Physiology

Dr. Muna H. AL-Saeed

Haematology

- **Blood:-** is a specialized bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells.

In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume) and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood. The blood cells are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellularly dissolved in plasma as bicarbonate ion.

Vertebrate blood is bright red when its hemoglobin is oxygenated. Some animals, such as crustaceans and mollusks, use hemocyanin to carry oxygen, instead of hemoglobin. Insects and some mollusks use a fluid called hemolymph instead of blood, the difference being that hemolymph is not contained in a closed circulatory system. In most insects, this "blood" does not contain oxygen-carrying molecules such as hemoglobin because their bodies are small enough for their tracheal system to suffice for supplying oxygen.

Vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Arthropods, using hemolymph, have hemocytes as part of their immune system.

Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled.

Medical terms related to blood often begin with ***hemo-*** or ***hemato-*** (also spelled ***haemo-*** and ***haemato-***) from the Greek word αἷμα (*haima*) for "blood". In terms of anatomy and histology, blood is considered a specialized form of connective tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen.

Function of blood:-

Blood performs many important functions within the body including:

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))
- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies

- Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding)
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature
- Hydraulic functions

▪ Constituents of blood:-

Blood accounts for 7% of the body weight with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3. The average adult has a blood volume of roughly 5 liters (1.3 gal), which is composed of plasma and several kinds of cells. These blood cells (which are also called *corpuscles* or "**formed elements**") consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%.

Whole blood (plasma and cells) exhibits non-Newtonian fluid dynamics; its flow properties are adapted to flow effectively through tiny capillary blood vessels with less resistance than plasma by itself. In addition, if all human hemoglobin were free in the plasma rather than being contained in RBCs, the circulatory fluid would be too viscous for the cardiovascular system to function effectively.

- **Erythrocytes:** Red blood cells contain the blood's hemoglobin and distribute oxygen. Mature red blood cells lack a nucleus and organelles in mammals. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoproteins that define the different blood types. The proportion of blood occupied by red blood cells is referred to as the hematocrit, and is normally about 45%. The combined surface area of all red blood cells of the human body would be roughly 2,000 times as great as the body's exterior surface.[6]
- **Leukocytes:** White blood cells are part of the body's immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances. The cancer of leukocytes is called leukemia.
- **Thrombocytes:**[7]: Also called platelets, thrombocytes are responsible for blood clotting (coagulation). They change fibrinogen into fibrin. This fibrin creates a mesh onto which red blood cells collect and clot, which then stops more blood from leaving the body and also helps to prevent bacteria from entering the body.

▪ Plasma :-

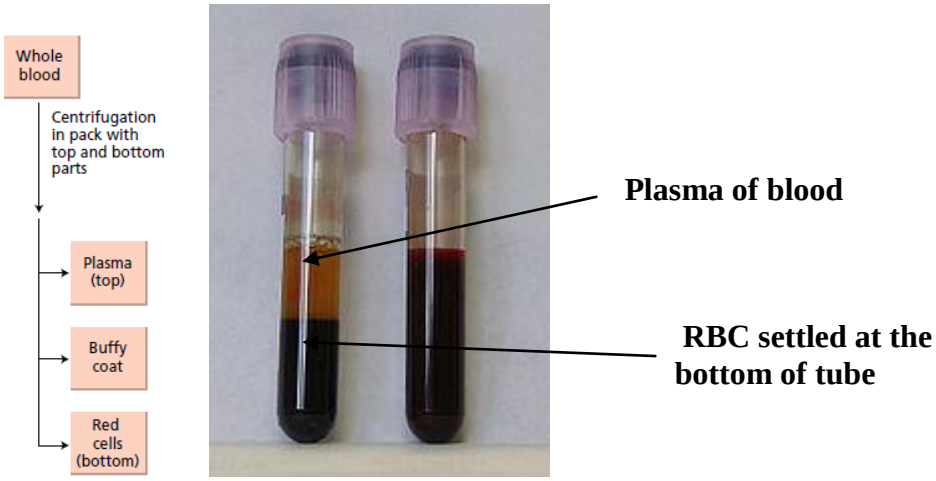
About 55% of blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid. Other important components include:

- Serum albumin
- Blood-clotting factors (to facilitate coagulation)
- Immunoglobulins (antibodies)

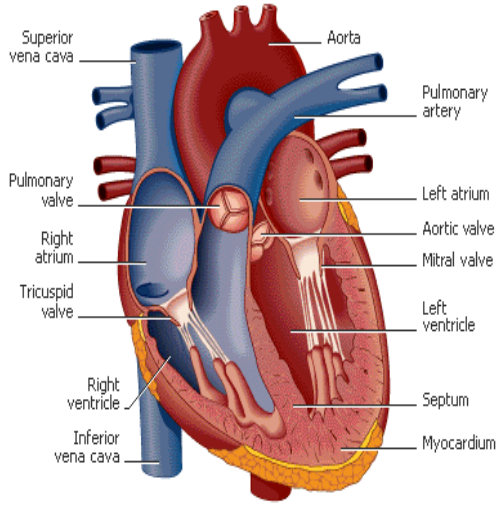
- lipoprotein particles
- Various other proteins

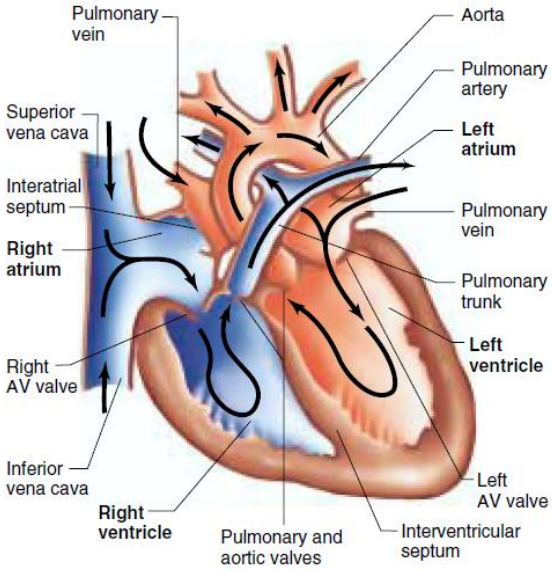
- Various electrolytes (mainly sodium and chloride)

The term **serum** refers to plasma from which the clotting proteins have been removed. Most of the proteins remaining are albumin and immunoglobulins.



- **Blood** is circulated around the body through blood vessels by the pumping action of the heart. Blood is pumped from the strong left ventricle of the heart through arteries to peripheral tissues and returns to the right atrium of the heart through veins. It then enters the right ventricle and is pumped through the pulmonary artery to the lungs and returns to the left atrium through the pulmonary veins. Blood then enters the left ventricle to be circulated again. Arterial blood carries oxygen from inhaled air to all of the cells of the body, and venous blood carries carbon dioxide, a waste product of metabolism by cells, to the lungs to be exhaled. However, one exception includes pulmonary arteries, which contain the most deoxygenated blood in the body, while the pulmonary veins contain oxygenated blood.
  Additional return flow may be generated by the movement of skeletal muscles, which can compress veins and push blood through the valves in veins toward the right atrium.





Dr. Muna H. AL-Saeed

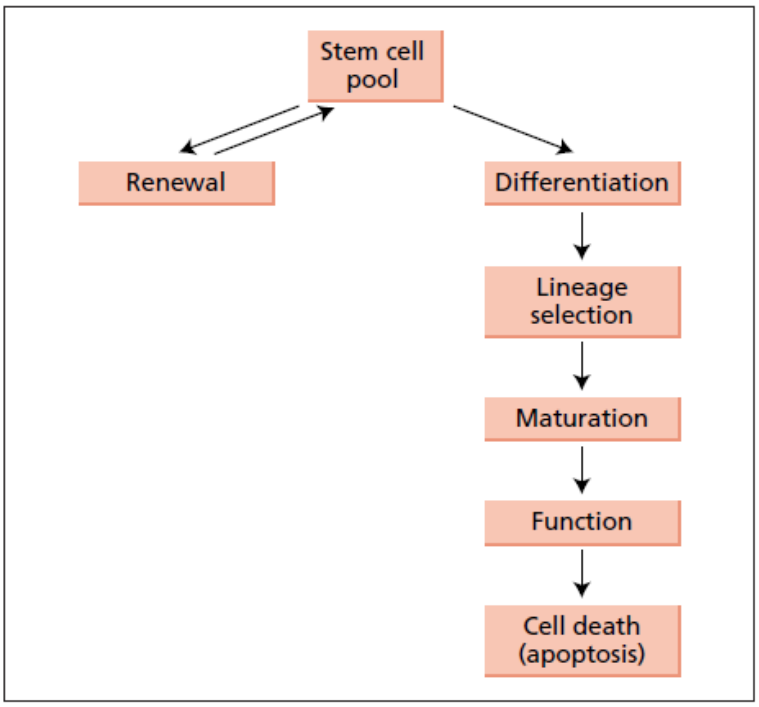
- **Production and degradation of blood cells:-**

In vertebrates, the various cells of blood are made in the bone marrow in a process called hematopoiesis, which includes erythropoiesis, the production of red blood cells; and myelopoiesis, the production of white blood cells and platelets.

