BLOOD PHYSIOLOGY

Objectives after studying this chapter, you should be able to . .
1. Describe the blood components and hemopoiesis.
2. Describe red blood cell, its function, and explain the mechanism for regulation of its production.
3. Expound the plasma proteins and their functions.
4. Understand the composition, types, forms, synthesis, destruction and abnormalities of Hb.
5. Characterize the types of anemia.
6. Explain the types, functions, and the formation of white blood cells.
7. Describe the types of immunity and the development of the immune system.
8. Understand the inflammatory process.
9. Expound blood types, blood incompatibility, and transfusion reaction.
10. Understand platelets and their functions.
11. Describe hemostasis and blood coagulation.
12. Explain fibrinolysis and the anticlotting mechanisms.

Blood is a viscous fluid that is pumped by the heart through a closed system of blood vessels. It is composed of cells (red blood cells, white blood cells, and platelets) which are suspended in a fluid portion, the plasma. Whole blood is 4.5 to 5.5 times as viscous as water. Plasma is 2.0 times as viscous as water

Functions of the blood include:

1. **Transport** of O_2, nutrients and hormones to the tissues and carries CO_2 to the lungs and other products of metabolism to the kidneys to be excreted.

2. It participates in the regulation of body **temperature**, the **pH** and **electrolyte** concentrations of interstitial fluid within the normal ranges through a constant exchange of molecules with the interstitial fluid.

3. Blood also serves essential **body protective functions**, such as combating invading microorganisms, mediating inflammation, initiating immune responses to foreign materials, and maintaining hemostasis.

The normal total circulating blood volume is about 8% of the body weight (5600 ml in a 70 kg man). About 55% of this volume is plasma.

Plasma: Plasma is a part of the extracellular fluid of the body. The normal plasma volume is about 4-5% of the body weight (3500 ml in a 70-kg man). Plasma consists of an aqueous solution of proteins, electrolytes, and small organic molecules.

Plasma Proteins: Its concentration is about 7 gm/dl. The major types of protein and their average normal concentrations present in the plasma are:

- **Albumin,** 4.5 g/dl, 58% of plasma proteins.
- **Globulins** (α_1, α_2, β_1, β_2, and γ), 2.5 g/dl 38% of plasma proteins.
- **Fibrinogen,** 0.3 g/dl, 4% of plasma proteins.

Functions of plasma proteins, in general, include:

1. Plasma proteins, mainly albumin, exert an **osmotic pressure** of about 25 mm Hg across the capillary wall. It is called the "colloid osmotic pressure" or "oncotic pressure". It tends to pull water into the blood.

2. Plasma proteins are responsible for 15% of the **buffering capacity of blood** by helping to keep the blood pH constant.

3. Some of the plasma proteins function in the **transport** of hormones and different substances in blood.

4. Circulating antibodies in the γ globulin fraction of the plasma proteins play a special role in providing the body with **immunity**.

5. Fibrinogen and other plasma proteins are concerned with **blood clotting**.

6. When the tissues become depleted of proteins, the plasma proteins can act as a source for rapid **replacement of the tissue proteins**.

Liver forms all the albumin and fibrinogen of the plasma proteins, as well as, 50-80% of the globulins. The lymphoid tissues form the remainders of the globulins. They are mainly the γ globulins that constitute the antibodies.

Hemopoiesis: Formation of blood cells (hemopoiesis) occurs at different anatomical sites during the course of development from embryonic to adult life.

➔ In the early few weeks of embryonic life, blood cells are produced in the yolk sac.

➔ After the third month of pregnancy, they are formed mainly in the liver and in the lymph nodes and the spleen.

➔ During the latter part of fetal life and after birth, blood cells are produced by the bone marrow of **all bones**.

➔ By the age of 20 and beyond, blood cells formation is restricted normally in the red marrow of flat or membranous bones (such as the vertebrae, sternum, ribs and pelvis) and the proximal ends of humerus and femur. This is because the active red marrow of long bones (except for the upper humerus and femur) has become inactive (yellow and fatty). Inactive yellow marrow produces no more blood cells.

• Even in these bones, the marrow becomes less productive as age increases.

However, maturation, activation, and some proliferation of lymphoid cells occur in secondary lymphoid organs (spleen, thymus, and lymph nodes).

• In certain pathological states, when there is increased demand for blood cell production, red marrow reappears in the shafts of the long bones, replacing the fat.

Diseases in which the bone marrow becomes destroyed, a significant hemopoietic activity occur in the liver, spleen and other sites, when it is referred to as **extramedullary hemopoiesis**.

• The bone marrow is one of the largest organs in the body and it is one of the most active. In the bone marrow, there are **multipotent uncommitted stem cells** (figure 1) from which all the cells in the circulating blood are derived.

The uncommitted stem cells have two properties:

1. Ability of cell division to give rise to new stem cells (self-renewal).

2. Ability to differentiate into **committed myloid stem cells** and **committed lymphoid stem cells.**

The committed myloid stem cells differentiated still further into:

• Committed stem cell that produces erythrocytes, the **colony-forming unit-erythrocyte (CFU-E)**.
• Committed stem cell that produces granulocytes and monocytes, the **colony-forming units-granulocytes, monocytes (CFU-GM)**
• Committed stem cell that produces megakaryocytes, the **colony-forming units-megakaryocytes (CFU-Meg)** (figure 3.1).

Factors regulating hemopoiesis (hemopoietic growth factors): Production of blood cells is regulated by growth factors, which are multiple proteins that control growth, differentiation, and function of cells in one or more of the lines of blood cell production. These factors include:

1. **Erythropoietin**,
2. **Thrombopoietin**
3. **Colony-stimulating factors (CSFs) & stem cell factor (SCF)**
4. **Interleukins** (IL-1, IL-3, and IL-6).

These growth factors affect their target cells through binding to specific receptors. Kidney cells produce erythropoietin, which is a circulating hormone. It is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial cells. Macrophages, activated T-lymphocytes, fibroblasts, and endothelial cells produce the other factors.

