

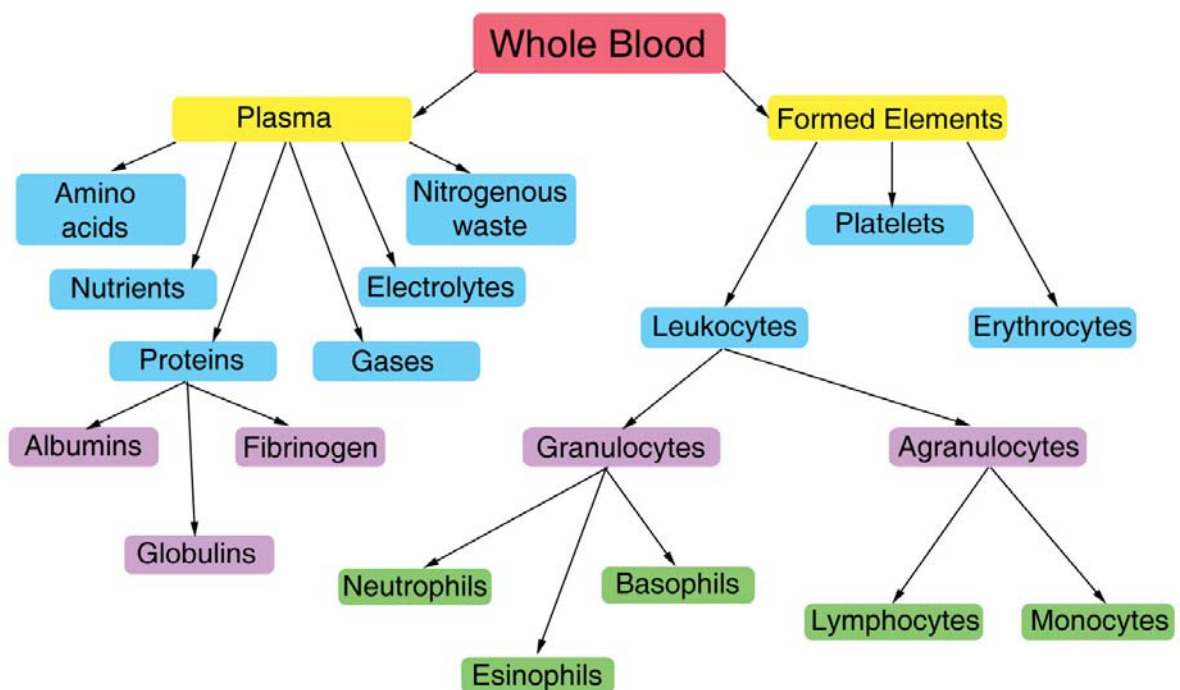
Haematology.

Learning Objectives.

At the end of this section, you should be able to :

1. discuss the structure and function of blood
2. know the sequence of events in the production of erythrocytes
3. understand the differences between the types of leucocyte
4. understand the role of platelets
5. describe the main stages in haemostasis
6. know the basic clinical tests employed in analysing blood

Blood is a type of connective tissue consisting of cells and fragments surrounded by a liquid matrix, which circulates through the heart and blood vessels. The cells and cell fragments are the formed elements, and the liquid is the plasma. The formed elements make up about 45% and plasma makes up about 55% of the total blood volume. The total blood volume in the average adult is about 4 - 5 litres in females and 5-6 litres in males. Blood makes up about 8% of the total weight of the body.



Cells require constant nutrition and waste removal because they are metabolically active. Most cells are located some distance from nutrient sources such as the digestive tract and sites of waste disposal such as the kidneys. The cardiovascular system, which consists of the heart, blood vessels, and blood, connects the various tissues. The heart pumps blood through blood vessels, which extend throughout the body, and the blood delivers nutrients and picks up waste products.

Functions

The functions of blood can be categorized as transportation, maintenance, and protection. Many of these functions can be placed in more than one category, eg, for blood cells to protect against microorganisms, they must be transported to sites of infection.

Transportation

Blood is the primary transport medium of the body. Oxygen enters blood in the lungs and is carried to cells; and carbon dioxide, produced by cells, is carried in blood to the lungs from which it is expelled. Ingested nutrients, electrolytes, and water are transported by the blood from the digestive tract to cells, and waste products are transported from cells to the kidneys for elimination in urine. In addition to transporting gases, nutrients, and waste products, blood transports other substances. For example, the precursor to vitamin D is produced in the skin and transported by the blood to the liver, and then to the kidneys for processing into active vitamin D. The active vitamin D is transported in blood to the small intestines, where it promotes the uptake of calcium. Another example is lactic acid produced by skeletal muscles during anaerobic respiration. The lactic acid is carried in blood to the liver and converted into glucose. Finally, many of the substances necessary for maintenance and protection must be transported throughout the body.

Maintenance

Blood plays a crucial role in maintaining homeostasis. Many of the hormones and enzymes that regulate body processes are found in blood, as are buffers, which help keep the blood's pH within its normal limits of 7.35-7.45. The osmotic composition of blood is also critical for maintaining the normal fluid and electrolyte balance. Because blood can hold heat, it is involved with temperature regulation, transporting heat from the interior to the surface of the body, where the heat is released. When blood vessels are damaged, the blood clots that form are the first step in tissue repair and the restoration of normal function.

Protection

Cells and chemicals of the blood constitute an important part of the immune system, protecting against foreign substances, such as microorganisms and toxins. Blood clotting also provides protection against excessive fluid and cell loss when blood vessels are damaged.

Plasma

Plasma is a pale yellow fluid that consists of about 91% water and 9% other substances, such as proteins, ions, nutrients, gases, and waste products. Plasma is a colloidal solution, which is a liquid containing suspended substances that do not

settle out of solution. Most of the suspended substances are plasma proteins, which include albumin, globulins, and fibrinogen. Albumin makes up 58% of the plasma proteins and is important in the regulation of water movement between tissues and blood. Because albumin does not easily pass from the blood into tissues, it plays an important role in maintaining the osmotic concentration of blood. Globulins account for 38% of the plasma proteins. Some globulins, such as antibodies and complement, are part of the immune system, whereas others function as transport molecules. Fibrinogen constitutes 4% of the plasma proteins and is responsible for the formation of blood clots. In addition to the suspended molecules, plasma contains a number of dissolved components, such as ions, nutrients, waste products, gases, and regulatory substances.

Plasma volume remains relatively constant. Normally, water intake through the digestive tract closely matches water loss through the kidneys, lungs, digestive tract, and skin. Oxygen enters blood in the lungs and carbon dioxide enters blood from the tissues. Other suspended or dissolved substances in the blood come from the liver, kidneys, intestines, endocrine glands, and immune tissues such as the spleen. These other substances are also regulated and maintained within narrow limits.