- **Stem cells and haemopoiesis:-**

The lifelong production of blood cells occurs in haemopoietic tissue. This involves a very high level of cell turnover, demanded by the need to replace mature circulating blood cells at a rapid rate, and is necessitated by the limited lifespan of the mature cells. Granulocytes survive for only a few hours and erythrocytes for a few months, so that some 10^{13} new cells must be replaced each day to maintain steady-state blood counts. This is equivalent to an annual number of cells approximating the total body weight, but the total bone marrow of an adult human contains around 10^{12} cells, 10-fold less than daily needs. From these estimates it is clear that the blood cells required for lifelong haemopoiesis cannot be preformed in the body**.**

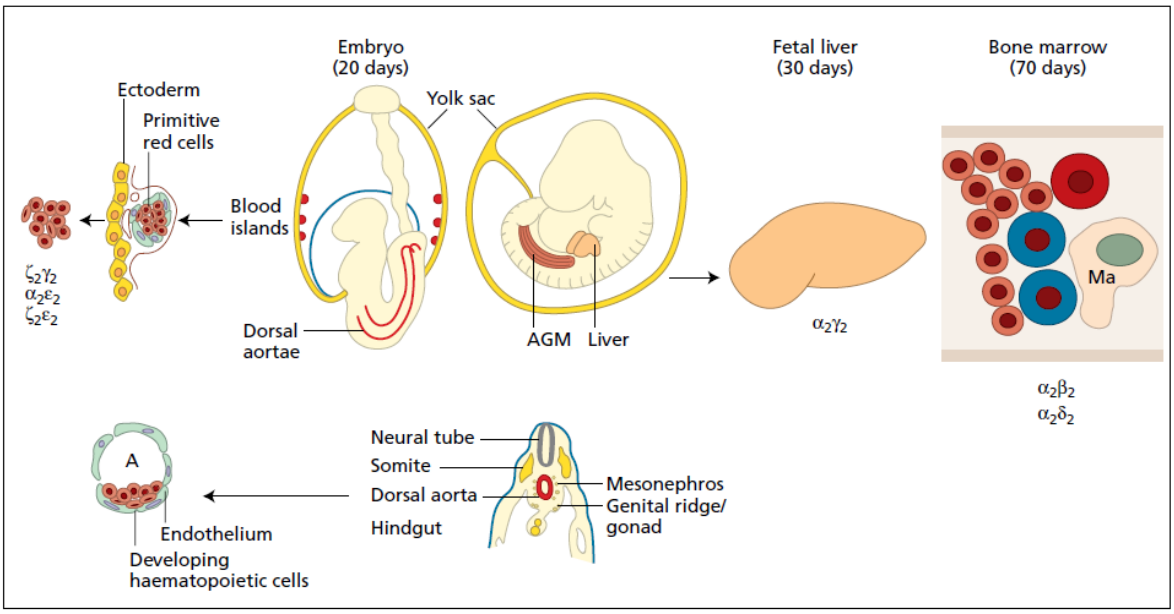
The bone marrow, which is the major site of haemopoiesis in adult humans, contains cells that represent the stages in the development of the different types of blood cells.



Stages in the haemopoietic cell development.

- Erythropoiesis:- This process includes all steps of haemopoiesis,starting with the initial specification of haemopoietic stem cells (HSCs) from mesoderm during embryogenesis. This continues with the decisions of these cells to undergo self-renewal or differentiation,through the process of lineage specification and proliferation to form committed erythroid progenitors. Finally, erythroblasts undergo terminal differentiation and post-mitotic maturation as they develop into red blood cells.

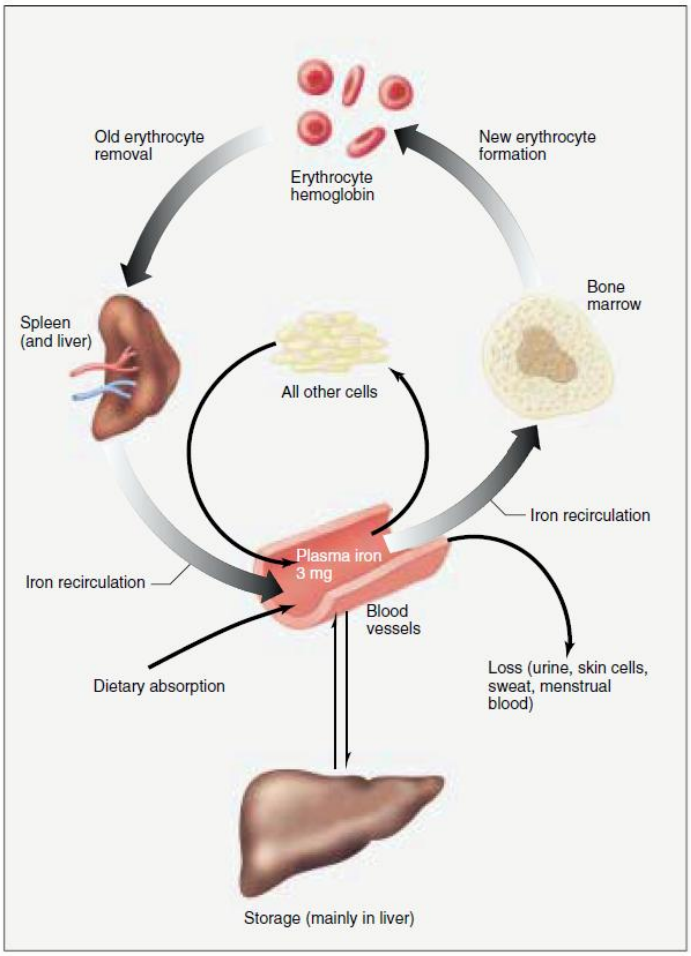
In a normal adult, the numbers of circulating red blood cells and their precursors remain more or less constant with a balance between the continuous loss of mature cells by senescence andnew red cell production in the marrow.



The origin and development of erythropoiesis during embryogenesis

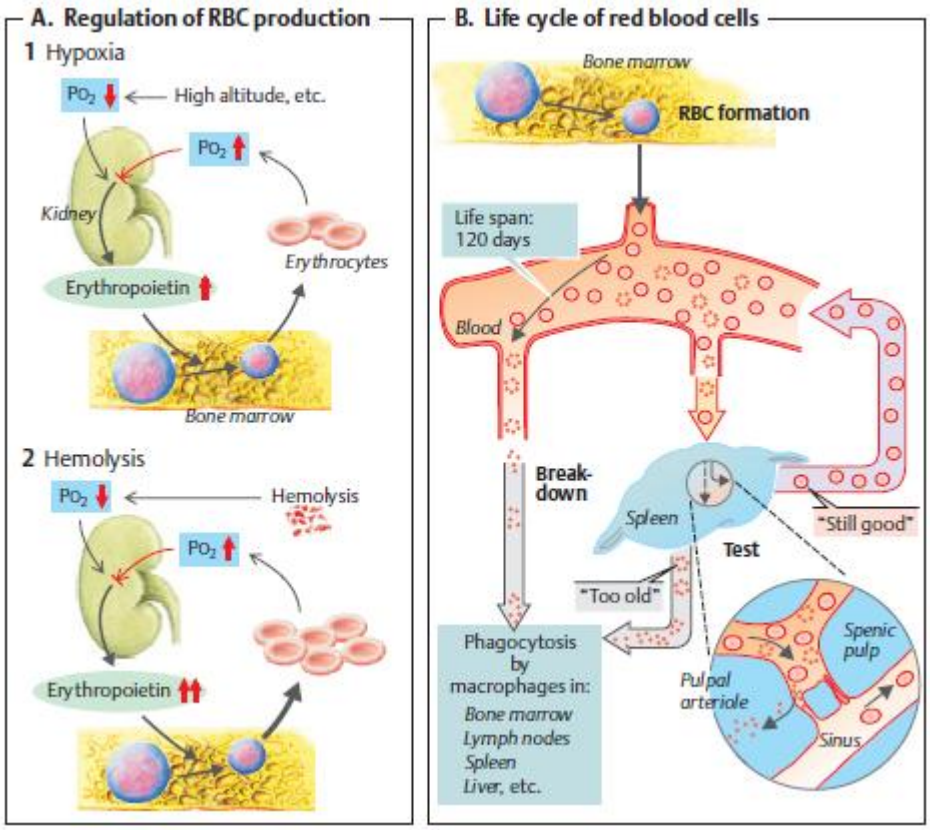
Erythrocytes have the shape of a biconcave disk—that is, a disk thicker at the edges than in the middle, like a doughnut with a center depression on each side instead of a hole. This shape and their small size (7 _m in diameter) impart to the erythrocytes a high surface-to-volume ratio, so that oxygen and carbon dioxide can diffuse rapidly to and from the interior of the cell. The plasma membrane of erythrocytes contains specific polysaccharides and proteins that differ from person to person, and these confer upon the blood its so-called type, or group. The site of erythrocyte production is the soft interior of bones called **bone marrow,** specifically the "red" bone marrow. With differentiation, the erythrocyte precursors produce hemoglobin but then they ultimately lose their nuclei and organelles—their machinery for protein synthesis. Young erythrocytes in the bone marrow still contain a few ribosomes, which produce a web-like (reticular) appearance when treated with special stains, an appearance that gives these young erythrocytes the name **reticulocyte.** Normally, only mature erythrocytes, which have lost these ribosomes, leave the bone marrow and enter the general circulation. In the presence of unusually rapid erythrocyte production, however, many reticulocytes do enter the blood, a fact of clinical diagnostic usefulness. Because erythrocytes lack nuclei and organelles, they can neither reproduce themselves nor maintain their normal structure for very long. The average life span of an erythrocyte is approximately 120 days, which means that almost 1 percent of the body's erythrocytes are destroyed and must be replaced every day. This amounts to 250 billion cells per day! Erythrocyte destruction normally occurs in the spleen and the liver. The most of the iron released in the process is conserved. The major breakdown product of hemoglobin is bilirubin, which, as noted above, gives plasma its color.