Stem Cells and Cancer Therapy: Many cancer therapies affect dividing cells, such as those found in tumors. An undesirable side effect of such therapies, however, can be the destruction of nontumor cells that are dividing, such as the stem cells and their derivatives 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 leukemia and genetic immune deficiency diseases can be treated with a bone marrow or stem cell transplant. To avoid the problems of tissue rejection, families with a history of these disorders can freeze the umbilical cord blood of their newborn children. The cord blood, which contains many stem cells, can be used instead of bone marrow
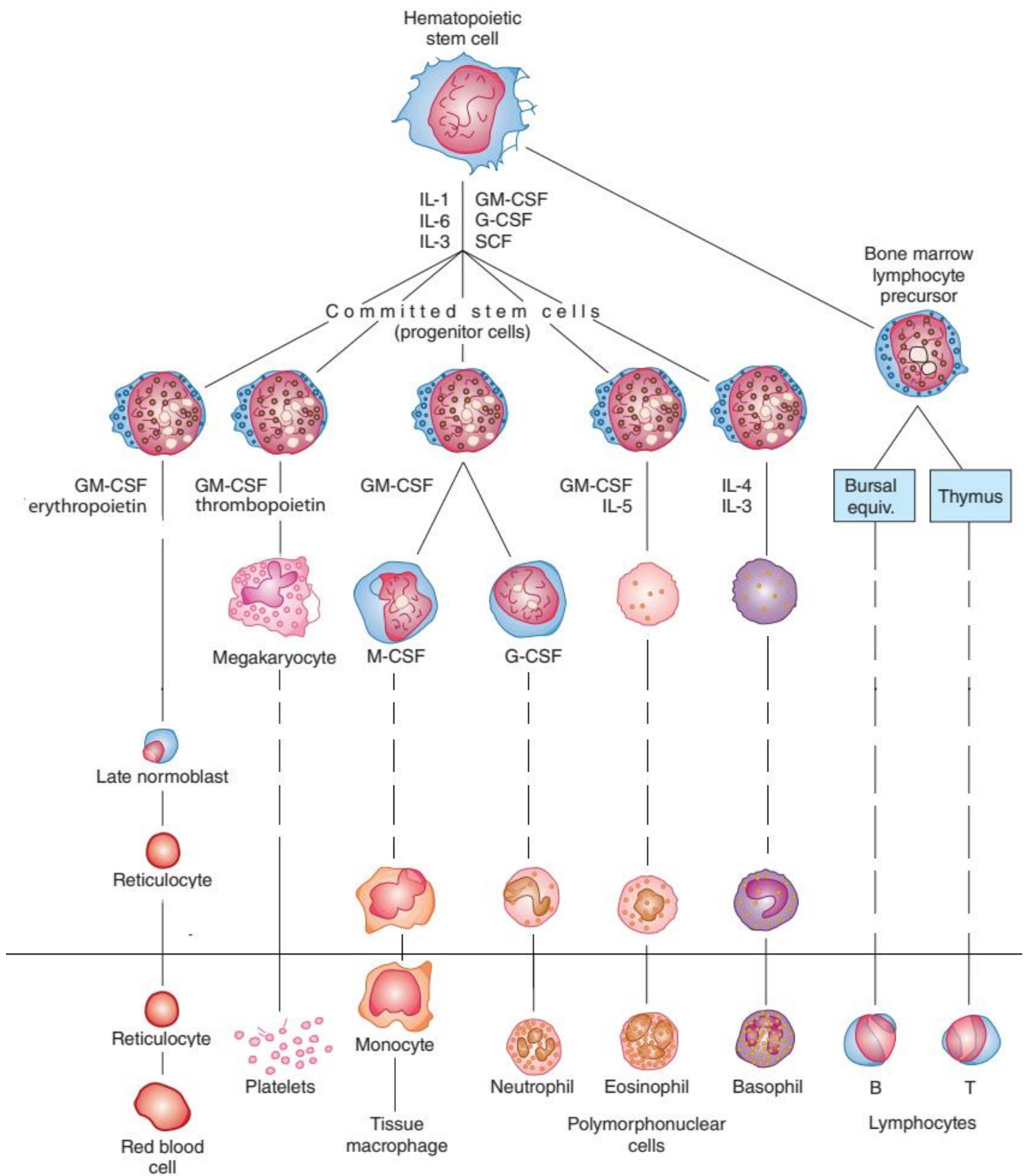
Development of various formed elements of the blond from bone marrow cells. Cells below the horizontal line are found in normal peripheral blood. The principal sites of action of erythropoietin [erythro] and the various colony-stimulating factors [C5F] that stimulate the differentiation of the components are indicated. G, granulocyte; M, macrophage; IL, interleukin; SCF, stem cell factor.

Red blood cells (erythrocytes): The primary functions of red blood cells are to transport oxygen from the lungs to the various tissues of the body, and to transport carbon dioxide from the tissues to the lungs. Red blood cells (RBCs) are non-nucleated, biconcave discs. Because they lack of mitochondria and ribosomes, RBCs are incapable of aerobic respiration. This prevents them from consuming the oxygen they are meant to transport to other tissues. The red cell membrane is flexible and exhibits a remarkable deformability; the RBC being able to change its shape as it passes through narrow capillaries and then return, undistorted, to its original biconcave shape. The biconcave shape of the RBC,

Figure 2: Red blood cell profile.

which provides a high surface to volume ratio, allows for **maximum surface area** (which facilitates gas transport) and **greatest deformability**. Cytoskeletal proteins (spectrin and actin) give membrane durability and flexibility to withstand the stretch and bend as squeezed through small capillaries Circulating erythrocytes live for about 120 days. As an RBC ages and its membrane proteins (especially spectrin) deteriorate, the membrane grows increasingly fragile. Without a nucleus or ribosomes, an RBC cannot synthesize new spectrin. Many RBCs die in the spleen, which has been called the "erythrocyte graveyard." The spleen has channels as narrow as 3 µm that severely test the ability of old, fragile RBCs to squeeze through the organ. Old cells become trapped, broken up, and destroyed. An enlarged and tender spleen may indicate diseases in which RBCs are rapidly breaking down.
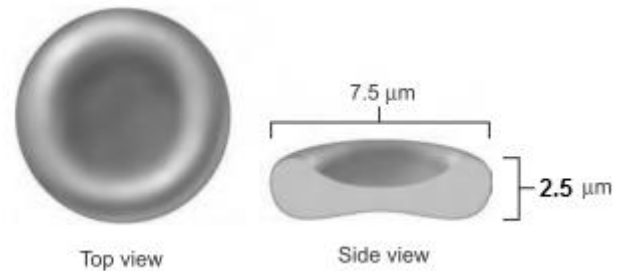
The **average normal RBC count** in adult male is 5,200,000 $\pm$ 300,000 per microliter of blood, and in adult female is 4,700,000 $\pm$ 300,000 per microliter of blood. At birth, the average RBC count is about 5,700,000 per microliter of blood. The number of RBCs varies with age, sex, and altitude. Each RBC has a mean diameter of about 7.5 micrometers and a thickness of 2.5 micrometers at the thickest point and 1 micrometer or less in the center (figure 2). The surface area of the RBC is about 140 square micrometers. The main constituent of the RBC is hemoglobin.

Erythropoiesis: It is the process of erythrocyte formation or production. Erythropoiesis occurs at different anatomical sites during the course of development from embryonic to adult life, and as mentioned above.

Stages of Erythropoiesis (Stages of differentiation of RBCs): Maturation and differentiation of RBC is shown in figure 1. Maturation proceeds with hemoglobin formation in the cytoplasm. After the cytoplasm of late normoblast is filled with hemoglobin and the nucleus is extruded from the cell and the endoplasmic reticulum is reabsorbed, at this stage the cell is called **reticulocyte**. During the reticulocyte stage, the cell passes to the blood and after 1-2 days in blood, it becomes mature erythrocyte. The concentration of reticulocytes among all the red cells of the blood is normally **0.5%-1.5%** in adults. **Reticulocyte count is used as a clinical measurement of erythropoietic activity**.

The basic substances needed for normal RBC and hemoglobin production are amino acids (proteins), iron, vitamin B_{12}, folic acid, and vitamin B_6.

Regulation of erythropoiesis: The main factor stimulating RBC production is **hypoxia** (O_2 deficiency inside the cells). Any condition that causes the quantity of O_2 transported to the tissues (O_2 carrying capacity of the blood) to decrease (decreased tissue oxygenation), increases the rate of RBC production. Examples on factors that decrease tissue oxygenation are:

1. At very high altitudes, O_2 quantity in air is greatly decreased, and insufficient O_2 is transported to the tissues.

2. Diseases of the heart and lungs.
3. Anemia.

On the other hand, when the rate of O_2 transport to the tissues rises above normal, the rate of RBC production is depressed.