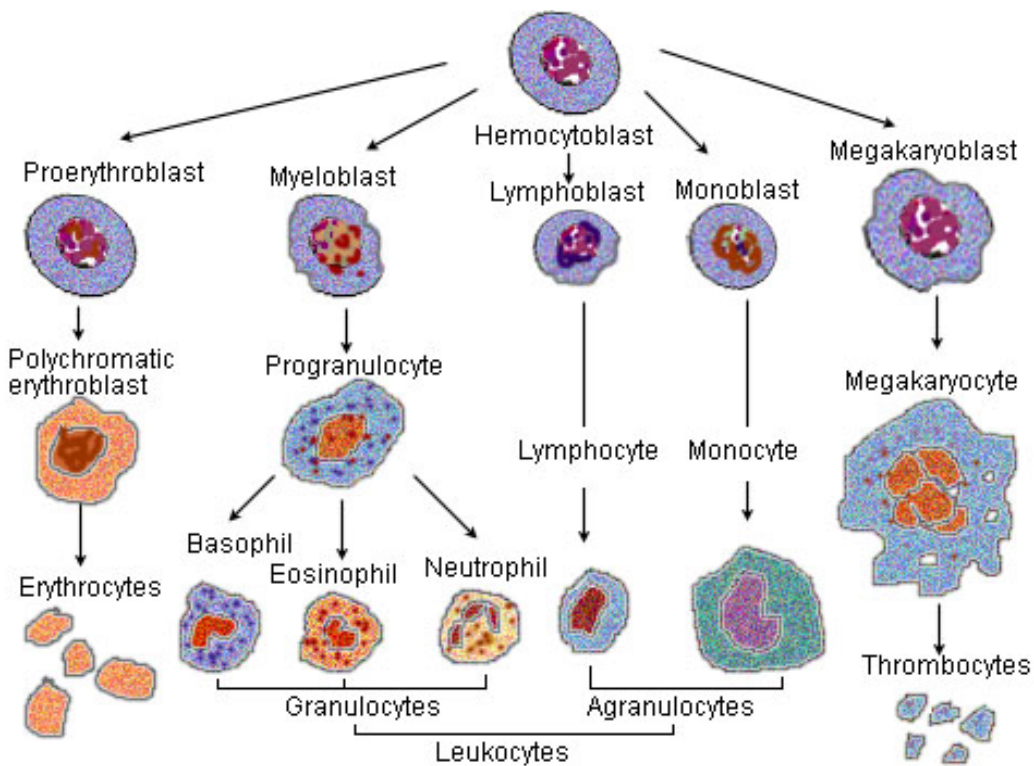
Formed Elements

About 95% of the volume of the formed elements consists of erythrocytes, or red blood cells. The remaining 5% consists of leukocytes, or white blood cells, and cell fragments called platelets (thrombocytes). In healthy adults, leukocytes are the only formed elements possessing nuclei, whereas erythrocytes and platelets have few organelles and lack nuclei.

Leukocytes are named according to their appearance in stained preparations. Leukocytes containing large cytoplasmic granules are granulocytes, and those with very small granules that cannot be seen easily with the light microscope are agranulocytes. The three types of granulocytes are named according to the staining characteristics of their cytoplasm: neutrophils, eosinophils, and basophils. There are two types of agranulocytes: monocytes, and lymphocytes.

Production of Formed Elements

The process of blood cell production, called hematopoiesis or hemopoiesis, occurs in the embryo and fetus in tissues such as the yolk sac, thymus, spleen, lymph nodes, and red bone marrow. After birth, hematopoiesis is confined primarily to red bone marrow with some lymphoid tissue helping in the production erythrocytes. In young children, nearly all the marrow is red bone marrow. In adults, however, red is confined to the skull, ribs, sternum, vertebrae, proximal femur, and proximal humerus. The red marrow in other locations is replaced by yellow marrow.



All the formed elements of the blood are derived from a population of stem cells located in the red bone marrow. Stem cells are precursor cells capable of dividing to produce daughter cells that can differentiate into other cell types. The hemopoietic stem cells produce the cells that give rise to the various types of blood cells: pro-erythroblasts, from which erythrocytes develop; myeloblasts, from which granulocytes develop; lymphoblasts, from which lymphocytes develop; monoblasts, from which monocytes develop; and megakaryoblasts, from which platelets develop. The development of each cell line is regulated by a specific growth factor. That is, the type of formed element derived from the stem cells and how many formed elements are produced are determined by the growth factor.

Many cancer therapies affect dividing cells such as those found in tumours. An undesirable side effect of such therapies, however, can be the destruction of non-tumour cells that are dividing, such as the cells in red bone marrow. After treatment for cancer, growth factors are used to stimulate the rapid regeneration of the red bone marrow. Although not a cure for cancer, the use of growth factors can speed recovery from the cancer therapy.

Some types of leukaemia and genetic immune deficiency diseases can be treated with a bone marrow transplant containing blood stem cells. To avoid problems of tissue rejection, families with a history of these disorders can freeze the umbilical cord blood of their newborn children. The cord blood contains many stem cells and can be used instead of a bone marrow transplant.

Erythrocytes

Erythrocytes are about 700 times more numerous than leukocytes and 17 times more numerous than platelets. Males have about 5.2 million erythrocytes per cubic millimetre of blood (range: 4.2-5.8 million), whereas females have about 4.5 million/mm³ (range: 3.6-5.2 million). Erythrocytes cannot move of their own accord and are passively moved by forces that cause the blood to circulate.



Structure

Normal erythrocytes are biconcave disks about 7.5 µm in diameter with edges that are thicker than the centre of the cell. Compared with a flat disk of the same size, the biconcave shape increases the surface area of the erythrocyte. The greater surface area makes the movement of gases into and out of the erythrocyte more rapid. In addition, the erythrocyte can bend or fold around its thin centre, decreasing its size and enabling it to pass more easily through small blood vessels.

Erythrocytes are highly specialized cells that lose their nuclei and nearly all their cellular organelles during maturation. The main component of the erythrocyte is the pigment protein haemoglobin, which occupies about one third of the total cell volume and accounts for its red colour. Other erythrocyte contents include lipids, adenosine triphosphate (ATP), and the enzyme, carbonic anhydrase.

Function

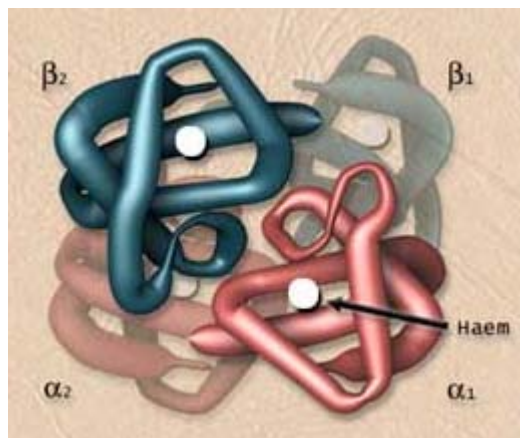
The primary functions of erythrocytes are to transport oxygen from the lungs to the various tissues of the body and transport carbon dioxide from the tissues to the lungs. A proximately 98.5% of the oxygen transported in the blood from the lungs to the tissues is transported in combination with the haemoglobin in the erythrocytes, and the remaining 1.5% is dissolved in the water part of the plasma. If erythrocytes rupture, the haemoglobin leaks out into the plasma and becomes non-functional because the shape of the molecule changes as a result of denaturation. Erythrocyte rupture followed by haemoglobin release is called haemolysis.