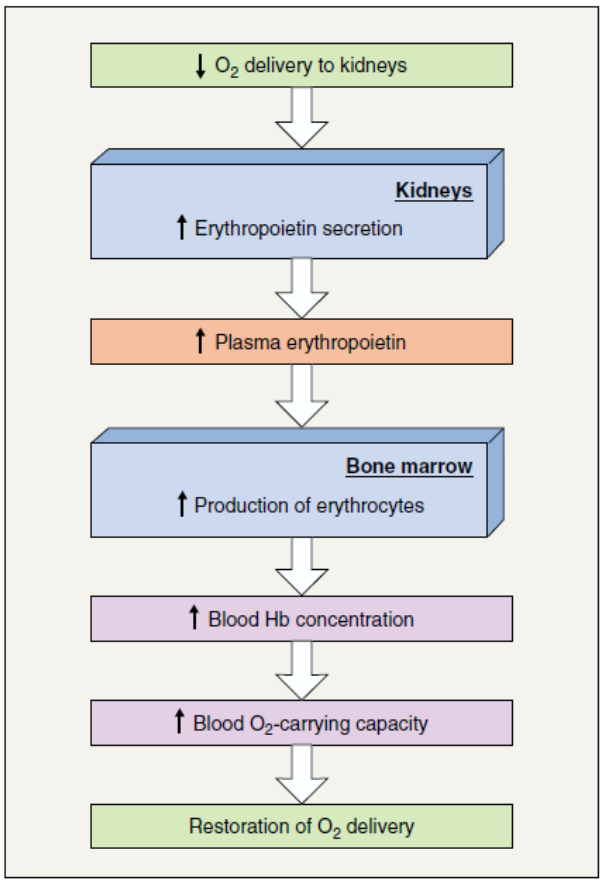
The production of erythrocytes requires the usual nutrients needed to synthesize any cell: amino acids, lipids, and carbohydrates. In addition, both iron and certain growth factors, including the vitamins folic acid and vitamin B12, are essential.

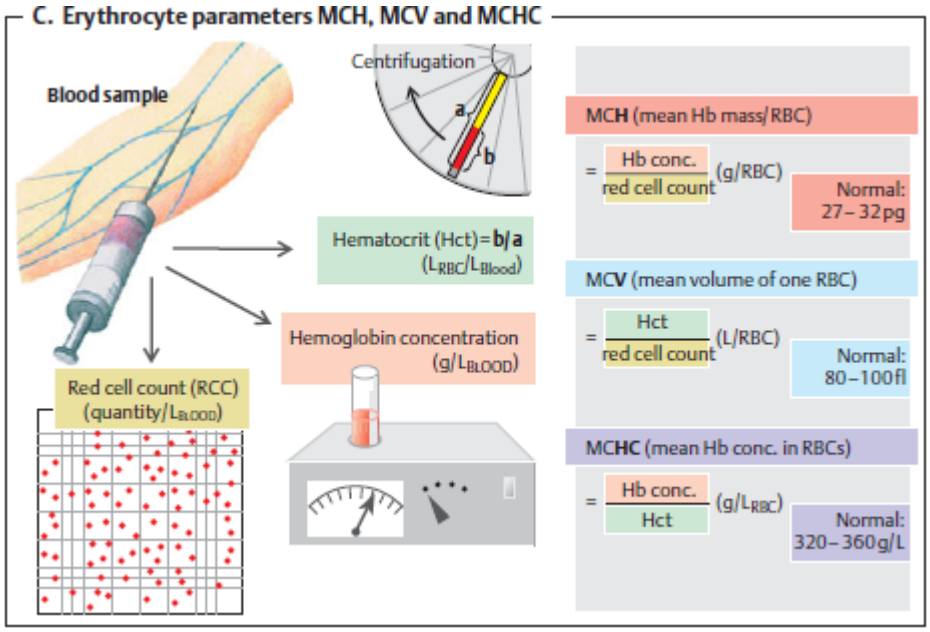


- **<u>Regulation of Erythrocyte Production</u>:-**

In a normal person, the total volume of circulating erythrocytes remains remarkably constant because of reflexes that regulate the bone marrow's production of these cells. The direct control of erythrocyte production (erythropoiesis) is exerted primarily by a hormone called **erythropoietin,** which is secreted into the blood mainly by a particular group of hormone-secreting connective-tissue cells in the kidneys (the liver also secretes this hormone, but to a much lesser extent). Erythropoietin acts on the bone marrow to stimulate
the proliferation of erythrocyte progenitor cells and their differentiation into mature erythrocytes. Erythropoietin is normally secreted in relatively small amounts, which stimulate the bone marrow to produce erythrocytes at a rate adequate to replace the usual loss. The erythropoietin secretion rate is increased markedly above basal values when there is a decreased oxygen delivery to the kidneys. Situations in which this occurs include insufficient pumping ofblood by the heart, lung disease, anemia (a decrease in number of erythrocytes or in hemoglobin concentration), and exposure to high altitude. As a result of the increase in erythropoietin secretion, plasma erythropoietin concentration, erythrocyte production, and the oxygen-carrying capacity of the blood all increase; therefore, oxygen delivery to the tissues returns toward normal.Testosterone, the male sex hormone, also stimulates the release of erythropoietin. This accounts, at least in part, for the higher hemoglobin concentration in men than in women.


A. Regulation of RBC production
1 Hypoxia
Po_2
High altitude, etc.
Po_2
Kidney
Erythrocytes
Erythropoietin
Bone marrow
2 Hemolysis
Po_2
Hemolysis
Po_2
Erythropoietin
B. Life cycle of red blood cells
Bone marrow
RBC formation
Life span: 120 days
Blood
Break-down
Spleen
"Still good"
Test
"Too old"
Phagocytosis by macrophages in:
Bone marrow
Lymph nodes
Spleen
Liver, etc.
Spenic pulp
Pulpal arteriole
Sinus


↓ O_2 delivery to kidneys
Kidneys
↑ Erythropoietin secretion
↑ Plasma erythropoietin
Bone marrow
↑ Production of erythrocytes
↑ Blood Hb concentration
↑ Blood O_2-carrying capacity
Restoration of O_2 delivery