Hypoxia increases the rate of RBC production by stimulating the secretion of the important regulating hormone "**erythropoietin**". So hypoxia does not act directly on bone marrow, but it causes marked increase in erythropoietin production and the erythropoietin stimulates RBC production until tissue hypoxia is relieved (figure 3A). In the normal person, about 90% of all erythropoietin is formed in the kidneys, and the remainder is formed in other tissues, mainly the liver. It has a half-life of hours and is broken down in the liver. When both kidneys are removed from a person or when the kidneys are destroyed by renal disease, the person invariably becomes very anemic, because only 10% of the normal erythropoietin formed in other tissues (mainly in the liver) which are insufficient to form RBC needed by the body.

Other factors stimulating erythropoietin production include **androgens, cobalt salts**, **epinephrine** and **norepinephrine**, and several of the **prostaglandins**. **Androgens** (male sex hormones) can also stimulate erythropoietin production, and it is for this reason that RBC count in male is more than in female.
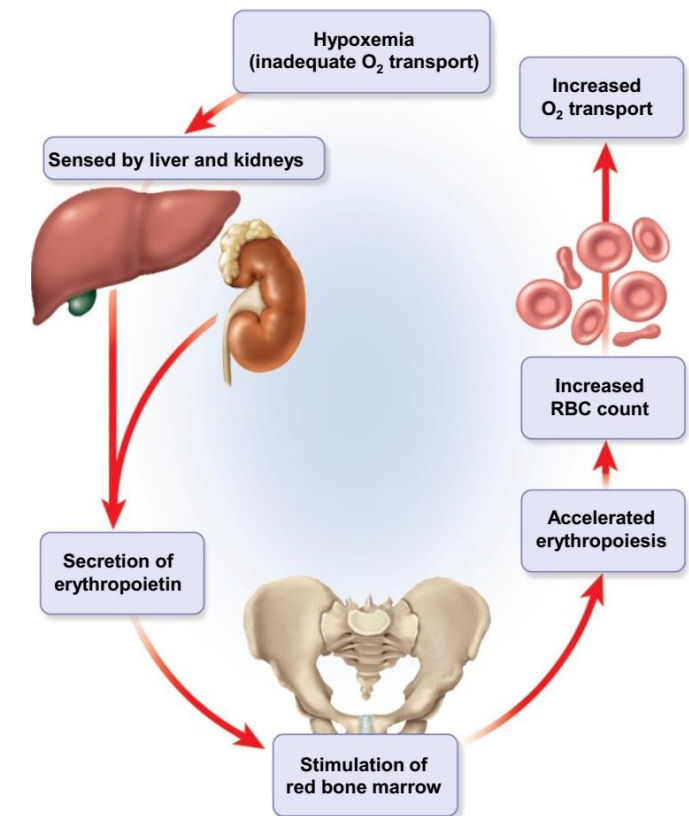

Figure 3A: Erythropoietin and erythropoiesis.

Effect of erythropoietin on erythropoiesis: Erythropoietin is a glycoprotein. It stimulates formation of proerythroblasts from committed stem cells (CFU-E) in bone marrow, and once these proerythroblasts are formed, the erythropoietin causes these cells to pass more rapidly through the different erythroblastic stages than normally, further speeding up the production of new cells. The rapid production of cells continues as long as the person remains in the low oxygen state or until enough red blood cells are produced to carry adequate amount of O_2 to the tissues despite the low oxygen. At this time, the rate of erythropoietin production decreases to a level that will maintain the required number of red cells but not an excess. **IL-1, IL-3, IL-6,** and **GM-CSF** also play part in erythropoiesis by their role in the development of the CFU-E stem cells (as explained earlier).

Human erythropoietin can be produced by recombinant deoxyribonucleic acid (DNA) technology. It is used for management of anemia in cases of chronic renal failure, for treatment of chemotherapy-induced anemia in persons with malignancies, and treatment of anemia in persons with human immune deficiency virus (HIV) infection who are being treated with zidovudine.

Erythrocyte metabolism: The RBC **anaerobic glycolysis** (figure 3B) is importance for the following reasons:
1. Provide energy, in terms of **ATP** molecules, to various biological activities of RBCs through anaerobic Embden-Meyerhof glycolysis pathway.
2. Provide Nicotinamide adenine dinucleotide phosphate (**NADPH**) against oxidative stress. NADPH is

the reduced form of NADP+. The only source of NADPH in RBCs is via the <u>Hexose monophosphate shunt</u>. Erythrocytes require NADPH to maintain normal levels of reduced glutathione (GSH) that is required to counteract against oxidative stress. This is because oxygen is toxic and without reduced glutathione, peroxides spontaneously formed from molecular oxygen would oxidize the lipid components of the red blood cell membranes. Oxidized membranes are significantly less flexible than normal membranes, and result in

Figure 3B: Erythrocyte Metabolic pathways.

damage to the red blood cells when the cells attempt to transit capillaries. In addition, peroxides tend to damage hemoglobin, resulting in precipitation of the protein. Insoluble aggregates of hemoglobin have severely impaired oxygen carrying capacity, and insoluble protein aggregates tend to be inflexible enough to prevent the normal deformations of the red blood cell.

3. Provides nicotinamide adenine dinucleotide (**NAD$^+$**) in red blood cells is required to keep the Hb in ferrous state. Oxygen tends to oxidize the hemoglobin iron from +2 to the more stable +3 oxidation state (resulting in methemoglobin). This is a problem: the +3 state of heme iron binds oxygen very poorly. NAD$^+$ is used to supply reducing equivalents to methemoglobin reductase, the enzyme that returns the hemoglobin to the +2 oxidation state.

4. To produce **2,3 diphosphoglycerate** through **Rapaport-Luebering pathway** for regulation of Hb affinity to O2.

Hemoglobin (Hb): It is the iron-containing oxygen-transport protein with a molecular weight of 64,450. It forms 33% of the RBC cytoplasm. In human beings, it is enclosed in the RBCs. If red blood cells rupture (**hemolysis**), the hemoglobin leaks out into the plasma and become nonfunctional because:

*   The shape of the molecule changes as a result of denaturation.
*   In addition, if it were free in the plasma, some of it leaks through the capillary membrane into the tissue spaces or through the glomerular membrane of the kidney into the glomerular filtrate, each time the blood passes through the capillaries.
*   Furthermore, high concentrations of free Hb in the plasma would increase blood viscosity and osmotic pressure. Therefore, for hemoglobin (Hb) to remain in the blood stream, it must exist in the RBCs.

Functions of Hb:

*   <u>Hb major function is to carry O$_2$</u> from the lungs to the tissues and to transport CO$_2$ from the tissues to the lungs.
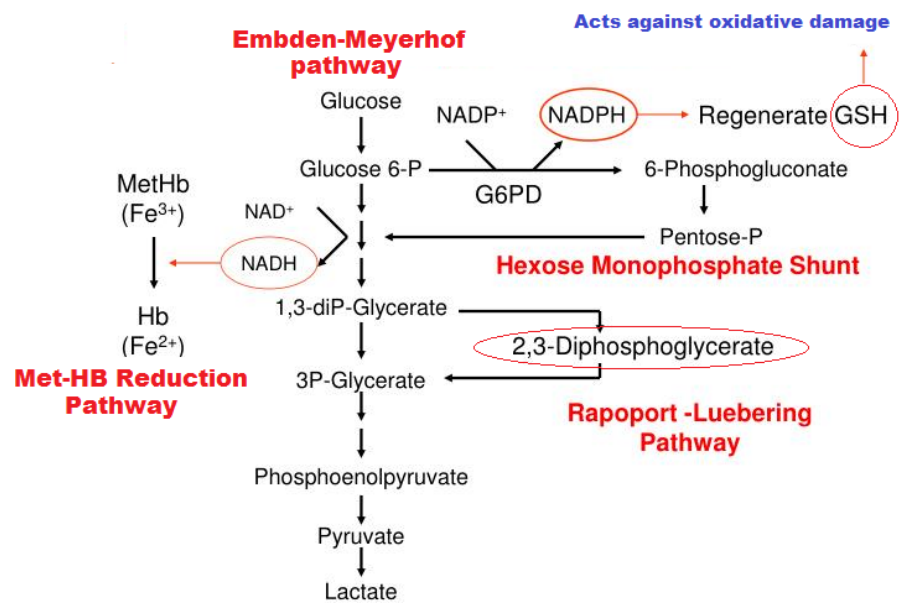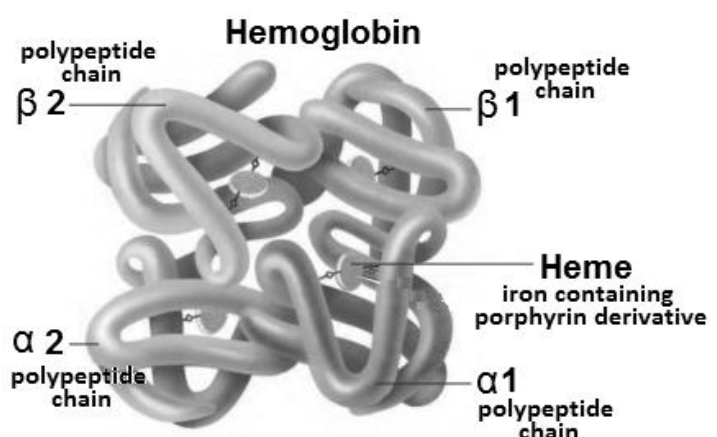

Figure 4: Hb molecule.

- Hb able to <u>bind to gaseous nitric oxide (NO)</u> produced by endothelial cells (causes vascular vasodilatation allowing them to expand which in effects helps the delivery of O2 to the cells).
- In addition, Hb, as a protein, acts as <u>buffer</u> against the intracellular pH changes.

Hb is a globular molecule (figure 4), made up of four subunits; each subunit contains a **heme** molecule and a **polypeptide chain**. Heme is an iron containing porphyrin derivative. The four polypeptides form the **globin** portion of Hb molecule. Therefore, in each Hb molecule there are two pairs of polypeptides; two of the subunits containing one type of polypeptide and two containing another. In each Hb molecule, four atoms of ferrous iron are present and each can bind to a molecule of O_2. Therefore, each Hb molecule can transport four oxygen molecules or eight oxygen atoms. The most important feature of the Hb molecule is its ability to combine loosely and reversibly with oxygen.

Normal Hb types:
Hb A: It is the normal adult Hb. The two types of polypeptide in Hb A are called the α chains, each of which contains 141 amino acids, and the β chains, each of which contains 146 amino acids. Thus Hb A is designated $\alpha_2 \beta_2$. Hb A is the predominant type of Hb in adults (95-97% of total Hb). There are small amounts of hemoglobin A derivatives that represent glycated hemoglobins. One of these, hemoglobin is called <u>A1c (HbA1c)</u>, has a glucose attached to the terminal valine in each β chain and is of special interest because the quantity of it in the blood is increased in poorly controlled diabetes mellitus
Hb A$_2$: In the normal adult about 2.5% of the total Hb is Hb A_2, in which β chains are replaced by delta (δ) chains and is designated $\alpha_2 \delta_2$. Each δ chain also contains 146 amino acids but 10 amino acids differ from those in the β chain.
Hb F (Fetal Hb): It is the main Hb in fetus and newborn. It is $\alpha_2 \gamma_2$. Gamma (γ) chain also has 146 amino acids but 37 amino acids differ from those in β chain. Adult Hb replaces Hb F gradually soon after birth, usually at about 6 months to one year of age the normal adult Hb predominates. In normal adults Hb F may be found in a level of less than 2% of the total Hb. Hb F has greater affinity for O_2 from Hb A. This facilitates movement of O_2 from maternal to fetal circulation. Therefore, the existence of Hb F is useful for the fetus during intrauterine life but becomes harmful to the infant after delivery because the higher affinity of Hb F to O_2, decreases O_2 delivery to the tissues.
Embryonic hemoglobins: Gower I Hb ($\zeta_2 \varepsilon_2$) contains two epsilon (ε_2) chains and two zeta (ζ_2) chains. Gower II Hb ($\alpha_2 \varepsilon_2$) is containing two alpha chains and two epsilon chains. These are found in young embryos and persist until approximately 12 weeks of gestation.

Variant forms of normal Hb: Hb may be found in the blood in different forms;
- **Oxyhemoglobin:** Hb combined with O_2. Each of the four iron atoms in Hb molecule can bind reversibly one O_2 molecule. The iron stays in the ferrous state, so that the reaction is an oxygenation, not an oxidation.
- **Carbaminohemoglobin:** Hb combined with CO_2.
- **Carboxyhemoglobin:** Hb combined with CO. A concentration of about 0.5% carboxyhemoglobin is produced by the normal degradation of Hb. Slightly increased levels can be found in blood of smokers and due to environmental pollution. Hb molecule becomes useless for O_2 transport.
- **Sulfhemoglobin:** Hb containing sulfur, and is incapable of transporting O_2. Certain oxidizing drugs form it.
- **Methemoglobin:** Ferrous iron is converted into ferric iron forming methemoglobin. Methemoglobin is dark-colored, and when it is present in large quantities in the circulation, it causes a dark discoloration of the skin resembling cyanosis. Some oxidation of Hb to methemoglobin occurs normally, but an enzyme system, **NADH-methemoglobin reductase** (see figure 3B), in the RBC

converts methemoglobin back to Hb. The congenital absence of this enzyme system is one cause of **hereditary methemoglobinemia**. Some drugs and oxidizing agents also cause formation of methemoglobin. Methemoglobin is incapable of combining with O_2. The O_2 binding site is occupied by water, in acidic solutions, and by an OH^- group, in alkaline solutions. Methemoglobin is normally found in the blood in a concentration of 1-2%.

- **Myoglobin** (symbol Mb or MB): It is an iron- and oxygen-binding protein found in the muscle tissue of vertebrates in general and in almost all mammals. It is related to hemoglobin, which is the iron- and oxygen-binding protein in blood, specifically in the red blood cells. In humans, myoglobin is only found in the bloodstream after muscle injury. It is an abnormal finding, and can be diagnostically relevant when found in blood. Myoglobin is the primary oxygen-carrying pigment of muscle tissues. High concentrations of myoglobin in muscle cells allow organisms to hold their breath for a longer period of time. Diving mammals such as whales and seals have muscles with particularly high abundance of myoglobin. Myoglobin is found in Type I muscle, Type II, but most texts consider myoglobin not to be found in smooth muscle. While myoglobin can only hold one oxygen, the affinity for that oxygen is very high compared to hemoglobin. Myoglobin is released from damaged muscle tissue (rhabdomyolysis), which has very high concentrations of myoglobin. The released myoglobin is filtered by the kidneys but is toxic to the renal tubular epithelium and so may cause Acute kidney injury. It is not the myoglobin itself that is toxic but the ferrihemate portion that is dissociated from myoglobin in acidic environments (e.g., acidic urine, lysosomes). Myoglobin is a sensitive marker for muscle injury, making it a potential marker for heart attack in patients with chest pain.