Carbon dioxide is transported in the blood in three major ways: approximately 7% is transported as carbon dioxide dissolved in the plasma, approximately 23% is transported in combination with blood proteins (mostly haemoglobin), and 70% is

transported in the form of bicarbonate ions. The bicarbonate ions (HCO_3^-) are produced when carbon dioxide (CO_2) and water combine to form carbonic acid (H_2CO_3), which dissociates to form hydrogen (H^+) and bicarbonate ions. The combination of carbon dioxide and water is catalysed by an enzyme, carbonic anhydrase, which is located primarily within erythrocytes.

Haemoglobin

Haemoglobin consists of four protein chains and four heme groups. Each protein, called a globin, is bound to one heme. Each heme is a red-pigment molecule containing one iron atom. A number of different types of globin exist, each having a slightly different amino acid composition. The four globins in normal adult haemoglobin consist of two alpha (α) chains and two beta (β) chains.



Embryonic and fetal globins appear at different times during development and are replaced by adult globin near the time of birth. Embryonic and fetal haemoglobins are more effective at binding oxygen than is adult haemoglobin. Abnormal haemoglobins are less effective at attracting oxygen than is normal haemoglobin and can result in anaemia.

Iron is necessary for the normal function of haemoglobin because each oxygen molecule that is transported is associated with an iron atom. The adult human body normally contains about 4 g of iron, two thirds of which is associated with haemoglobin. Small amounts of iron are regularly lost from the body in waste products such as urine and faeces. Females lose additional iron as a result of menstrual bleeding and therefore require more dietary iron than do males. Dietary iron is absorbed into the circulation from the upper part of the intestinal tract. Acid from the stomach and vitamin C in food increase the solubility of iron in the alkaline environment of the small intestine, thus facilitating the absorption of iron in the small intestine. Iron absorption is regulated according to need, and iron deficiency can result in anaemia.

Various types of poisons affect the haemoglobin molecule. Carbon monoxide (CO) such as occurs in incomplete combustion of gasoline binds to the iron of haemoglobin, forming the relatively stable carboxyhaemoglobin. As a result of the stable binding of carbon monoxide, haemoglobin cannot transport oxygen, and death may occur. Cigarette smoke also produces carbon monoxide, and the blood of smokers can contain 5% - 15% carboxyhaemoglobin.

When haemoglobin is exposed to oxygen, one oxygen molecule can become associated with each heme group. This oxygenated form of haemoglobin is called oxyhaemoglobin. Haemoglobin containing no oxygen is called deoxyhaemoglobin. Oxyhaemoglobin is bright red, whereas deoxyhaemoglobin has a darker red colour. Haemoglobin also transports carbon dioxide, which does not combine with the iron atoms but is attached to amino groups of the globin molecule. This haemoglobin form is carbaminohaemoglobin.

A relatively-recently discovered function of haemoglobin is the transport of nitric oxide, which is produced by the endothelial cells lining the blood vessels. In the lungs, at the same time that heme picks up oxygen, in each β -globin a sulphur-containing amino acid, cysteine, picks up a nitric oxide molecule to form S-nitrosothiol. When oxygen is released in tissues, so is the nitric oxide, which functions as a chemical signal that induces the smooth muscle of blood vessels to relax. By affecting the amount of nitric oxide in tissues, haemoglobin may play a role in regulating blood pressure because relaxation of blood vessels results in a decrease in blood pressure.

Life-span of Erythrocytes

Under normal conditions about 2.5 million erythrocytes are destroyed every second, representing 0.00001% of the total 25 trillion erythrocytes contained in the normal adult circulation. In addition, these 2.5 million erythrocytes are being replaced by of an equal number of erythrocytes every second.

The process by which new erythrocytes is produced is called erythropoiesis, and the time required for the production of a single erythrocyte is about 4 days. Stem cells, from which all blood cells originate, give rise to pro-erythroblasts. After several mitotic divisions, pro-erythroblasts become early (basophilic) erythroblasts. Early erythroblasts give rise to intermediate (polychromatic) erythroblasts. As haemoglobin is synthesised it accumulates in the cytoplasm. Intermediate erythroblasts continue to produce haemoglobin, and then most of their ribosomes and other organelles degenerate. The resulting late erythroblasts have a reddish colour because about one third of the cytoplasm is now haemoglobin.

The late erythroblasts lose their nuclei by a process of extrusion to become immature erythrocytes, which are called reticulocytes, which become mature erythrocytes when the remaining ribosomes degenerate. Mature erythrocytes and reticulocytes are released from the bone marrow into the circulating blood, which normally consists of mature erythrocytes and 1-3% reticulocytes.

Cell division requires the vitamins folate and B12, necessary for the synthesis of DNA. Haemoglobin production requires iron. Consequently, a lack of folate, B12, or iron will interfere with normal erythrocyte production.

Production is stimulated by low blood oxygen levels, typical causes of which are low erythrocyte numbers, decreased or defective haemoglobin, pathologies relating to the lungs, increased altitudes, or cardio-vascular problems. Low blood oxygen levels stimulate production by increasing the formation of the glycoprotein erythropoietin by the kidneys. The erythropoietin stimulates red bone marrow to produce more erythrocytes by increasing the number of proerythroblasts formed and by decreasing the time required for erythrocytes to mature. Conversely, if blood oxygen levels increase, less erythropoietin is released, and erythrocyte production decreases.

Erythrocytes normally stay in the circulation for about 120 days in males and 110 days in females. These cells have no nuclei and therefore cannot produce new proteins. As their existing proteins, enzymes, cell membrane components, and other structures degenerate, the erythrocytes become old and abnormal in form and function. Erythrocytes can also be damaged in various ways while passing through the circulation.

Old, damaged, or defective erythrocytes are removed from the blood by macrophages located in the spleen, liver, kidney, and other lymphatic tissues. Within the macrophage, lysosomal enzymes break open erythrocytes and begin to digest haemoglobin. Globin is broken down into its component amino acids, most of which are reused in the production of other proteins. Iron atoms also are released for recycling. The heme groups are converted to biliverdin and then to bilirubin, which is released into the plasma. Bilirubin binds to albumin and is transported to liver cells. This bilirubin is called free (indirect) bilirubin even though it binds to albumin. The free bilirubin is taken up by the liver cells and is conjugated, or joined, to glucuronic acid to form conjugated (direct) bilirubin, which is more water-soluble than free bilirubin. Some of the conjugated bilirubin escapes back into the blood and is excreted by the kidneys. Most of the conjugated bilirubin becomes part of the bile, which is the fluid secreted from the liver into the small intestine. In the intestines, bacteria convert bilirubin into the pigments that give the faeces its characteristic brownish colour. Some of these pigments are absorbed from the intestine, modified in the kidneys, and excreted in

the urine, contributing to the characteristic yellowish colour of urine. A yellowish staining of the skin and sclerae by bile pigments, associated with a build up of bilirubin in the circulation and interstitial spaces, is known as jaundice.

KEY LEARNING POINT.