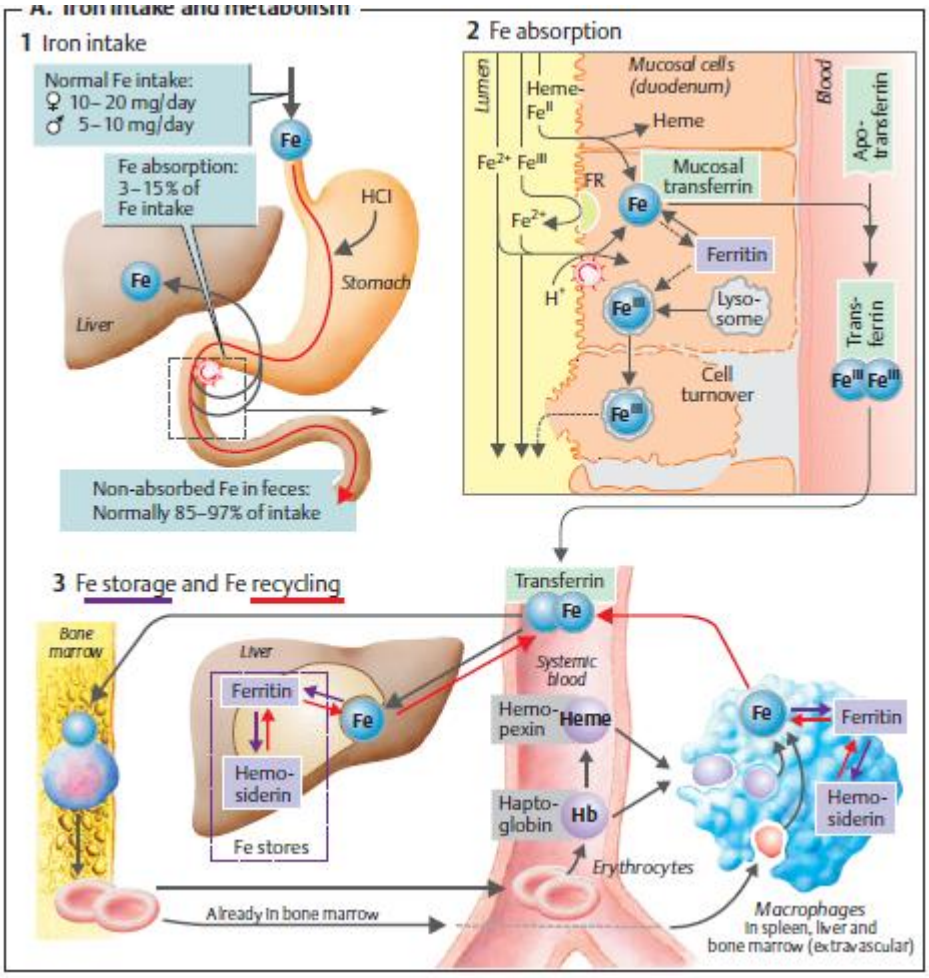


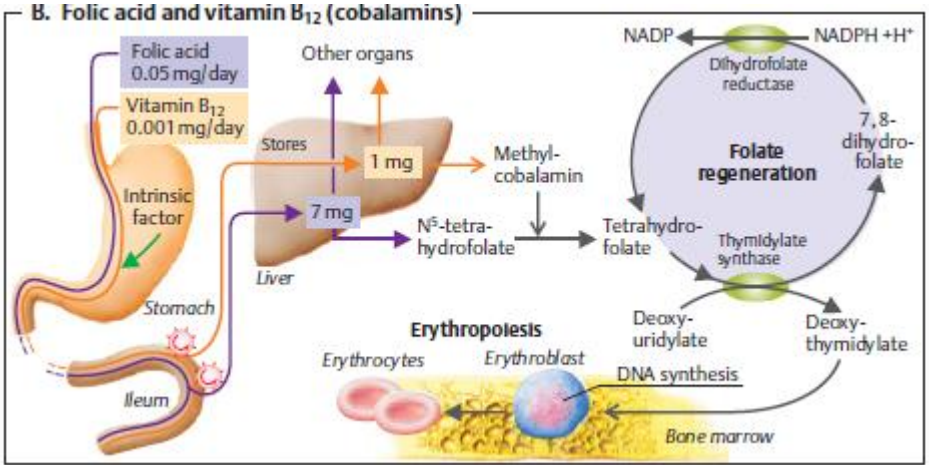
Iron Metabolism and Erythropoiesis

The body's **iron pool** (ca. 2 g in women and 5 g in men) is bound to *hemoglobin* (Hb). About 1/4 exists as *stored iron* (ferritin, hemosiderin),the rest as *functional iron* (myoglobin,iron-containing enzymes). **Iron losses** from the body amount to about 1 mg/day in men and up to 2 mg/day in women due to menstruation, birth, and pregnancy. **Iron absorption** occurs mainly in the duodenum and *varies according to need*. The absorption of iron supplied by the diet usually amounts to about 3 to 15% in healthy individuals, but can increase to over 25% in individuals with iron deficiency. A minimum daily **iron intake** of at least 10–20 mg/day is therefore recommended (women ! children ! men).

Iron absorption Fe(II) supplied by the diet (hemoglobin,myoglobin found chiefly in meat and fish) is absorbed relatively efficiently as a **heme-Fe(II)** upon protein cleavage. With the aid of *heme oxygenase*, Fe in mucosal cells cleaves from heme and oxidizes to Fe(III). The triferric form either remains in the mucosa as a ferritin-Fe(III) complex and returns to the lumen during cell turnover or enters the bloodstream. **Non-heme-Fe** can only be absorbed as Fe2+. Therefore, non-heme Fe(III) must first be reduced to Fe2+ by *ferrireductase*(and ascorbate on the surface of the luminal mucosa. Fe2+ is probably absorbed through secondary active transport via an Fe2+-H+ symport carrier (DCT1) (competition with Mn2+, Co2+, Cd2+, etc.). A *low chymous pH* is important since it (a) increases the H+gradient that drives Fe2+ via DCT1 into the cell and (b) frees dietary iron from complexes. The absorption of iron into the bloodstream is *regulated by the intestinal mucosa*. When an iron deficiency exists, *aconitase* (an iron-regulating protein) in the cytosol binds with ferritin-mRNA, thereby inhibiting mucosal ferritin translation. As a result, larger quantities of absorbed Fe(II) can enter the bloodstream. Fe(II) in the blood is oxidized to Fe(III) by ceruloplasmin (and copper). It then binds to *apotransferrin*, a protein responsible for **iron transport in plasma**. *Transferrin* (= apotransferrin loaded with 2 Fe(III)), is taken up by endocytosis into erythroblasts and cells of the liver, placenta, etc. with the aid of *transferrin receptors*. Once iron has been released to the target cells, apotransferrin again becomes available for uptake of iron from the intestine and macrophages (see below). **Iron storage and recycling** (!**A3**). *Ferritin*, one of the chief forms in which iron is stored in the body, occurs mainly in the intestinal mucosa, liver, bone marrow, red blood cells, and plasma. It contains binding pockets for up to

4500 Fe3+ ions and provides rapidly available stores of iron (ca. 600 mg), whereas iron mobilization from *hemosiderin* is much slower (250mg Fe in macrophages of the liver and bone marrow). Hb-Fe and heme-Fe released from malformed erythroblasts (so-called inefficient erythropoiesis) and hemolyzed red blood cells bind to haptoglobin and hemopexin, respectively. They are then engulfed by macrophages in the bone marrow or
in the liver and spleen, respectively, resulting in 97% iron recycling .
An **iron deficiency** inhibits Hb synthesis, leading to hypochromic microcytic anemia:
MCH "26 pg, MCV "70 fL, Hb "110 g/L. The primary causes are: ! blood loss (most common cause); 0.5mg Fe are lost with each mL of blood; ! insufficient iron intake or absorption; ! increased iron requirement due to growth,pregnancy, breast-feeding, etc.;
! decreased iron recycling (due to chronic infection); ! apotransferrin defect (rare cause).
Iron overload most commonly damages the liver, pancreas and myocardium (hemochromatosis). If the iron supply bypasses the intestinal tract (iron injection

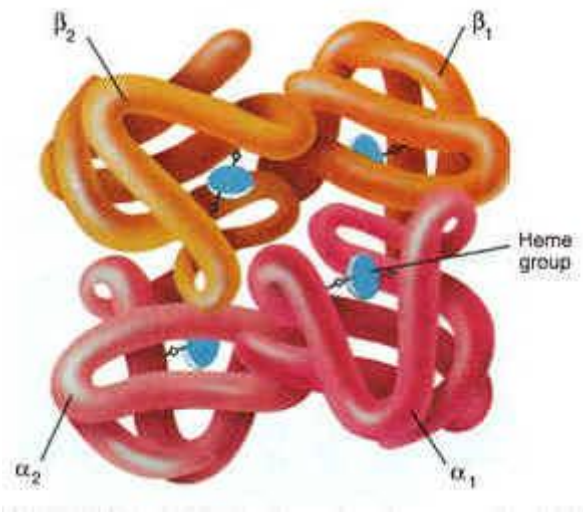



B. Folic acid and vitamin B_{12} (cobalamins)
Folic acid 0.05 mg/day
Vitamin B_{12} 0.001 mg/day
Intrinsic factor
Stomach
Ileum
Other organs
Stores
1 mg
7 mg
Liver
Methyl-cobalamin
N^5-tetra-hydrofolate
Tetrahydro-folate
NADP
NADPH +H+
Dihydrofolate reductase
7,8-dihydro-folate
Folate regeneration
Thymidylate synthase
Deoxy-uridylate
Deoxy-thymidylate
Erythropoiesis
Erythrocytes
Erythroblast
DNA synthesis
Bone marrow

Physiology

Dr. Muna H. AL-Saeed

Haemoglobin



Haemoglobin(Hb):- is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body. In mammals, the protein makes up about 97% of the red blood cells' dry content (by weight), and around 35% of the total content (including water). Hemoglobin has an oxygen binding capacity of 1.34 mL O_2 per gram of hemoglobinwhich increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules. Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen. Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism. Hemoglobin and hemoglobin-like molecules are also found in many invertebrates, fungi, and plants. In these organisms, hemoglobins may carry oxygen, or they may act to transport and regulate other things such as carbon dioxide, nitric oxide, hydrogen sulfide and sulfide. A variant of the molecule, called leghemoglobin, is used to scavenge oxygen away from anaerobic systems, such as the nitrogen-fixing nodules of leguminous plants, before the oxygen can poison the system.

Synthesis:- Hemoglobin (Hb) is synthesized in a complex series of steps. The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol. Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals,

residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name).

Structure:- Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins. Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.