- **Neuroglobin:** It is an intracellular hemoprotein expressed in the central and peripheral nervous system, cerebrospinal fluid, retina and endocrine tissues. Neuroglobin is a monomer that reversibly binds oxygen with an affinity higher than that of hemoglobin. It also increases oxygen availability to brain tissue and provides protection under hypoxic or ischemic conditions, potentially limiting brain damage. In vitro studies and cell culture experiments imply that neuroglobin may detoxify reactive oxygen or nitric oxide.

Normal values of Hb concentration:
In adults: Normal range in male is 13.5-17.5 g/dl, and in female 11.5-15.5 g/dl. At birth, the Hb concentration is high (an average of about 21 g/dl).

Hb synthesis: Cell division and protein synthesis require vitamins folic acid (folate) and B_{12}, which are necessary for the synthesis of DNA. Hemoglobin production, in addition, requires iron. Consequently, adequate amounts of folate, vitamin B_{12}, and iron are necessary for normal red blood cell production. It begins in proerythroblasts and continues slightly even into the reticulocyte stage. **Mature RBCs cannot synthesize Hb.**

- Heme synthesis ➜ occurs mainly in the <u>mitochondria</u> from succinyl-CoA and glycine.
- Polypeptides of globin ➜ are produced in <u>ribosomes</u>.

Genetic abnormalities of Hb (hemoglobinopathies): The amino acid sequences in the polypeptide chains of Hb are genetically determined. Because of abnormal gene, abnormal types of polypeptides with different amino acid sequences are formed resulting in abnormal hemoglobins. Abnormalities of the Hb chains can alter the physical characteristics of Hb molecule. One important example is **sickle cell anemia**, in which abnormal Hb (sickle cell Hb or Hb S) is produced. Hb S has normal α chains but in each β chain glutamic acid at the sixth position is replaced by valine (figure 5). HbS does not bind oxygen well, and when deoxygenated becomes insoluble and this cause the RBCs to become rigid, sickle shaped, loses their deformability, Clump together and block small blood vessels and hemolyze easily. Heterozygotes (only one sickle cell allele) are resistant to malaria.

In another type of inherited disorders of Hb, the amino acid sequence is normal but polypeptide chain production is impaired or absent, these are the **thalassaemias**. The α and β thalassaemias are defined by decreased or absent α and β polypeptides, respectively.

Destruction of red blood cells and catabolism of hemoglobin (see figure below): RBCs circulate in the blood for an average of 120 days, after that old RBCs are destroyed by macrophages in the **mononuclear phagocyte system** (formerly called **reticuloendothelial system, RES**), in many parts of the body especially in the liver, spleen, and bone marrow. Inside the mononuclear phagocyte system, the Hb is broken into its constituents (globin and heme). **Globin** is catabolized within mononuclear phagocyte system into its constituent amino acids and enters the circulating amino acid pool. The **heme** is converted into **biliverdin**. The enzyme involved is heme oxygenase, and **CO** is formed in the process. The **iron** from the heme is released into the blood to be carried to the bone marrow for production of new RBCs or to the liver and other tissues for storage. Biliverdin is converted into **bilirubin** which is released into the blood where it combines to albumin and later it is detached from albumin and enters the liver where it is conjugated and becomes water soluble bilirubin and is secreted by the liver into the bile which empties into the intestine. The **normal level of plasma bilirubin** is less than 1 mg/dl.
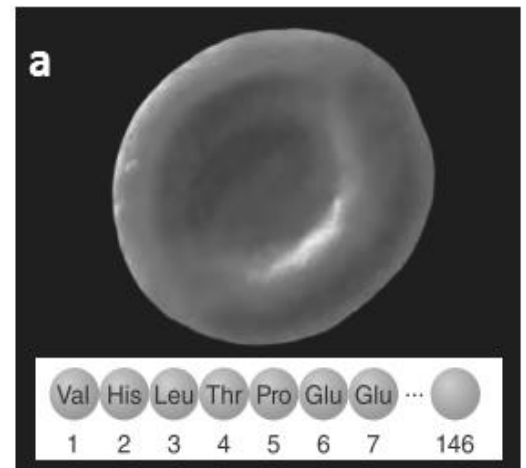
(a) Normal erythrocyte has normal hemoglobin amino acid sequence in the beta chain.
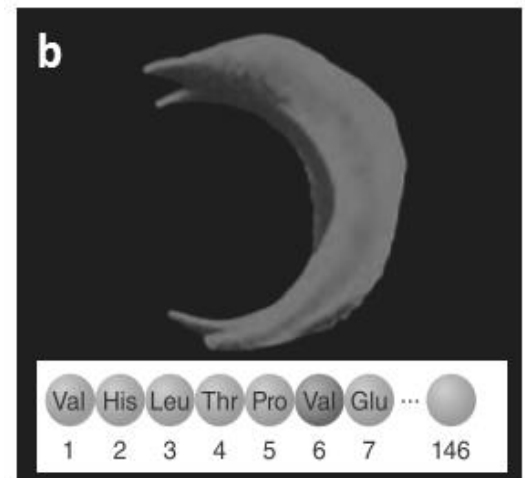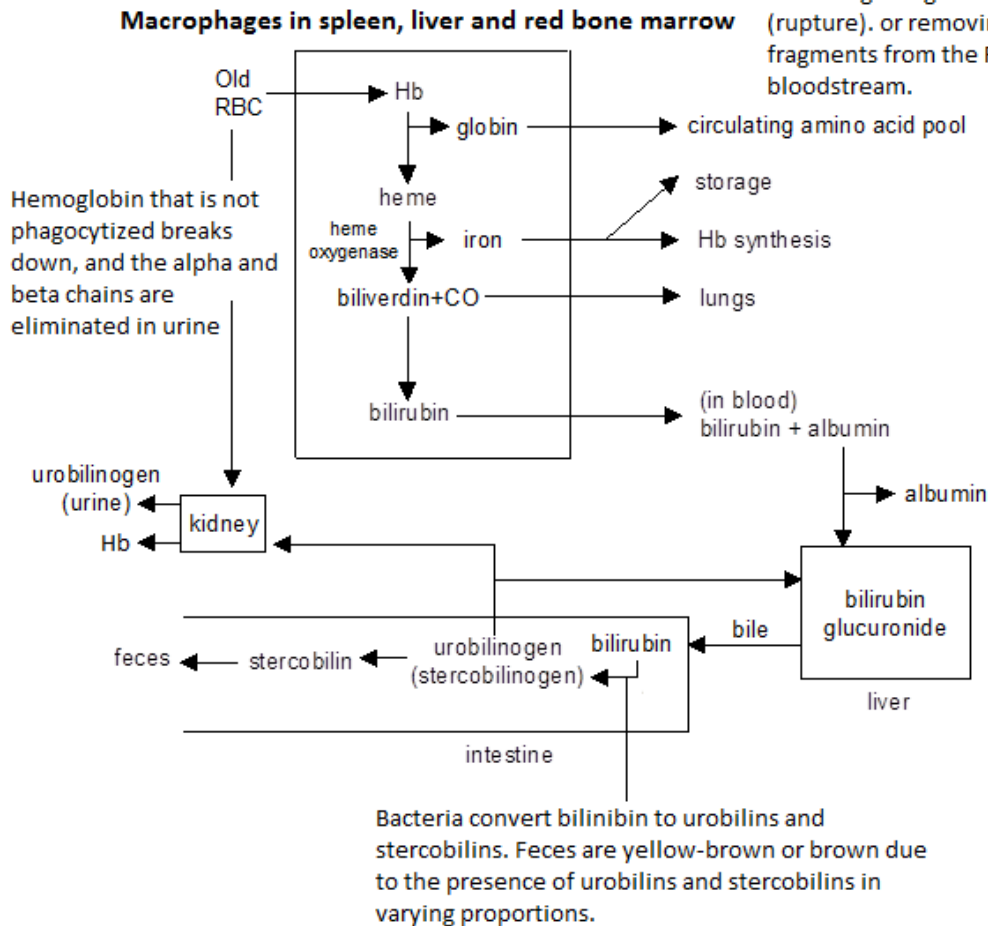
(b) Sickled erythrocyte results from a single amino acid change in the beta chain of hemoglobin.