- 1. Blood is a connective tissue consisting of a liquid matrix, suspended cells, and fragments.**
- 2. The formed elements contribute 45%, whilst the plasma contributes 55% of blood volume.**
- 3. The function of the blood is to transport oxygen and nutrients, maintain homeostasis, and contribute to the function of the immune system.**
- 4. Plasma is 91% water, the other 9% consisting of proteins, ions, nutrients, and dissolved gases. The most important plasma protein is albumin.**
- 5. The formed elements consist of red and white blood cells, at 95% and 5% respectively.**
- 6. Haemopoiesis is the formation of blood cells.**
- 7. Erythrocytes are biconcave discs, without a nucleus, being active up to 120 days in the circulation.**
- 8. Their primary function is the transport of oxygen, via the protein haemoglobin.**
- 9. Haemoglobin is made up of heme and globin units, and requires iron so that oxygen can be carried.**
- 10. Haemoglobin is also involved in the transport of nitric oxide, essential for maintenance of vascular tone.**
- 11. Erythrocytes are both destroyed and produced at a rate of approx 2.5 million per second.**
- 12. Erythropoiesis is the formation of new erythrocytes.**
- 13. Old and damaged erythrocytes are removed from the circulation by macrophages in the spleen, liver, kidney, and lymphatic tissues. The globin is broken down and re-used, the iron is recycled, and heme is converted to bilirubin and biliverdin, and excreted.**

Leukocytes

Leukocytes, or white blood cells, are clear or whitish coloured cells that lack haemoglobin but have a nucleus. In stained preparations, leukocytes attract stain, whereas erythrocytes remain relatively unstained.

Leukocytes protect the body against invading microorganisms and remove dead cells and debris from the body. Most leukocytes are motile, exhibiting amoeboid movement, which is the ability to move like an amoeba by putting out irregular cytoplasmic projections. Leukocytes leave the circulation and enter tissues by diapedesis, a process in which they become thin and elongated and slip between or in some cases through the cells of blood vessel walls. The leukocytes can then be attracted to foreign materials or dead cells within the tissue by chemotaxis. At the site of an infection, leukocytes accumulate and phagocytise bacteria, debris, and dead cells; then they die. The accumulation of dead leukocytes, along with fluid and cell debris, is called slough. The five types of leukocytes are neutrophils, eosinophils, basophils, lymphocytes, and monocytes.

Neutrophils



Neutrophils are the most common type of leukocytes in the blood, and have small cytoplasmic granules that stain with both acidic and basic dyes. Their nuclei are commonly tri-lobed, but the number of lobes varies from two to five. Neutrophils often are called polymorphonuclear neutrophils, or PMNs, to indicate that their nuclei can occur in more than one (poly) form (morph). Neutrophils usually remain in the circulation for about 10-12 hours and then move into other tissues, where they become motile and seek out and phagocytise bacteria, antigen-antibody complexes (antigens and antibodies bound together), and other foreign matter. Neutrophils also secrete a class of enzymes called lysozymes, which are capable of destroying certain bacteria. Neutrophils usually survive for 1-2 days after leaving the circulation.

Eosinophils



Eosinophils contain cytoplasmic granules that stain bright red with eosin, an acidic stain. They are motile cells that leave the circulation to enter the tissues during an inflammatory reaction. They are most common in tissues undergoing an allergic response, and their numbers are elevated in the blood of people with allergies or certain parasitic infections.

Eosinophils apparently reduce the inflammatory response by producing enzymes that destroy inflammatory chemicals such as histamine. Eosinophils also phagocytise antigen-antibody complexes formed during the allergic response, although they are not as important in this function as neutrophils.

Basophils



Basophils are the least common of all leukocytes, and contain large cytoplasmic granules that stain blue or purple with basic dyes. Basophils, like other granulocytes, leave the circulation and migrate through the tissues, where they play a role in both allergic and inflammatory reactions. Basophils contain large amounts of histamine, which they release within tissues to increase inflammation. They also release heparin, which inhibits blood clotting.

Lymphocytes



The smallest leukocytes are lymphocytes, most of which are slightly larger in diameter than erythrocytes. The lymphocytic cytoplasm consists of only a thin, sometimes imperceptible, ring around the nucleus.

Although lymphocytes originate in bone marrow, they migrate through the blood to lymphatic tissues where they can proliferate, producing more lymphocytes. The majority of the body's total lymphocyte population is in the lymphatic tissues: the lymph nodes, spleen, tonsils, lymph nodules, and thymus. Although they cannot be identified using standard microscopic examination, a number of different kinds of lymphocytes play important roles in immunity. For example, B-lymphocytes can be stimulated by bacteria or toxins to divide, forming cells that produce proteins called antibodies. The antibodies can attach to the bacteria and activate mechanisms that result in destruction of the bacteria. T-lymphocytes protect against viruses by attacking and destroying cells in which viruses are reproducing. In addition, T-lymphocytes are involved with the destruction of tumour cells and tissue graft rejections.

Monocytes



Monocytes are typically the largest of the leukocytes. Monocytes normally remain in the circulation for about 3 days, leave the circulation, become transformed into macrophages, and migrate through the various tissues. They phagocytise bacteria, dead cells, cell fragments, and other debris within the tissues. An increase in the number of monocytes is often associated with chronic infections. In addition, macrophages can break down phagocytised foreign substances and present the processed substances to lymphocytes, which results in activation of the lymphocytes.

Platelets



Platelets, or thrombocytes, are minute fragments of cells consisting of a small amount of cytoplasm surrounded by a plasma membrane. The surface of platelets has glycoproteins and proteins that allow platelets to attach to other molecules, such as collagen in connective tissue. Some of these surface molecules, as well as molecules released from granules in the platelet cytoplasm, play important roles in controlling blood loss. The platelet cytoplasm also contains actin and myosin that can cause contraction of the platelet.

Platelets are roughly disc-shaped and average about 3 μm in diameter. Even though they are about 40 times more common in the blood than leukocytes, platelets often are not counted in typical blood smears because they tend to form clumps and become difficult to distinguish.

The life expectancy of platelets is about 5-9 days. Platelets are produced within the marrow and are derived from megakaryocytes, which are extremely large cells with diameters up to 100 μm . Small fragments of these cells break off and enter the circulation as platelets.

Platelets play an important role in preventing blood loss in two ways:

- (1) the formation of platelet plugs, which seal holes in small vessels;
- (2) the formation of clots, which help seal off larger wounds in the vessels.

KEY LEARNING POINT.



1. Leukocytes lack haemoglobin, but have nuclear material.
2. Their role is to protect the body against invading microorganisms, and remove dead cells and debris.
3. Leukocytes have the ability to leave the vasculature by changing their shape, and moving into the extra-vascular space.
4. Neutrophils are the commonest type of leukocyte, and they phagocytise bacteria, antigen-antibody complexes, and other foreign material. They produce lysosomes to aid in this process.
5. Eosinophils leave the circulation to enter tissues as part of the inflammatory process. They are commonly found in tissues undergoing an allergic response. They produce enzymes which destroy histamine.
6. Basophils are the least common type of leukocyte, playing a role in allergic and inflammatory reactions. They release heparin, which inhibits blood clotting.
7. There are a number of types of lymphocyte, but all are involved in the immune response.
8. Monocytes are the largest white blood cells, transforming into macrophages to ingest cell debris and bacteria.
9. Platelets are small fragments of cells which have an important role in haemostasis.