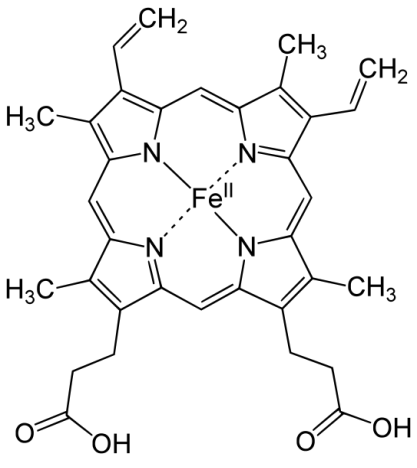
In most vertebrates, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement, so called because this arrangement is the same folding motif used in other heme/globin proteins such as myoglobin. This folding pattern contains a pocket that strongly binds the heme group.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methine bridges) with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the imidazole ring of F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond, completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is actually bound to the protein chains of the structure.

The iron ion may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporarily turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable of binding oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

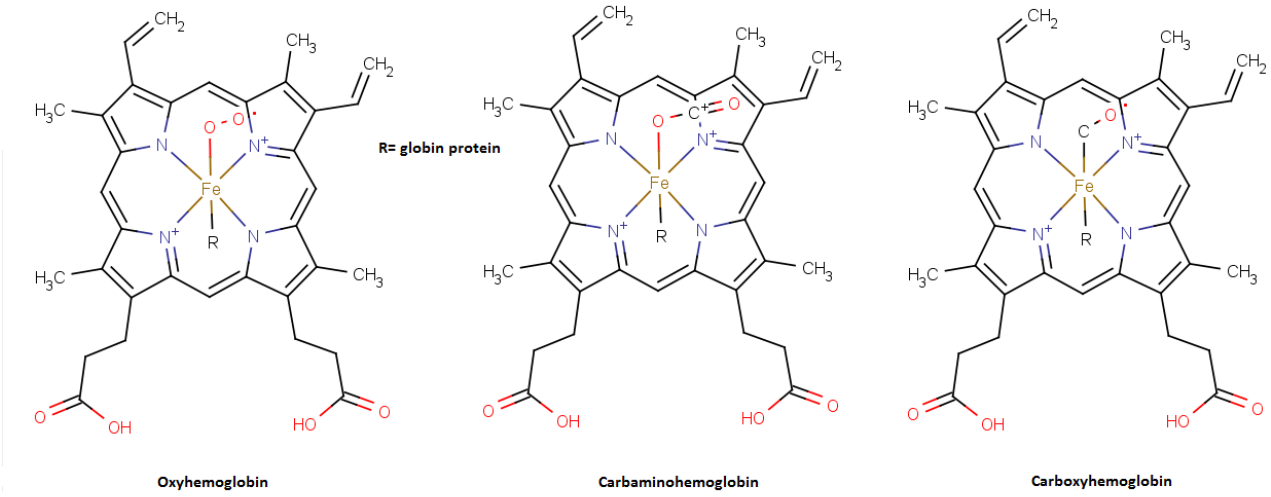
In adult humans, the most common hemoglobin type is a tetramer (which contains 4 subunit proteins) called *hemoglobin A*, consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$. The subunits are structurally similar and about the same size.



Dr. Muna H. AL-Saeed

Oxygen saturation:- In general, hemoglobin can be saturated with oxygen molecules (oxyhemoglobin), or desaturated with oxygen molecules (deoxyhemoglobin).

Oxyhemoglobin:- *Oxyhemoglobin* is formed during physiological respiration when oxygen binds to the heme component of the protein hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the blood stream to be dropped off at cells where it is utilized as a terminal electron acceptor in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease in blood pH. Ventilation, or breathing, may reverse this condition by removal of carbon dioxide, thus causing a shift up in pH. Hemoglobin exists in two forms, a *taut (tense) form* (T) and a *relaxed form* (R). Various factors such as low pH, high CO_2 and high 2,3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. Conversely, a high pH, low CO_2, or low 2,3 BPG favors the relaxed form which can better bind oxygen. The partial pressure of the system also affects O_2 affinity where, at high partial pressures of oxygen (such as those present in the alveoli), the relaxed (high affinity, R) state is favoured. Inversely, at low partial pressures (such as those present in respiring tissues), the (low affinity, T) tense state is favoured. Additionally, the binding of oxygen to the Iron-II heme pulls the iron into the plane of the porphryn ring, causing a slight conformational shift. The shift encourages oxygen to bind to the three remaining hemes within hemoglobin (thus, oxygen binding is cooperative).



Deoxygenated hemoglobin:- Deoxygenated hemoglobin is the form of hemoglobin without the bound oxygen. The absorption spectra of oxyhemoglobin and deoxyhemoglobin differ. The oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm its absorption is slightly higher. This difference is used for measurement of the amount of oxygen in patient's blood by an instrument called pulse oximeter. This difference also accounts for the presentation of cyanosis, the blue to purplish color that tissues develop during hypoxia**.**

Types of Hemoglobin:- Hemoglobin variants are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Some well-known hemoglobin variants such as sickle-cell anemia are responsible for diseases, and are considered hemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants**.**

- In the embryo
- Gower 1 ($\zeta_2\varepsilon_2$)
- Gower 2 ($\alpha_2\varepsilon_2$) (PDB 1A9W)
- Hemoglobin Portland I ($\zeta_2\gamma_2$)
- Hemoglobin Portland II ($\zeta_2\beta_2$).

In the fetus:

- Hemoglobin F ($\alpha_2\gamma_2$) (PDB 1FDH).

In adults:

- Hemoglobin A ($\alpha_2\beta_2$) (PDB 1BZ0) – The most common with a normal amount over 95%
- Hemoglobin A_2 ($\alpha_2\delta_2$) – δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5–3.5%
- Hemoglobin F ($\alpha_2\gamma_2$) – In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia

Degradation of hemoglobin in animals:-

When red cells reach the end of their life due to aging or defects, they are broken down in spleen, the hemoglobin molecule is broken up and the iron gets recycled. This process also produces one molecule of carbon monoxide for every molecule of heme degraded. This is one of the few natural sources of carbon monoxide production in the human body, and is responsible for the normal blood levels of carbon monoxide even in people breathing pure air. The other major final product of heme degradation is bilirubin. Increased levels of this chemical are detected in the blood if red cells are being destroyed more rapidly than usual. Improperly degraded hemoglobin protein or hemoglobin that has been released from the blood cells too rapidly can clog small blood vessels, especially the delicate blood filtering vessels of the kidneys, causing kidney damage. Iron is removed from heme and salvaged for later use, it is stored as hemosiderin or ferritin in tissues and transported in plasma by beta globulins as transferins. When the porphyrin ring is broken up, the fragments are normally secreted as a yellow pigment called bilirubin, which is secreted into the intestines as bile. Intestines metabolise bilirubin into urobilinogen. Urobilinogen leaves the body in faeces, in a pigment called stercobilin. Globulin is metabolised into amino acids which are then released into circulation.

white blood cell, also called leukocyte or white corpuscle, a cellular component of the blood that lacks hemoglobin, has a nucleus, is capable of motility, and defends the body against infection and disease by ingesting foreign materials and cellular debris, by destroying infectious agents and cancer cells, or by producing antibodies. A healthy adult human has between 4,500 and 11,000 white blood cells per cubic millimetre of blood. Fluctuations in white cell number occur during the day; lower values are obtained during rest and higher values during exercise. Intense physical exertion may cause the count to exceed 20,000 per cubic millimetre. White cell count also may increase in response to convulsions, strong emotional reactions, pain, pregnancy, labour, and certain disease states, such as infections and intoxications.

Although white cells are found in the circulation, most occur outside the circulation, within tissues, where they fight infections; the few in the bloodstream are in transit from one site to another. As living cells, their survival depends on their continuous production of energy. The chemical pathways utilized are more complex than those of red blood cells and are similar to those of other tissue cells. White cells, containing a nucleus and able to produce ribonucleic acid (RNA), can synthesize protein. White cells are highly differentiated for their specialized functions, and they do not undergo cell division (mitosis) in the bloodstream; however, some retain the capability of mitosis. On the basis of their appearance under a light microscope, white cells are grouped into three major classes—lymphocytes, granulocytes, and monocytes—each of which carries out somewhat different functions.

Lymphocytes, which are further divided into B and T cells, are responsible for the specific recognition of foreign agents and their subsequent removal from the host. B lymphocytes secrete antibodies, which are proteins that bind to foreign microorganisms in body tissues and mediate their destruction. Typically, T cells recognize virally infected or cancerous cells and destroy them, or they serve as helper cells to assist the production of antibody by B cells. Also included in this group are natural killer (NK) cells, so named for their inherent ability to kill a variety of target cells. In a healthy person, about 25 to 33 percent of white blood cells are lymphocytes.