Figure 5: Morphology of normal and sickled RBC.

Jaundice: It is the yellowish discoloration of skin and mucous membranes resulting from an increased bilirubin concentration in the body fluids. It is detectable when plasma bilirubin level rises above 2 mg/dl. Jaundice can be of different types; **hemolytic jaundice** (because of excessive destruction of RBCs), **hepatic jaundice** (because of damage to the liver), and **obstructive jaundice** (as in cases of obstruction of bile ducts).

Catabolism of hemoglobin

Macrophages monitor the condition of ciculating RBCs. engulfing them before they hemotyze (rupture). or removing Hb molecules. iron, and cell fragments from the RBCs that hemolyze in the bloodstream.

Bacteria convert bilinibin to urobilins and stercobilins. Feces are yellow-brown or brown due to the presence of urobilins and stercobilins in varying proportions.

Anemia: It is defined as a reduction in blood Hb level and/or in RBC count below the normal range for the patient's age and sex.

Classification of anemia: Anemia can be classified, **according to the cause**, into anemia due to:
1. Inadequate production of normal RBCs by the bone marrow.
2. Excessive destruction of RBCs (hemolysis).
3. Blood loss (hemorrhage).

I. Inadequate production of normal RBCs by the bone marrow: Examples:
1. Due to deficiency of essential factors (iron, vitamin B_{12} and folic acid).
2. Aplastic anemia (bone marrow aplasia).

Vitamin B12 and **Folic acid** are required for DNA synthesis, so they are important for maturation of RBCs. If B_{12} or folic acid is deficient, DNA synthesis and nuclear maturation is slowed, whereas cytoplasmic maturation (largely dependent on RNA function) is relatively unimpeded. Consequently, erythropoiesis is delayed with production of the erythroblastic cells in the bone marrow, which grow but

cannot divide rapidly and become larger than normal (called megaloblasts). The production of larger than normal erythrocytes (called **macrocytes**) which are abnormal in shape and break easily leading to decreased number of RBCs in blood and anemia develops which is called **megaloblastic anemia** or maturation failure anemia.

B_{12} deficiency can occur due to lack of B_{12} in diet or more commonly due to lack of a factor (intrinsic factor) which is secreted by gastric mucosa and is bound to B_{12} so that to protect it from digestive enzymes and also assists in absorption of vitamin B_{12} in the ileum. In a condition called "**pernicious anemia**" there is failure of secretion of intrinsic factor by stomach due to atrophy of gastric mucosa, so megaloblastic anemia develops.

In **aplastic anemia**, bone marrow may be destroyed and become unable to produce blood cells, such as following excessive x-ray exposure or the use of certain drugs that cause bone marrow aplasia (lack of functioning bone marrow).

II. Excessive destruction of RBCs (hemolysis): Hemolytic anemia results from abnormalities of red cell membrane or Hb, or other causes. In which there is excessive destruction of RBCs. Examples are hereditary spherocytosis (a common membrane defect, in which the RBCs are small and spherical in shape, and fragile), sickle cell anemia, deficiency of G6PD (misleadingly also called favism), and erythroblastosis fetalis (explained later).

III. Blood loss (hemorrhage):

Acute hemorrhage is loss of large volume of blood over a short period. After rapid hemorrhage, the body replaces plasma within 1-3 days, but this leaves a low concentration of RBCs, which will return to normal within 3-4 weeks if no more hemorrhage occurs.

Chronic hemorrhage is loss of small volume of blood over long period. Therefore, this person needs continuous formation of new RBCs, so he needs more iron than normal, with time if this person does not receive extra iron, store of iron is going to be depleted, and then the person will suffer from iron deficiency anemia.

Anemia can also be classified, **according to the blood indices:** The red blood cells indices are used to define the **size** and **hemoglobin content** of the red blood cells. They consist of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

1. Mean corpuscular volume (MCV): Indicates the average volume of a single RBC in femtoliter (ft).

$$MCV = \frac{PCV\ (in\ \%)}{RBC\ count\ /\ L}$$

Normal range= 80 – 95 ft

2. Mean corpuscular hemoglobin (MCH): Indicates the average weight of Hb in a single RBC in pg.

$$MCH = \frac{Hb\ (g\ /\ L)}{RBC\ count\ /L}$$

Normal range =27 -32 pg

Example: Hb 15.6 g / 100 ml, RBC count =5,340,000 cell /µl
MCH= 156 / [5.34 ×10^{12} cell /L] =29.2 × 10^{-12} gm =29.2 pg.
One pictogram = 10^{-12} gram = 1 micro microgram.

MCH expresses the amount in absolute units, without taking the cell size in account. Therefore, it is of limited use and less useful in classification of anemia than the MCHC.

3. **Mean corpuscular hemoglobin concentration (MCHC):** indicates the average concentration of Hb in the RBC and it is expressed as%.

$$MCHC = \frac{Hb\ (gm/dl)}{PCV\ (\%)} \times 100$$

Normal range 32-36%

Example: Hb 15.6 gm/100 ml, PCV=45%, so MCHC=[15.6/45] ×100=34.7%
If MCHC is normal, the RBC is called **normochromic** as in acute blood loss anemia, while if it is below normal, the RBC is **hypochromic** as in iron deficiency anemia.

Medical application:
Low blood Hb + Low MCV + Low MCH & MCHC➔ Microcytic hypochromic anemia may indicate iron deficiency anemia, thalassemia, and chronic diseases.
Low blood Hb + High MCV + High MCH + Normal MCHC ➔ Macrocytic normochromic anemia or Megaloblastic anemia may indicate vitamin B_{12} or folate deficiency anemia, liver diseases, and posts splenectomy.
Low blood Hb + Normal MCV + Normal MCH & MCHC ➔ Normocytic normochromic anemia may indicate hemolysis or blood loss anemia, marrow aplasia or infiltration or leukemia.

Anemia has potential consequences:
1. The tissues suffer **hypoxia** (oxygen deprivation). Severe anemic hypoxia can cause life-threatening necrosis of brain, heart, and kidney tissues.
2. Blood osmolarity is reduced. More fluid is thus transferred from the blood stream to the intercellular spaces, resulting in **edema**. It is suggested that the low concentration of Hb in patients with anaemia causes a reduced inhibition of basal endothelium-derived relaxing factor (NO) activity and leads to generalised vasodilatation and consequently low blood pressure. The consequent of low blood pressure may be the stimulus for neurohormonal activation and salt and water retention.
3. Blood **viscosity is reduced**.
4. The **heart and lungs also must work harder** to compensate for the blood's low capacity to carry oxygen. Because the heart has to work harder to get blood and oxygen to the tissues, anemia, particularly severe anemia, can result in cardiac failure and arrest.