Haemostasis

Haemostasis, the arrest of bleeding, is very important to the maintenance of homeostasis. If not stopped, excessive bleeding from a cut or torn blood vessel can result in a positive-feedback pathway, consisting of ever-decreasing blood volume and blood pressure, leading away from homeostasis, and resulting in death.

Fortunately, when a blood vessel is damaged, a number of events occur that help prevent excessive blood loss. Haemostasis can be divided into three stages: vascular spasm, platelet plug formation, and coagulation.

Vascular Spasm

Vascular spasm is an immediate but temporary closure of a blood vessel resulting from contraction of smooth muscle within the wall of the blood vessel. In small vessels, this constriction can close the vessels completely and stop the flow of blood through the vessels. Vascular spasm is produced by nervous system reflexes and by chemicals. For example, during the formation of a platelet plug, platelets release thromboxanes, derived from prostaglandins, and endothelial cells release the peptide endothelin, which both contribute to vascular spasm.

Platelet Plug Formation

A platelet plug is an accumulation of platelets that can seal up small breaks in blood vessels. Platelet plug formation is very important in maintaining the integrity of the circulatory system because small tears occur in the smaller vessels and capillaries many times each day, and platelet plug formation quickly closes them. People who lack the normal number of platelets tend to develop numerous small haemorrhages in their skin and internal organs.

The formation of a platelet plug can be described as a series of steps, but in actuality many of the events occur simultaneously.

1. Platelet adhesion occurs when platelets bind to collagen exposed by blood vessel damage. Von Willebrand's factor, produced and secreted by blood vessel endothelial cells, binds to platelet surface receptors and collagen, causing the platelets to adhere to the collagen. In addition, other platelet surface receptors can bind directly to collagen.
2. After platelets adhere to collagen, they become activated, and in the platelet release reaction, adenosine diphosphate (ADP), thromboxanes, and other chemicals are extruded from the platelets by exocytosis. The ADP and thromboxanes stimulate other platelets to become activated and release additional chemicals, producing a cascade of chemical release by the platelets. Thus more and more platelets become activated.

3. As platelets become activated they also express surface receptors that can bind to fibrinogen, a plasma protein. In platelet aggregation, the fibrinogen forms a bridge between the surface receptors of different platelets, resulting in the formation of a platelet plug.
4. Activated platelets express phospholipids (platelet factor III) and coagulation factor V, which are an important part of clot formation.

The production of prostaglandins is very important to platelet plug formation, as is demonstrated by the effect of aspirin. Because aspirin inhibits prostaglandin synthesis, so the production of thromboxanes, which are derived from prostaglandins, is also inhibited, resulting in reduced platelet activation. If an expectant mother ingests aspirin near the end of pregnancy, prostaglandin synthesis is inhibited and several effects are possible. Two of these effects are firstly, the mother can experience excessive postpartum haemorrhage because of decreased platelet function, and secondly, the baby can exhibit numerous localized haemorrhages called petechiae over the surface of its body as a result of decreased platelet function. If the quantity of ingested aspirin is large, the infant, mother, or both may die as a result of haemorrhage. On the other hand, in a stroke or heart attack, platelet plugs and clots can form in vessels and threaten the life of the individual. Small amounts of aspirin taken daily can help prevent such vascular problems.

Coagulation

Vascular spasms and platelet plugs alone are not sufficient to close large tears or cuts. When a blood vessel is severely damaged, coagulation, or blood clotting, results in the formation of a clot. A blood clot is a network of threadlike protein fibres, called fibrin, that traps blood cells, platelets, and fluid. The formation of a blood clot depends on a number of proteins found within plasma called coagulation factors. Normally the coagulation factors are in an inactive state and do not cause clotting. After injury the clotting factors are activated to produce a clot. This activation is a complex process involving many chemical reactions, some of which require calcium ions and molecules on the surface of activated platelets, such as phospholipids and coagulation factor V.

The activation of clotting proteins can be summarized in three main stages :

Stage 1 consists of the formation of prothrombinase,

Stage 2 is the conversion of prothrombin to thrombin by prothrombinase,

Stage 3 consists of the conversion of soluble fibrinogen to insoluble fibrin by thrombin.

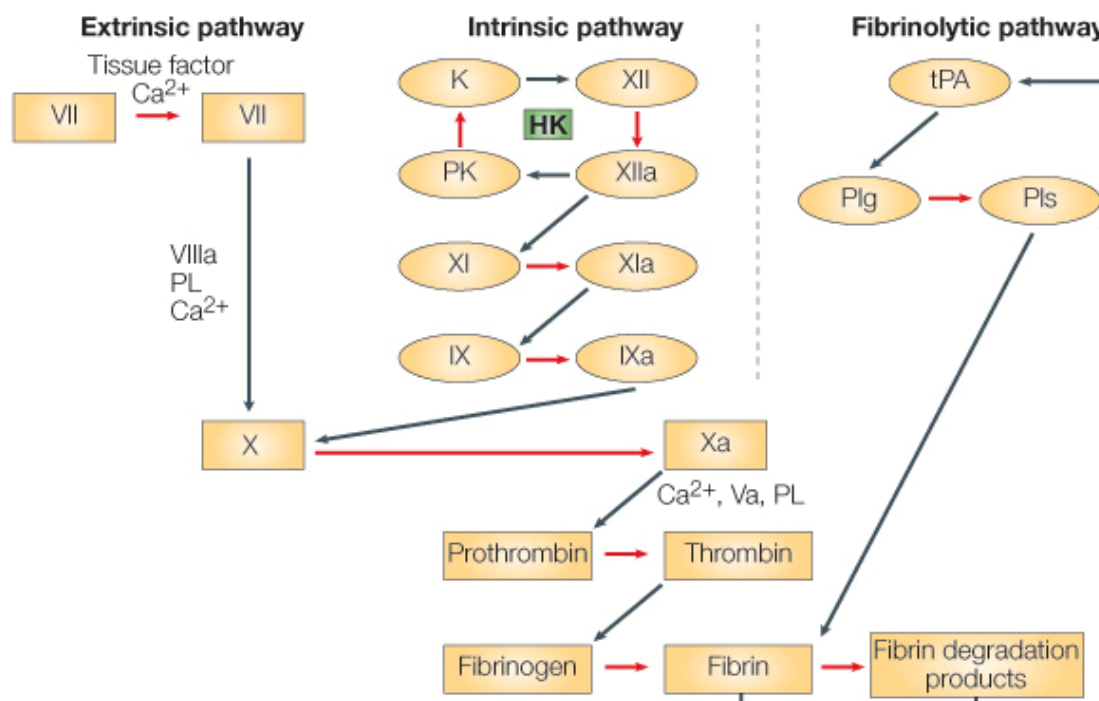
Depending on how prothrombinase is formed in stage 1, two separate pathways for coagulation have been described: the extrinsic clotting pathway and the intrinsic clotting pathway.