Granulocytes, the most numerous of the white cells, rid the body of large pathogenic organisms such as protozoans or helminths and are also key mediators of allergy and other forms of inflammation. These cells contain many cytoplasmic granules, or secretory vesicles, that harbour potent chemicals important in immune responses. They also have multilobed nuclei, and because of this they are often called polymorphonuclear cells. On the basis of how their granules take up dye in the laboratory, granulocytes are subdivided into three categories: neutrophils, eosinophils, and basophils. The most numerous of the granulocytes—making up 50 to 80 percent of all white cells—are neutrophils. They are often one of the first cell types to arrive at a site of infection, where they engulf and destroy the infectious microorganisms through a process called phagocytosis. Eosinophils and basophils, as well as the tissue cells called mast cells, typically arrive later. The granules of basophils and of the closely related mast cells contain a number of chemicals, including histamine and leukotrienes, that are important in inducing allergic inflammatory responses. Eosinophils destroy parasites and also help to modulate inflammatory responses.

Monocytes, which constitute between 4 and 8 percent of the total number of white blood cells in the blood, move from the blood to sites of infection, where they differentiate further into macrophages. These cells are scavengers that phagocytose whole or killed microorganisms and are therefore effective at direct destruction of pathogens and cleanup of cellular debris from sites of infection. Neutrophils and macrophages are the main phagocytic

cells of the body, but macrophages are much larger and longer-lived than neutrophils. Some macrophages are important as antigen-presenting cells, cells that phagocytose and degrade microbes and present portions of these organisms to T lymphocytes, thereby activating the specific acquired immune response.

Specific types of cells are associated with different illnesses and reflect the special function of that cell type in body defense. A fall in white cell count, which is called leukopenia, occurs in states such as debilitation, anaphylaxis, and overwhelming infection. In general, newborns have a high white blood cell count that gradually falls to the adult level during childhood. An exception is the lymphocyte count, which is low at birth, reaches its highest levels in the first four years of life, and thereafter falls gradually to a stable adult level. *See also* blood cell formation.

WBC Normal values:

- WBC Count = 5,000 – 10,000/cu mm or
  $5 – 10 \times 10^9/L$
- Differential Count:
- Neutrophil = 50 – 70 %
  Segmenter = 50 – 65 % ; Stab = 0 – 5 %
- Eosinophil = 0 – 3 %
- Basophil = 0 – 1 %
- Lymphocytes = 20 – 40 %
- Monocytes = 2 – 6 %

Quantitative abnormalities

- Leucocytosis – substantial increase in the WBC count.

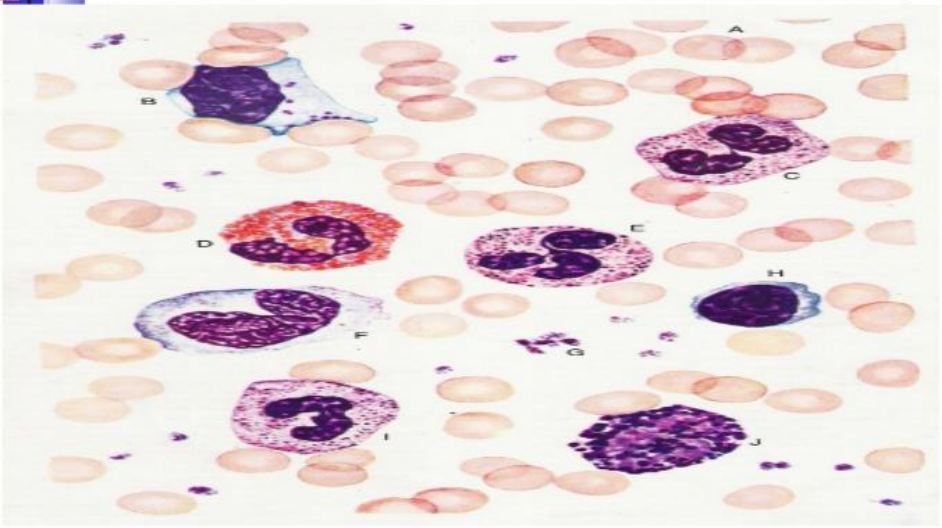
  - Physiologic increase (no trauma/injury)
  - Pathologic increase (trauma/pathology)

- Leucopenia – substantial decrease in the WBC count.
- N.V. = 5,000 – 10,000/cu mm

Differential Count

Neutrophil	50 – 75 %
segmenter	50 – 65 %
stab	0 – 5 %
Eosinophil	0 – 3 %
Basophil	0 – 1 %
Lymphocyte	20 – 40 %
Monocyte	2 – 6 %

The 5 WBC types



Neutrophilia
(> 7 – 8 x10^9/L)

- Infections, Inflammation, Metabolic disorders
- Acute hemorrhage, corticosteroids
- Stress, post-surgery, burns, HDN
- Lithium drugs, neoplasms

Neutropenia
(<1.75 – 1.8$10^9$/L)

- Decreased production
  - Inherited/acquired stem cell disorder
  - Benzene toxicity, cytotoxic drugs
- Increased destruction
  - Immune mechanism, sequestration
- BM depression, IM, varicella, Typhoid
- SLE, hepatitis or any viral infections

(> 0.7 x 10^9/L)

- Allergic disorders (asthma)
- Parasitic infections (nematodes)
- Skin disease (eczema)
- Hodgkin's disease
- Scarlet Fever
- Pernicious anemia

Eosinopenia (< 0.05 x 10^9/L)

- Stress due to trauma or shock
- Mental distress
- Cushing's syndrome
- ACTH administration

Basophil (0.3 x 10^9/L)

BASOPHILIA	Chronic myelocyic leukemia
	Polycythemia vera
	Hodgkin's disease
BASOPENIA	Hyperthyroidism
	Pregnancy

Lymphocytosis (>4.0 x 10^9/L)

- Viral infections (German measles)
- Infectious Mononucleosis (kissing dis.)
- Mumps (parotitis), pertussis
- Tuberculosis, syphilis, thyrotoxicosis

Lymphopenia

- Congestive heart failure, SLE
- Renal failure
- Advanced Tuberculosis
- High levels of adrenal corticosteroids

Monocytosis ($>0.9 \times 10^9/L$)

- SBE, Syphilis, Tuberculosis
- Protozoan infections
- Mycotic or fungal infections
- Malaria, Systemic lupus erythematosus
- Rheumatoid arthritis

Monocytopenia

- Lymphocytic leukemia
- Aplastic anemia

QUALITATIVE CHANGES-WBC

- Morphologic abnormalities involving either the nucleus or cytoplasm
- Functional abnormalities

- Inherited or Acquired
- Examination of peripheral blood or a bone marrow evaluation

The White blood cells:

- Nucleus details:

  - Mononuclear or Polymorphonuclear

- Granules present:

  - Granulocytic or Agranulocytic

- Function:

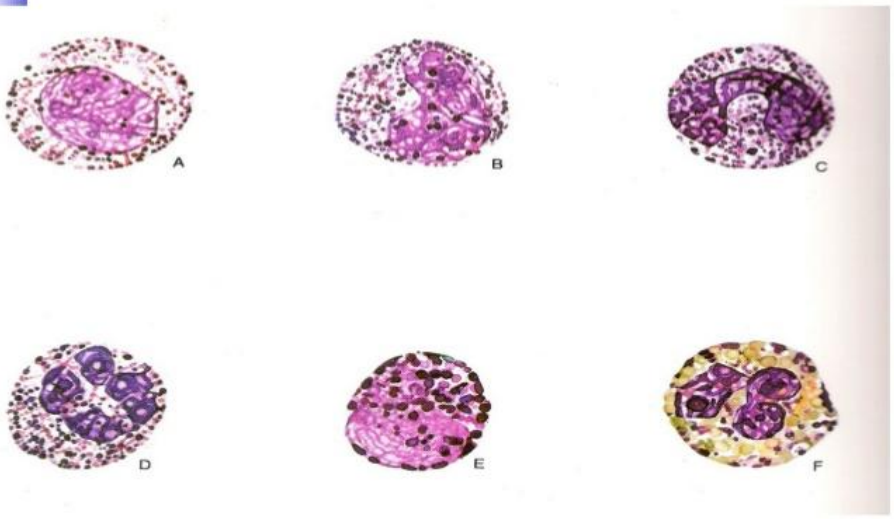
  - Phagocytic or Immunocytic

Abnormal granulocyte morphology (inherited)

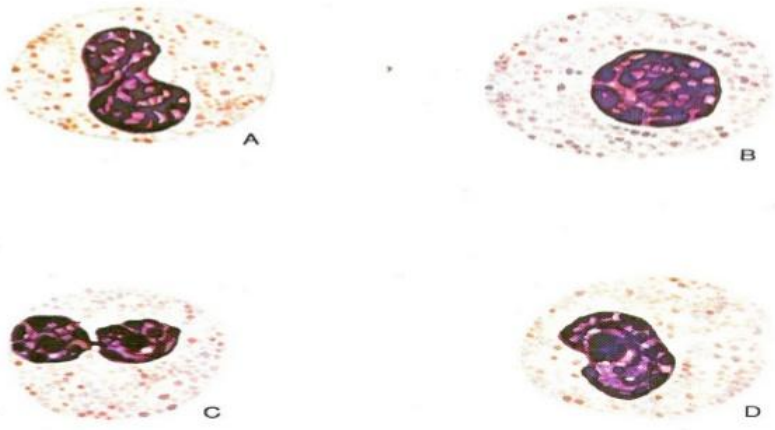
- Alder-Reilly anomaly - dense azurophilic granules, mucopolysaccharoidoses
- May-Hegglin anomaly - Giant platelets, Dohle-bodies like inclusions seen even in monocytes
- Pelger Huet anomaly – failure of normal segmentation of nucleus, bi-lobed nucleus or stab forms only,