Polycythemia: It is an increased concentration of erythrocytes in the circulating blood that is above normal for sex and age. Polycythemia could be:
1. **Relative polycythemia** is due to reduction in plasma volume. This may occur because of dehydration that occurs in conditions such as diarrhea.
2. **Absolute or true polycythemia**, which could be;
    a. **Secondary polycythemia** that is related to increased erythropoietin production. It is seen for example in those living at high altitudes.
    b. **Primary polycythemia** (**polycythemia vera**) is caused by a gene aberration that occurs in the cell line that produces the blood cells. The blast cells continue producing red cells even when too many cells are already present. This causes excess production of red cells without erythropoietin stimulus, and usually there is excess production of white blood cells and platelets as well.

The principal dangers of polycythemia are increased blood volume, blood pressure, and viscosity. Blood volume can double in primary polycythemia and cause the circulatory system to become tremendously engorged. Blood viscosity may rise to three times normal. Circulation is poor, the capillaries are clogged with viscous blood, and the heart is dangerously strained.

Factors affecting blood viscosity:

■ The plasma proteins and formed elements (red cells, white cells, and platelets) increase the viscosity of blood. Of these formed elements, red cells have the greatest effect on viscosity. Therefore, viscosity is strongly dependent on Hct, as Hct increases, there is a disproportionate increase in viscosity.

■ Temperature: As temperature decreases, viscosity increases (increases ~ 2% for each °C decrease in temperature)

■ Flow rate of blood: Low flow rates ➔ marked increased in viscosity ➔ Increased cell-to-cell and protein-to-cell adhesive interactions ➔ Erythrocytes adhere to one another (rouleau formation)

■ Vessel diameter: Small vessel diameters (e.g., in arterioles less than 300 microns), there is a paradoxical decrease in blood viscosity (Fahraeus-Lindqvist effect). This occurs because the hemotocrit decreases in small vessels relative to the hemotocrit of large feed arteries.

White blood cells (leukocytes): Acting together, the white blood cells (WBCs) provide the body with powerful defenses against tumors, and viral, fungal, bacterial, and parasitic infections. WBCs are nucleated cells. They are classified by several ways. One type of classification is according to the
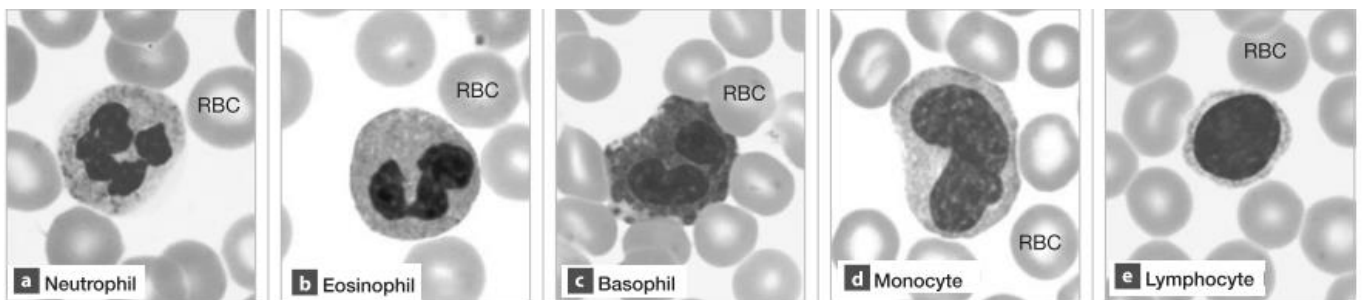

Figure 8: Types of blood leukocytes.

presence or absence of specific-staining granules in the cytoplasm.
Accordingly, WBCs are divided into (figure 8):
1. Granulocytes which are 10-15 micrometers in diameter, are of three types according to the nature of their specific staining granules: **neutrophils** (granules stain with acidic and basic dyes), **eosinophils** (granules stain with acidic dyes), and **basophils** (granules stain with basic dyes). Their nuclei are lobulated. They are also called polymorphonuclear leukocytes.
2. Non granulocytes which are:
- The small and large **lymphocytes** (6-16 micrometers in diameter). The lymphocyte has a large nucleus and scanty cytoplasm. There are three main types: T-lymphocytes, B-lymphocytes, & natural killer (NK) lymphocytes.
- The **monocytes** (15-20 micrometers in diameter).The monocyte is the largest WBC and has abundant cytoplasm and kidney-shaped or round nucleus.

Formation of WBCs (leukopoiesis): After birth, the **granulocytes** and **monocytes** are formed and stored within the marrow until they are needed in the circulatory system. Then when the need arises, various factors cause them to be released. However, **lymphocytes** are formed also in the bone marrow but maturation, activation, and some proliferation of lymphocytes occur in secondary lymphoid organs (spleen, thymus, and lymph nodes). The lymphocytes are mostly stored in the various areas of lymphoid

tissue except for the small number of lymphocytes that are temporarily being transported in the blood. They enter the blood stream for the most part via the lymphatics. The stages of WBC formation shows in figure 1. The production of WBCs is regulated with great precision, and multiple growth factors are involved, as explained earlier.

Normal Values: The normal range of **total WBC count** in adults is 4000-11000 per microliter (mm^3) of blood. The normal percentages of the different types of WBCs are:
- **Neutrophils** ➔ 50-70%,
- **Lymphocytes** ➔ 20-40%,
- **Monocytes** ➔ 2-8%,
- **Eosinophils** ➔ 1-4%, and
- **Basophils** ➔ 0.4%.

There is some diurnal variation in the total WBC count. In the newborn, the total WBC count is high, about 18000 per microliter of blood.

Leukocytosis and leukopenia: When total WBC count is lower than 4000/microliter of blood, the condition is known as **leukopenia** which occurs for example as a result of <u>viral infection</u>, <u>typhoid fever</u>, and in <u>bone marrow aplasia</u> such as exposure to ionizing radiation or certain drugs. When total WBC count is higher than 11000/ microliter of blood, the condition is known as **leukocytosis,** which could be due to <u>pathological causes</u> (for example bacterial infection) or <u>physiological causes</u> (for example in exercise, emotional stress and anxiety, and pregnancy). The cause of physiological leukocytosis is because at rest, when the blood flow is slow through the tissues, large number of WBCs especially neutrophils adhere or stick to the walls of the capillaries (this process is known as **margination**) and are sequestered from the usual circulation. Hard exercise or stimulation of the circulation by norepinephrine, with rapid blood flow through the capillaries can mobilize the leukocytes and their number increase in the blood leading to **physiological leukocytosis.**

Leukemias: The term leukemia, literally "white blood," refers to a group of cancerous conditions involving overproduction of abnormal white blood cells. As a rule, the abnormal white blood cells are members of a single clone (descendants of a single cell) that remain unspecialized and proliferate out of control, impairing normal red bone marrow function. The leukemias are named according to the cell type primarily involved. For example, myeloid leukemia involves myeloblast descendants, whereas lymphocytic leukemia involves the lymphocytes. Leukemia is acute (quickly advancing) if it derives from stem cells, and chronic (slowly advancing) if it involves proliferation of later cell stages.

Immunity: Is the ability to resist damage from foreign substances, such as microorganisms; harmful chemicals; and internal threats, such as cancer cells. Immunity is categorized as **innate immunity** (also called nonspecific immunity) and **adaptive immunity** (also called specific immunity) (table 3.1), although the two systems are fully integrated in the body.

Table 3.1: The differences between innate and adaptive immunity.	
Innate immunity	**Adaptive immunity**
The body recognizes and destroys certain foreign substances, but the response to them is <u>the same</u> each time the body is exposed to them, because specificity and memory of previous encounters are not present.	The body recognizes and destroys foreign substances, but the response to them <u>improves each time</u> the foreign substance is encountered, because the immune system remembers the bacteria from the first exposure..
<u>No Specificity</u> (the ability to recognize a specific substance) and <u>no memory</u> (to "remember" previous exposure to a particular substance)	<u>Have Specificity and memory</u>

Innate immunity (non-specific immunity): The main components of innate immunity are:

(1) mechanical barriers to prevent the entry of microbes into the body or that physically remove them from body surfaces in several ways; such as the intact skin and mucous membranes.

