tissue factor (III) and calcium ions (IV). A deficiency or defect in any one of the coagulation factors can cause a bleeding disorder, i.e., patients who have a defective factor VIII or factor IX suffer from *haemophilia.*

There are two pathways leading to the conversion of prothrombin to thrombin. The *intrinsic pathway* involves only constituents circulating in the blood. An abnormal surface, i.e., damaged endothelium, causes activation of factor XII which activates factor XI. Activated factor XI in turn activates factor IX, which in the presence of calcium ions, platelet phospholipid and factor VIII, activates factor X. Factor X can also be activated in the *extrinsic pathway* by a complex of tissue factor (factor III, released from damaged cells), factor VII and calcium ions. In a *final common pathway,* activated factor X together with factor V, phospholipid, and calcium ions convert prothrombin to thrombin. Recent work has shown that there are interactions between the internal and external pathways suggesting that the distinction between them is somewhat arteficial.

The following points should be noted:

1. Both internal and external pathways are required for normal haemostasis and, excepting factor XII, a deficit of any of the coagulation factors, if it is severe enough, can result in a bleeding disorder.

2. Coagulation requires calcium ions. Substances such as *citrate, oxalate* and *ethylenediaminetetraacetic acid (EDTA)* when added to blood bind or precipitate calcium ions, and therefore prevent coagulation.

3. Sequential enzymatic activation of coagulation factors allows amplification and acceleration so that a small stimulus results in an 'explosive' response.

4. Coagulation is controlled by *antithrombins.* Antithrombin III, a plasma protein, is the most powerful of the circulating inhibitors of thrombin. It combines with thrombin and also with activated factors XI, X and IX to inactivate them and thus stop coagulation. This action is potentiated by the naturally-occurring anticoagulant, *heparin.*

5. Vitamin K is required for gamma-carboxylation of glutamic acid residues of four of the coagulation factors (II, VII, IX and X). This is necessary if these factors are to bind calcium ions and form a complex with phospholipid. Attachment to phospholipid accelerates their activation. Antagonists of vitamin K (i.e., warfarin) are used therapeutically as anticoagulants.

Fibrinolytic System

Fibrin is eventually broken down by a proteolytic enzyme, *plasmin.* An inactive precursor, *plasminogen,* found in blood and tissue fluid is converted to plasmin by activators present in blood vessel walls and tissues. Activated factor XII also activates plasminogen. Plasmin can digest fibrinogen, fibrin, factors V and VIII, and other proteins. There is almost certainly a slow release of plasminogen activator from capillaries and venules keeping these small vessels free from fibrin deposits. Plasmin, like thrombin, is a potent proteolytic enzyme. Its actions is limited by *antiplasmins* circulating in the blood.