  “pince-nez nucleus”

Alder Reilly anomaly



Pelger Huet anomaly



Other abnormalities:

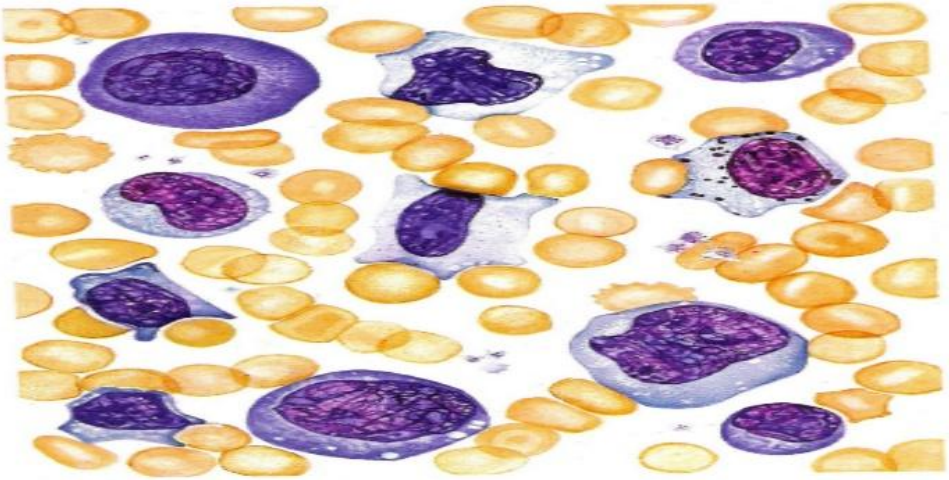
- Smudge or basket cells – squash-degenerated nucleus of WBCs
- Jordan's anomaly – fat-containing vacuoles in WBC cytoplasm, Ichthyosis
- Twinning deformity
- Auer rod – rod-like structure seen in the cytoplasm of myeloblasts, diagnostic for Acute myeloblastic leukemia (AML)

- Plasmacytoid lymphocyte or Turk's irritation cell
- Downey cell (atypical lymphocyte)
- Transformed lymphocyte (reticular or pyroninophilic cell)
- Reider cell – "clover-leaf like nucleus"
- Plasma cells

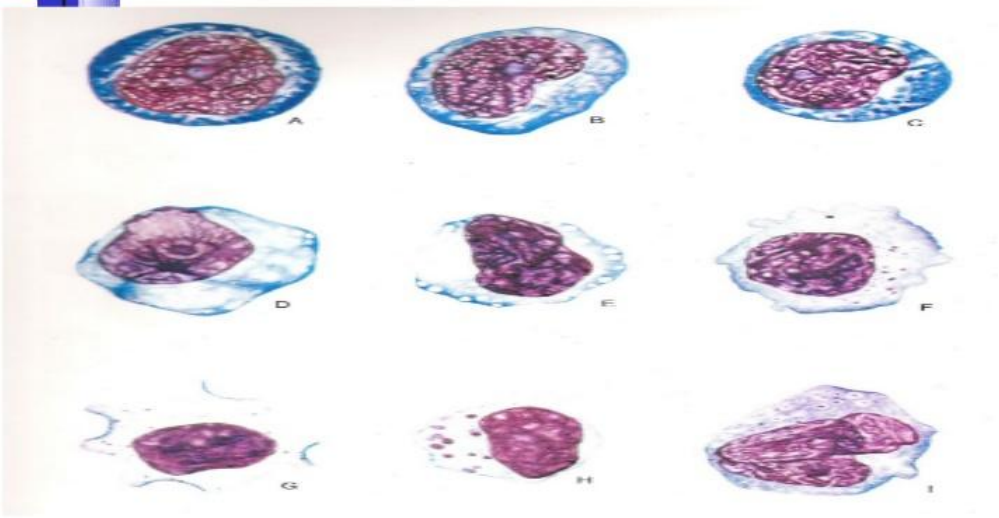
Downey cell

- Hallmark cell seen in cases of Infectious mononucleosis (kissing disease)
- Atypical lymphocyte (stress lymphocyte)
- “ballerina skirt cell”

Reactive lymphocytes



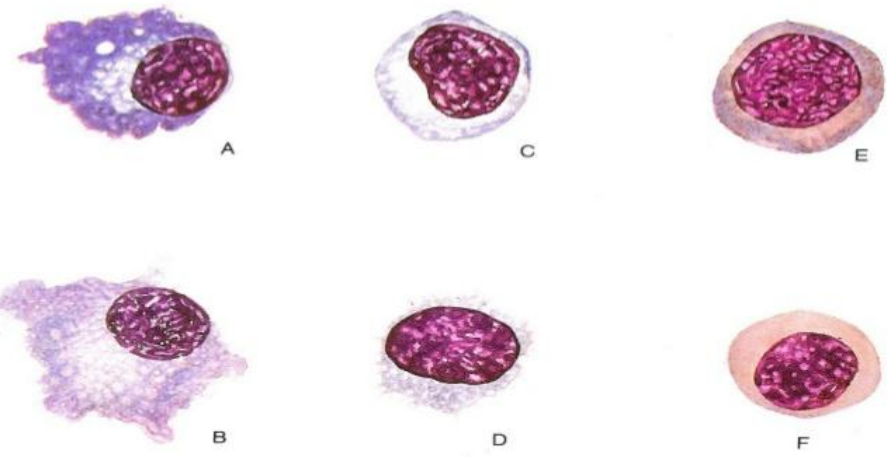
Infectious Mononucleosis



Plasma cells

- Ovoid or fibrillary shaped
- Eccentric location of nucleus
- Perinuclear halo
- “cart-wheel pattern or spoke of the wheel pattern of nucleus”
- basophilic cytoplasm

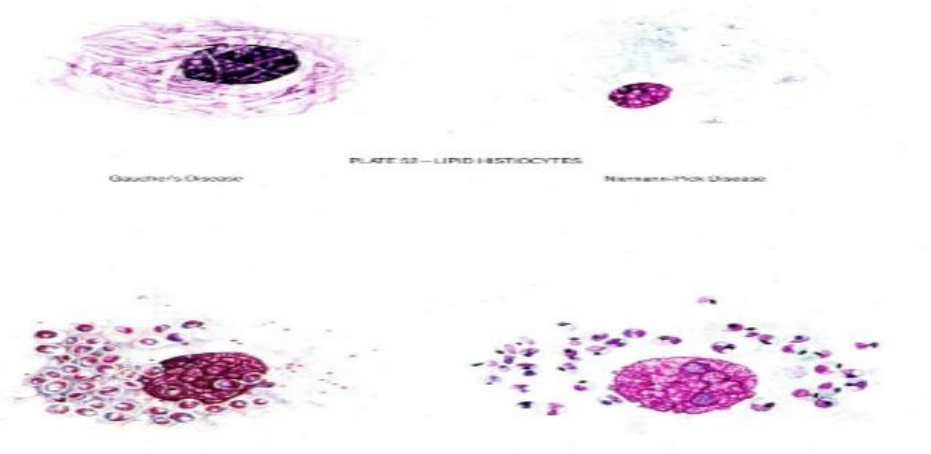
Comparative morphology of plasma cells, lymphocytes and NRBC



Inherited abnormalities involving Monocyte-macrophage group

- MUCOPOLYSACCHAROIDOSES
  - Hunter syndrome, Hurler's disease
- LIPIDOSES – lipid accumulation
  - Gaucher's disease – accumulation of glucocerebroside due to lack of beta-glucosidase enzyme
  - Neimann Pick disease – sphingomyelin and cholesterol accumulation due to lack of the enzyme sphingomyelinase

Monocyte-macrophage abnormality



WBC functions

- Neutrophil – phagocytic
- Eosinophil – phagocytic and damage to larval stages of parasite.
- Basophil – storage of histamine, involved in immediate hypersensitivity reaction.
- Monocyte – phagocytic, cellular and humoral immunity

Disorder In WBC:- Increased of its leukocytosis which may be physiological like active of muscle in pregnant or through infection to resistance for infection but abnormal increase in its know leukemia because of bone marrow infected. Decrease of its know leukopenia,more decreased in some infected like pneumonia, typhoid fever and also in drugs(analgesic drugs).

The Factor Effect to Stimulate WBC:-

Leukocyte inducing factor (Plasma factor).