Extrinsic Clotting Pathway

The extrinsic clotting pathway is so-named because it begins with chemicals that are outside of or extrinsic to the blood. In stage 1, damaged tissues release a mixture of lipoproteins and phospholipids called tissue factor (TF), also known as thromboplastin, or Factor III. Tissue factor, in the presence of calcium ions, forms a complex with Factor VII, which activates Factor X. On the surface of platelets, activated Factor X, Factor V, platelet phospholipids, and calcium ions complex to form prothrombinase. In stage 2, the soluble plasma protein prothrombin is converted to the enzyme thrombin by prothrombinase. During stage 3, the soluble plasma protein fibrinogen is converted to the insoluble protein fibrin by thrombin. The fibrin forms the fibrous network of the clot. Thrombin also stimulates factor XIII activation, which is necessary to stabilise the clot.

Intrinsic Clotting Pathway

The intrinsic clotting pathway is so named because it begins with chemicals that are inside or intrinsic to the blood. In stage 1, damage to blood vessels can expose collagen in the connective tissue beneath the epithelial lining of the blood vessel. When plasma factor XII comes into contact with collagen, it is activated and stimulates Factor XI, which in turn activates Factor IX. Activated Factor IX joins with Factor VIII, platelet phospholipids, and calcium ions to activate Factor X. On the surface of platelets, activated Factor X, Factor V, platelet phospholipids, and calcium ions complex to form prothrombinase. Stages 2 and 3 then are activated, and a clot results.



Although once considered distinct pathways, it is now known that the extrinsic pathway can activate the clotting proteins in the intrinsic pathway. The TF-VII complex from the extrinsic pathway can stimulate the formation of activated Factors IX and X in the intrinsic pathway. When tissues are damaged, tissue factor also rapidly leads to the production of thrombin, which can activate many of the clotting proteins in the intrinsic pathway, such as Factor XI and prothrombin. Thus thrombin is part of a positive-feedback system in which thrombin production stimulates the production of additional thrombin. Thrombin also has a positive-feedback effect on coagulation by stimulating platelet activation.

Many of the factors involved in clot formation require vitamin K for their production. Vitamin K deficiency can result in haemorrhages such as frequent nosebleeds. Vitamin K is produced by intestinal bacteria, and those bacteria can be killed by large doses of oral antibiotics, resulting in insufficient coagulation ability. Newborns lack these intestinal bacteria, and a vitamin K injection is routinely given to infants at birth. Infants can also obtain vitamin K from food such as milk. Because cow's milk contains more vitamin K than does human milk, breast-fed infants are more susceptible to haemorrhage than bottle-fed infants.

Vitamin K is absorbed from the small intestine into the circulation and is necessary for the synthesis of several clotting factors by the liver. The absorption of vitamin K from the intestine requires the presence of bile. Certain disorders such as obstruction of bile flow to the intestine can interfere with vitamin K absorption and lead to insufficient clotting. Liver diseases that result in the decreased synthesis of clotting factors also can lead to insufficient clot formation.

Control of Clot Formation

Without control, coagulation would spread from the point of initiation to the entire circulatory system. Furthermore, vessels in a normal person contain rough areas that can stimulate clot formation, and small amounts of prothrombin are constantly being converted into thrombin. To prevent unwanted clotting, the blood contains several anticoagulants which prevent coagulation factors from initiating clot formation. Only when coagulation factor concentrations exceed a given threshold does coagulation occur. At the site of injury so many coagulation factors are activated that the anticoagulants are unable to prevent clot formation. Away from the injury site, however, the activated coagulation factors are diluted in the blood, anticoagulants neutralise them, and clotting is prevented.

Examples of anticoagulants in the blood are antithrombin, heparin, and prostacyclin. Antithrombin, a plasma protein produced by the liver, slowly inactivates thrombin. Heparin, produced by basophils and endothelial cells,

increases the effectiveness of antithrombin because heparin and antithrombin together rapidly inactivate thrombin. Prostacyclin is a prostaglandin derivative produced by endothelial cells. It counteracts the effects of thrombin by causing vasodilation and by inhibiting the release of coagulation factors from platelets.

Anticoagulants are also important outside the body, preventing the clotting of blood used in transfusions and laboratory blood tests. Examples include heparin, ethylenediaminetetraacetic acid (EDTA), and sodium citrate. EDTA and sodium citrate prevent clot formation by binding to calcium ions, making them inaccessible for clotting reactions.

When platelets encounter damaged or diseased areas on the walls of blood vessels or the heart, an attached clot called a thrombus can form. A thrombus that breaks loose and begins to float through the circulation is called an embolus. Both thrombi and emboli can result in death if they block vessels that supply blood to essential organs such as the heart, brain, or lungs. Abnormal coagulation can be prevented or hindered by the injection of anticoagulants such as heparin, which acts rapidly, or warfarin, which acts more slowly than heparin. Warfarin prevents clot formation by suppressing the production of vitamin K-dependent coagulation Factors (II, VII, IX, and X) by the liver. Warfarin was first used as a rat poison, causing rats to bleed to death. In small doses warfarin is a proven, effective anticoagulant in humans. Caution is necessary with anticoagulant treatment however, because the patient can haemorrhage internally or bleed excessively when cut.

Clot Retraction and Dissolution

The fibrin meshwork constituting the clot adheres to the walls of the vessel. Once the clot has formed, it begins to condense into a denser, compact structure through a process known as clot retraction. Platelets contain contractile proteins, actin and myosin, which operate in a similar fashion to the actin and myosin in smooth muscle. Platelets form small extensions that attach to fibrin. Contraction of the extensions pulls on the fibrin and is responsible for clot retraction. As the clot condenses, a fluid called serum is squeezed out of it. Serum is plasma from which fibrinogen and some of the clotting factors have been removed.

Consolidation of the clot pulls the edges of the damaged vessel together, which can help to stop the flow of blood, reduce infection, and enhance healing. The damaged vessel is repaired by the movement of fibroblasts into the damaged area and the formation of new connective tissue. In addition, epithelial cells around the wound proliferate and fill in the damaged area.

The clot usually is dissolved within a few days after clot formation by a process called fibrinolysis, which involves the activity of plasmin, an enzyme that hydrolyses fibrin. Plasmin is formed from inactive plasminogen, which is a normal blood protein. It is activated by thrombin, Factor XII, tissue plasminogen activator (t-PA), urokinase, and lysosomal enzymes released from damaged tissues. In disorders that are caused by blockage of a vessel by a clot, such as a heart attack, dissolving the clot can restore blood flow and reduce damage to tissues. For example, streptokinase (a bacterial enzyme), tissue plasminogen activator, or urokinase can be injected into the blood or introduced at the clot site by means of a catheter. These substances convert plasminogen to plasmin, which breaks down the clot.

KEY LEARNING POINT.