White blood cell growth factors, also called hematopoietic (blood-forming) colony-stimulating factors (CSFs), are proteins that help the body make white blood cells. White blood cells help fight infection. Many cancer treatments, such as chemotherapy, damage white blood cells. This can cause neutropenia, a very low level of white blood cells that increases the risk of getting an infection. When neutropenia occurs with a fever, it is called febrile neutropenia and may be a sign of an infection. Infections can be very serious for people with cancer because they often do not have enough white blood cells to fight the infection on their own and will usually need to be treated in the hospital with antibiotics. CSFs increase levels of white blood cells to help a person avoid infection. However, most patients receiving chemotherapy will not need CSFs. This is because most chemotherapy is only associated with less severe neutropenia, and the risk of severe neutropenia can usually be predicted ahead of time.

Platelets

It's a fregment of cell from megakaryocytes which add to the blood after RBCs (1-3µ) no nucli, occure after distruction of megakaryocyte250000-400000.

1-make plug to wall of vein (platetate piug).

2-release of thromboplastin activator which act to produce coagulation.

3-serotonine release (vasoconstruction) decrease the blood slow in the destroyed wall.(vascular spasm).

4-ADP release (actin retruction of clott)(blood coagulation this above hemastasis.

Haemostasis:-

Aims to decrease the amount of blood and also keep the injury wound clean.

Blood clott

Its convers of protein (fibrinogen) to gel.

If the number of platelets is too low, excessive bleeding can occur. However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy, which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis). There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) or thrombotic thrombocytopenic purpura (TTP) that typically cause thromboses, or clots, instead of bleeding.

Platelets release a multitude of growth factors including platelet-derived growth factor (PDGF), a potent chemotactic agent, and TGF beta, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local

application of these factors in increased concentrations through Platelet-rich plasma (PRP) has been used as an adjunct to wound healing for several decades.

- The physiological range for platelets is $(150 – 400) \times 10^3$ per mm^3.
- Platelets are produced in blood cell formation (thrombopoiesis) in bone marrow, by budding off from megakaryocytes.
- Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone usually produced by the liver and kidneys.
- Each megakaryocyte produces between 5,000 and 10,000 platelets.
- Around 10^{11} platelets are produced each day by an average healthy adult.
- Reserve platelets are stored in the spleen, and are released when needed by sympathetically induced splenic contraction.
- The lifespan of circulating platelets is 5 to 9 days.
- Old platelets are destroyed by phagocytosis in the spleen and by Kupffer cells in the liver.

Thrombus formation:-

The function of platelets is the maintenance of hemostasis. This is achieved primarily by the formation of thrombi, when damage to the endothelium of blood vessels occurs. Conversely, thrombus formation must be inhibited at times when there is no damage to the endothelium. These processes are regulated through thromboregulation.

Activation

The inner surface of blood vessels is lined with a thin layer of endothelial cells that, in normal hemostasis, acts to inhibit platelet activation by producing nitric oxide, endothelial-ADPase, and PGI_2. Endothelial-ADPase clears away the platelet activator, ADP.

Endothelial cells produce a protein called von Willebrand factor (vWF), a cell adhesion ligand, which helps endothelial cells adhere to collagen in the basement membrane. Under physiological conditions, collagen is not exposed to the bloodstream. vWF is secreted constitutively into the plasma by the endothelial cells, and is stored in granules within the endothelial cell and in platelets.

When the endothelial layer is injured, collagen, vWF and tissue factor from the subendothelium is exposed to the bloodstream. When the platelets contact collagen or vWF, they are activated (e.g. to clump together). They are also activated by thrombin (formed with the help of tissue factor). They can also be activated by a negatively charged surface, such as glass. Non-physiological flow conditions (especially high values of shear stress) caused by arterial stenosis or artificial devices (Mechanical Heart Valves, blood pumps etc.) can also lead to platelet activation.

Platelet activation further results in the scramblase-mediated transport of negatively charged phospholipids to the platelet surface. These phospholipids provide a catalytic surface (with the charge provided by phosphatidylserine and phosphatidylethanolamine) for the tenase and prothrombinase complexes. Calcium ions are essential for binding of these coagulation factors.

Mechanism of clot

After injury there will be vasoconstriction due to decrease to blood flow by influence on sympathetic stimulation (adrenaline). This secretion will act as stronger vasoconstriction platelets will accumulate in injury site and make temporary plug to prevent more bleeding. Then coagulation occur.

Coagulation (homeostasis):-

Three stages (phases) of coagulation occur:-

1- **Enzymatic phase :** The enzyme play important role after injury thromboplastine release which occur from two sources:-

a- from damage tissue which are more active rapidly after release.(skin factor).

b- from destroyed of platelets itself (inactive enzyme). So there is must factor to inflence on this enzyme to make it active.

*Antihemophilic factor.

* Plasma thromboplastine anticidant +Vit K also help this (above to produce it).

Then thromboplastine activated.

c- Convert of prothrobine to thrombin:-

Prothrombine manfuctured in liver with cooperative Ca++ with thromboplastine activator act to convert prothrombine to thrombin with help from two factors found in plasma.

1-Exciterater globulin.

2-proconvertive.

d-Convert the fibrinogen to fibrin by thrombin.

2-**Clotting phase:**

The fibrin like not have RBC& WBC & platelets adhesion with fibrin fiber so given clot(plug) in site of injury.

3-**rettration Phase:**

ADP play important role which lead retraction of clot & squeeze the serum.

*Antithromboplastine found in blood circulation which prevent for thromboplastine formation or make it inactive.

*Antithrombin: even thromboplastine activator the antithrombin will prevent prothrombin convert to thrombin.

* heparin:-anticoagulant produce in liver & lung but not in basophile.

Heparin anti convert prothrmbine to thrombin.

α_2-Macroglobin simillar to heparin & antithrombin. Its bind with coagulant factors & prevent there protyolytive.

If thrombus occur:-

And will analysis of thrombus in bloodvcirculation by plasmin secretion. Tis plasmin occur from plasminogen which found in plasma which activated by after enzyme called enterokinase. So plasmine will analysis of thrombus. Trypsine will sometime digest the thrombus.

How to prevent coagulation in blood:- (in vitro)

1- Isolate fibrin by remove of blood glass rod the fibrin fiber separated from blood to glass rod and the blood called defibirinated.

Some type used metalic net float in blood & accumulation of fibers on it.

2- Oxalate ⟶ precipitation of Ca++ ⟶ prevent the aggulatation.(oxalated blood).

3- Dicumarine:-

Its important when animal feed on spoiled clover which lead to death due to bleed (no blood aggulation).(so its use in human fraction).

4- Heparin:- may be used commonly in blood transfusion because of its harmless & strong.

5- EDTA:- ethyline ditetraacetic acid.

Disorder in aggulation Process

1- May forma thrombus in blood circulatory.
2- Aggulation process may not happen naturally because avoid of thromboplastin activator which release from platelets. In this stage hemophilia will occur due to an activator of thromboplastine (antihemophilic factor depressed factor VIII).

Hemophilia:-Heredity disease in genes occur in male and female will carrier.

3-Reduce of platelates

Thrombocytopenia purpura:-This occur when blood leave its capillary to be & give blue color.

Cyanosis :- Occur in case of polycythemia.

Blood Volume

7% of total body weight are blood.(70cm^3/kg)

Blood volume = $\frac{\text{plasma volume}}{\text{Hematocrit (PCV)}}$

Two ways to calculate blood volume:-

1-Direct way:-

Depend on animal bleeding until death and calculate blood volume so little blood found in blood vessels so wash it by injection of normal saline and then calculate it with bleeding blood.(some remain in heart).

2-Dilution way(Indirect):-

Depend on

Dilution Volume= $\frac{\text{adding substance(mg)}}{\text{Substance concentration in dilution(mg/cm}^3\text{)}}$

So the blood volume can calculated when know plasma volumeor RBC volume (haematocrit PCV) in adult 3L

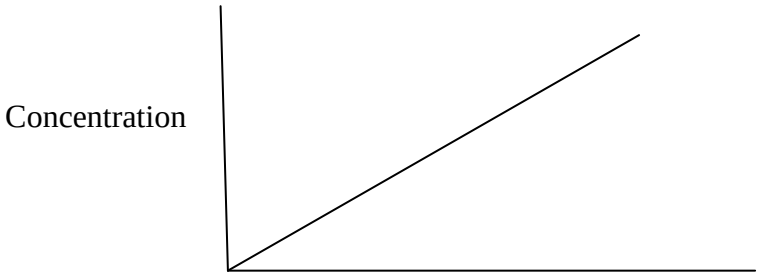
%of plasma then blood volume = $\frac{\text{plasma volume}}{\text{Plasma percentage in blood}} \times 100$

% of blood

Blood volume= $\frac{\text{RBC volume}}{\text{RBC percentage in blood}} \times 100$

Plasma volume calculate by two ways:-

1- Dilution :-by use dye (Evan blue) or endocynin green injection of one theses dye in peripheral blood volume and leave it for 10 min to be mixture with all blood and do standard dilution from the same dye to do the comparism collect blood sample along the exam period to determind the concentration then draw aline.



Disorder in WBC:-