- 1. Haemostasis is the arrest of bleeding - a number of events which occur in this process.**
- 2. Vascular spasm is an immediate but temporary closure of a blood vessel via contraction of smooth muscle.**
- 3. Platelet plug formation occurs as platelets accumulate at the site of damage.**
- 4. Platelets adhere to exposed collagen fibres, after which they become activated, beginning a chemical cascade to promote the clotting process.**
- 5. This encourages the binding of fibrinogen, further strengthening the platelet plug.**
- 6. Coagulation factors are normally inactive, but become activated via the action of calcium ions, clotting factors, and platelet surface proteins.**
- 7. The extrinsic and intrinsic clotting pathways both play a role in the clotting cascade, and bring about the same end result, but in different ways.**
- 8. Coagulation is controlled by anticoagulation factors already present in the blood, such as antithrombin, heparin, and prostacyclin.**
- 9. Blood clots are usually dissolved within a few days of formation by fibrinolysis, which involves the enzyme plasmin. This activated from its inactive form (plasminogen) by the action of thrombin, Factor XII and tissue plasminogen activating factor.**
- 10. Dissolution of the clots re-establishes normal blood flow, and prevents damage to the tissues.**

Blood Grouping

If large quantities of blood are lost during surgery or in an accident, the blood volume must be increased, or the patient can go into shock and die. A transfusion is the transfer of blood or other solutions into the blood of the patient. In many cases the return of blood volume to normal levels is all that is necessary. This can be accomplished by the transfusion of plasma or plasma expanders, which are prepared solutions having the proper amounts of solutes. When large quantities of blood are lost, however, erythrocytes must also be replaced that the oxygen-carrying capacity of the blood is restored.

Early attempts to transfuse blood from one person to another were often unsuccessful because they resulted in transfusion reactions, which included clotting within blood vessels, kidney damage, and death. It is now known that transfusion reactions are caused by interactions between antigens and antibodies. In brief, the surfaces of erythrocytes have molecules called antigens, and in the plasma there are molecules called antibodies. Antibodies are very specific, meaning that each antibody can combine only with a certain antigen. When the antibodies in the plasma bind to the antigens on the surfaces of the erythrocytes, they form molecular bridges that connect the erythrocytes. As a result, agglutination, or clumping, of the cells occurs. The combination of the antibodies with the antigens can also initiate reactions that cause haemolysis, or rupture of the erythrocytes. Because the antigen-antibody combinations can cause agglutination, the antigens are often called agglutinogens, and the antibodies are called agglutinins.

The antigens on the surface of erythrocytes have been categorized into blood groups, and more than 35 blood groups, most of which are rare, have been identified. The ABO and Rh blood groups are among the most important. Other well-known groups include the Lewis, Duffy, MNSs, Kidd, Kell, and Lutheran groups.

ABO Blood Group

In the ABO blood group, type A blood has type A antigens, type B blood has type B antigens, type AB blood has both types of antigens, and type O blood has neither A nor B antigens. In addition, plasma from type A blood contains type B antibodies, which act against type B antigens, whereas plasma from type B blood contains type A antibodies, which act against type A antigens. Type AB blood has neither type of antibody, and type O blood has both A and B antibodies.

The presence of A and B antibodies in blood is not clearly understood. Antibodies normally do not develop against an antigen unless the body is exposed to that antigen. This means, for example, that a person with type A blood should not have

type B antibodies unless he or she has received a transfusion of type B blood, which contains type B antigens. People with type A blood do have type B antibodies, however, even though they have never received a transfusion of type B blood. One possible explanation is that type A or B antigens on bacteria or food in the digestive tract stimulate the formation of antibodies against antigens that are different from one's own antigens. Thus a person with type A blood would produce type B antibodies against the B antigens on the bacteria or food. In support of this hypothesis the observation that A and B antibodies are not found in the blood until about 2 months after birth.

Usually a donor can give blood to a recipient if they both have the same blood type. Historically, people with type O blood have been called universal donors because they usually can give blood to other ABO blood types without causing an ABO transfusion reaction. Their erythrocytes have no ABO surface antigens and therefore do not react with the recipient's A or B antibodies.

The term universal donor is misleading, however. The transfusion of type O blood can produce a transfusion reaction two reasons. First, other blood groups can cause a transfusion reaction. Second, antibodies in the blood of the donor react with antigens in the blood of the recipient. For example, type O blood has type A and B antibodies. If type O blood is transfused into a person with type A blood, the A antibodies (in the type O blood) react against the A antigens (in the type A blood). Usually such reactions are not serious because the antibodies in the donor's blood are diluted in the blood of the recipient, and few reactions take place. Because type O blood sometimes causes transfusion reactions it is given to another person with another blood type only in life-or-death emergency situations.

Rh Blood Group

Another important blood group is the Rhesus blood group, so named because it was first studied in the rhesus monkey. People are Rh-positive if they have a certain Rh antigen (the D antigen) on the surface of their erythrocytes, and people are Rh-negative if they do not have this Rh antigen. About 85% of white people and 88% of black people in the UK are Rh-positive. The ABO blood type and the Rh blood type usually are designated together. For example, a person designated as A positive is type A in the ABO blood group and Rh-positive. The rarest combination in the UK is AB negative, which occurs in less than 1% of the population.

Antibodies against the Rh antigen do not develop unless an Rh-negative person is exposed to Rh-positive blood. This can occur through a transfusion or by transfer of blood between a mother and her fetus across the placenta. When an Rh-negative person receives a transfusion of Rh-positive blood, the recipient becomes sensitized

to the Rh antigen and produces Rh antibodies. If the Rh-negative person is unfortunate enough to receive a second transfusion of Rh positive blood after becoming sensitised, a transfusion reaction results.

Rh incompatibility can pose a major problem in some pregnancies when the mother is Rh-negative and the fetus is Rh-positive. If fetal blood leaks through the placenta and mixes with the mother's blood, the mother becomes sensitized to the Rh antigen. The mother produces Rh antibodies that cross the placenta and cause agglutination and haemolysis of fetal erythrocytes. This disorder is called haemolytic disease of the newborn (HDN), or erythroblastosis fetalis, and it may be fatal to the fetus. In the woman's first pregnancy there is usually no problem. The leakage of fetal blood is usually the result of a tear in the placenta that takes place either late in the pregnancy or during delivery. Thus there is not enough time for the mother to produce enough Rh antibodies to harm the fetus. In later pregnancies, however, a problem can arise because the mother has already been sensitised to the Rh antigen. Consequently, if the fetus is Rh-positive and if there is any leakage of fetal blood into the mother's blood, she rapidly produces large amounts of Rh antibodies, and HDN develops.

HDN can be prevented if the Rh-negative woman is given an injection of a specific type of antibody preparation, called Rh_o(D) immune globulin (RhoGAM). The injection can be administered during the pregnancy or before or immediately after each delivery or abortion. The injection contains antibodies against Rh antigens. The injected antibodies bind to the Rh antigens of any fetal erythrocytes that may have entered the mother's blood. This treatment inactivates the fetal Rh antigens and prevents sensitization of the mother.

If HDN develops, treatment consists of slowly removing the newborn's blood and replacing it with Rh-negative blood. The newborn can also be exposed to fluorescent light because the light helps to break down the large amounts of bilirubin formed as a result of erythrocyte destruction. High levels of bilirubin are toxic to the nervous system and can damage brain tissue.

Diagnostic Blood Tests

Type and Cross-match

To prevent transfusion reactions the blood is typed, and a cross-match is made. Blood typing determines the ABO and Rh blood groups of the blood sample. Typically, the cells are separated from the serum. The cells are tested with known antibodies to determine the type of antigen on the cell surface. For example, if a patient's blood cells agglutinate when mixed with type A antibodies but do not agglutinate when mixed with type B antibodies, it is concluded that the cells have type A antigen. In a similar fashion the serum is mixed with known cell types (antigens) to determine the type of antibodies in the serum.

Normally donor blood must match the ABO and Rh type of the recipient. Because other blood groups can also cause a transfusion reaction, however, a crossmatch is performed. In a crossmatch the donor's blood cells are mixed with the recipient's serum, and the donor's serum is mixed with the recipient's cells. The donor's blood is considered safe for transfusion only if there is no agglutination in either match.

Complete Blood Count

The complete blood count (CBC) is an analysis of the blood that provides much information. It consists of a red blood cell count, haemoglobin and haematocrit measurements, and a white blood cell count.

Red Blood Cell Count

Blood cell counts usually are done electronically with a machine, but they can also be done manually with a microscope. The normal range for a red blood cell count (RBC) is 4.2 - 5.8 million erythrocytes per cubic millimetre of blood for a male, and 3.6 - 5.2 million/mm³ of blood for a female. Polycythaemia is an overabundance of erythrocytes. It can result from a decreased oxygen supply which stimulates erythropoietin production, or from red bone marrow tumours. Because erythrocytes tend to stick to one another, increasing the number of erythrocytes makes it more difficult for blood to flow. Consequently, polycythaemia increases the workload of the heart. It also can reduce blood flow through tissues and, if severe, can result in plugging of small blood vessels (capillaries).

Haemoglobin Measurement

The haemoglobin measurement determines the amount of haemoglobin in a given volume of blood, usually expressed as grams of haemoglobin per 100 mL, of blood. The normal haemoglobin count for a male is 14-18 g/100 mL, of blood, and for a female it is 12-16 g/100 mL, of blood. Abnormally low haemoglobin is an indication

of anaemia , which is a reduced number of erythrocytes per 100 mL of blood or a reduced amount of haemoglobin in each erythrocyte.

Haematocrit Measurement

The percentage of total blood volume composed of erythrocytes is the haematocrit. One way to determine haematocrit is to place blood in a tube and spin the tube in a centrifuge. The formed elements are heavier than the plasma and are forced to one end of the tube. The erythrocytes account for 44%-54% of the total blood volume in males and 38%-48% in females. Because the haematocrit measurement is based on volume, it is affected by the number and size of erythrocytes. For example, a decreased haematocrit can result from a decreased number of normal-sized erythrocytes or a normal number of small-sized erythrocytes. The average volume of an erythrocyte is calculated by dividing the haematocrit by the red blood cell count. A number of disorders cause erythrocytes to be smaller or larger than normal. For example, inadequate iron in the diet can impair haemoglobin production. Consequently, during their formation erythrocytes do not fill with haemoglobin, and they remain smaller than normal.

White Blood Cell Count

A white blood cell count (WBC) measures the total number of leukocytes in the blood. There are normally 5000-10,000 Leukocytes per cubic millimetre of blood. Leukopenia is a lower-than-normal WBC count and can indicate depression or destruction of the red marrow by radiation, drugs, tumour, or a deficiency of vitamin B, or folate. Leukocytosis is an abnormally high white blood cell count. Leukaemia (a tumour of the red marrow) and bacterial infections often cause leukocytosis

White Blood Cell Differential Count

A white blood cell differential count determines the percentage of each of the five kinds of leukocytes in the white blood cell count. Normally neutrophils account for 60%-70%; lymphocytes, 20%-30%; monocytes, 2%-8%; eosinophils, 1%--4%; and basophils, 0.5%- 1%. A white blood cell differential count can provide much insight about a patient's condition. For example, in patients with bacterial infections the neutrophil count is often greatly increased, whereas in patients with allergic reactions the eosinophil and basophil counts are elevated.

Clotting

Two measurements that test the ability of the blood to clot are the platelet count and the prothrombin time.

Platelet Count

A normal platelet count is 250,000-400,000 platelets per cubic millimetre of blood. Thrombocytopenia is a condition in which the platelet count is greatly reduced, resulting in chronic bleeding through small vessels and capillaries. It can be caused by decreased platelet production as a result of hereditary disorders, lack of vitamin B12 (pernicious anaemia), drug therapy, or radiation therapy.

Prothrombin Time Measurement

Prothrombin time measurement is a measure of how long it takes for the blood to start clotting, which normally is 9-12 seconds. Because many clotting factors must be activated to form prothrombin, a deficiency of any one of them can cause an abnormal prothrombin time. Vitamin K deficiency, certain liver diseases, and drug therapy can cause an increased prothrombin time.

The following gives the normal reference range (for approx 95% of adults) of measurements of various haematological values in normal subjects.

Quantity	Value	Units
Basophils	0.01-0.10	$\times 10^9/l$
Bleeding time (Ivy)	< 11	minutes
Body fluid, total	50 (lean)-70 (obese)	% bodyweight
Body fluid, intracellular	30-40	% bodyweight
Body fluid, extracellular	20-30	% bodyweight
Blood volume, red cells	25-35 (M), 20-30 (F)	ml/kg
Blood volume, plasma	40-50	ml/kg
Cyanocobalamin (vitamin B12)	160-925	ng/l
Eosinophils	0.04-0.40	$\times 10^9/l$
Erythrocyte sedimentation rate (ESR)	< 6 (normal), 6-20 (suspicious), > 20 (abnormal)	mm/hour
Fibrinogen	1.5-4.0	g/l
Folate, serum	2-20	ug/l
Folate, red cell	> 100	ug/l
Haematocrit	40-54 (M), 35-47 (F)	% blood volume
Haemoglobin	13-18 (M), 11.5-16.5 (F)	g/dl
Leucocytes, total	4.0-11	$\times 10^9/l$
Lymphocytes	1.0-3.5	$\times 10^9/l$
Mean corpuscular volume (MCV)	78-98	fl
Monocytes	0.2-0.8	$\times 10^9/l$
Platelets	150-400	$\times 10^9/l$
Prothrombin time	11-15	sec
Red cells	4.5-6.5 (M), 3.8-5.8 (F)	$\times 10^{12}/l$
Red cell life span (mean)	120	days
Red cell half life (by Cr-51)	25-35	days
Reticulocytes	10-100	$\times 10^9/l$
Neutrophils	2.5-7.5	$\times 10^9/l$

KEY LEARNING POINT.



- 1. The most commonly-used systems for blood grouping are the ABO system, and the Rhesus system.**
- 2. In the ABO system, the presence of A and B antigens are detected to establish groupings. Type O blood has neither A nor B antigens.**
- 3. Plasma from type A blood contains B antibodies, type B plasma contains A antibodies, AB blood has neither, whilst O blood has both.**
- 4. The transfusion of blood with incompatible antibodies will result in a transfusion reaction.**
- 5. Rhesus markers refer to the presence of an antigen of the surface of erythrocytes. Rh positive means that the antigen is present.**
- 6. Rhesus positive blood cannot be given to a person with Rh negative blood as a transfusion reaction will occur – the recipient will produce antibodies against the Rh antigen.**