(2) chemical barriers that act directly against microorganisms or that activate other mechanisms, leading to the destruction of the microorganisms. Examples of such chemical mediators are **sebum**, and **mucus**, the **strong acidity of gastric juice** (pH 1–2) & other chemical substances that help to kill many microorganisms before they can invade the cells of the body.

(3) cells involved in target cell destruction and phagocytosis, these processes are associated with the production of chemicals that participate in the response of the immune system. The cells involved are granulocytes mainly the **neutrophils,** and non-granulocytes mainly the **Natural killer (NK) lymphocytes** and **macrophages**.

(4) Fever: Fever, or abnormally high body temperature, is a systemic response to invading microorganisms (infection). Fever is an adaptive response that seems to benefit the body.

- An elevated body temperature (fever) enhances the body's defense mechanisms. This is done by stimulating the motion, activity, multiplication of white blood cells, and increases the production of antibodies.
- At the same time, elevated heat levels may directly kill or inhibit the growth of some bacteria and viruses that can tolerate only a narrow temperature range.
- Fever increases the metabolic rate of tissue cells in general, speeding up repair processes.

   When leukocytes and macrophages are exposed to foreign substances in the body, they release chemicals called pyrogens. These pyrogens act on the body's thermostat—a cluster of neurons in the hypothalamus—raising the body's temperature above normal [37°C].

(5) Leukocytes: The leukocytes use the blood to migrate from bone marrow or other production sites to the tissues where they function.

- **Granulocytes:** Once released from the bone marrow <u>stay in the blood for few hours</u> and another <u>4-5 days in the tissues</u>. However, when there is serious tissue infection, this total life span is often shortened to only few hours, because the granulocytes then proceed rapidly to the infected area, perform their functions and eventually are destroyed.

   Neutrophils: The most important function of neutrophils is phagocytosis; cellular ingestion of the offending agent. The neutrophils are mature cells that can attack and destroy bacteria, viruses and other

injurious agents even in the circulating blood.

Eosinophils migrate into tissues diseased by <u>parasites</u>, and attach themselves by way of special surface molecules to the parasites and release substances that <u>kill many of them</u>. They also migrate toward <u>inflamed allergic tissue</u> and <u>detoxify some of the substances released during the allergic reactions</u>. Circulating eosinophil level is elevated in allergic diseases and parasitic infections.

Basophils contain **heparin** and **histamine** and play a role in allergic reactions.

- **Monocytes** are immature cells while still in the blood, stay in the blood for <u>few days</u>, before passing through the capillary membranes into the tissues. At their new residences, monocytes continue to mature and greatly enlarge, becoming the large tissue phagocytes known as **tissue macrophages** (present in all tissues). The tissue macrophages include Kupffer cells in the liver, pulmonary alveolar macrophages, osteoclasts, microglia in the brain, and tissue macrophages in lymph nodes, spleen, and bone marrow. In the past they have been called **reticuloendothelial system**, but **monocyte-macrophage system** or **mononuclear phagocyte system** seem more appropriate. A macrophage's life span may range from <u>months to years</u> unless it is destroyed sooner while performing its phagocytic activity.

- **Natural killer (NK)** cells, it is often called the non-T, non-B cell and also called "police"cells. They are large lymphocytes with a nonspecific role (about 10%) of the total lymphocytes (figure 9). They attack and lyse host cells (cells of one's own body) that either have turned cancerous or become Infected with viruses, as well as bacteria and cells of transplanted tissues. The continual "patrolling" of the body by NK cells "on the lookout" searching for abnormal cells by NK cells is called immunological surveillance. When an NK cell recognizes an abnormal cell, it secretes proteins called **perforins**, which bind to the enemy cell surface and make holes in its membrane. This has generally been thought to destroy host cells by rupturing the membrane; although there is a newer theory, that perforin induces target cell apoptosis.
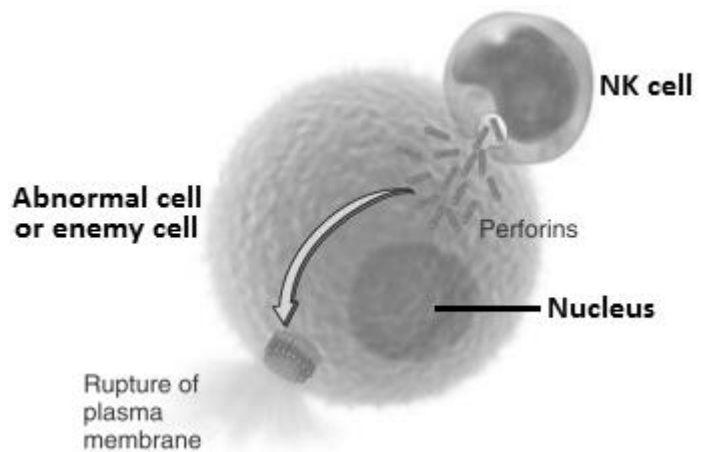
Figure 9: Natural killer (NK) cells

Inflammation: It is a mechanism of innate immunity. Inflammation is part of the body protective response to harmful agent (such as pathogens, damaged cells, or irritants) attempting to remove the injurious stimuli and to initiate the healing process. Inflammation is a localized response to infection.

The classical signs of acute inflammation are **pain**, **heat**, **redness**, **swelling**, and **loss of function**. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Inflammation is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. The process of acute inflammation is initiated by cells already present in all tissues, mainly:

- **Tissue macrophages** (Histiocytes),
- **Mast cells.** A mast cell is a resident granulocyte of several types of tissues (especially concentrated in the skin, bronchioles, and intestinal mucosa.) that contains many granules rich in **histamine** and **heparin**. The mast cell is very similar in both appearance and function to the basophil, a type of white blood cell. However, they are not the same, as they arise from different cell lines.

The sequence of events of acute inflammation: (figure 10):
1. Activation of tissue macrophages and mast cells
2. Release of chemical mediators from tissue macrophages and mast cells

3. The response to the chemical mediators
4. Chemotaxis and phagocytosis

1. Activation of tissue macrophages and mast cells: At the onset of an <u>infection</u>, <u>burn</u>, <u>toxins</u>, or other <u>injuries</u>, **tissue macrophages** and **mast cells** undergo activation and release of chemical mediators (**cytokines**, as well as **other substances,** both together are called the **products of inflammation)** responsible for the inflammatory events and the clinical signs of inflammation. The chemical mediator molecules thus released into the extracellular environment.

2. The response to chemical mediators from tissue macrophages and mast cells: The chemical mediators cytokines and other substances (such as NO, substance P, histamine, serotonin, bradykinin, thromboxane, prostaglandin, leukotriene, platelet-activating factor) act on the bone marrow and on vascular system of the tissues and bone marrow capillaries leading to:

- <u>Greatly increase   production of both granulocytes and monocytes by the bone marrow</u>. This occurs within a few hours after the onset of acute severe inflammation.

- <u>Changes in vascular caliber (vasodilatation) and increased blood flow</u>: This leads to more granulocytes and monocytes to pass from the bone marrow to the blood. The stored cells in the bone marrow mobilize immediately into the circulating blood. Consequently, the number of neutrophils and monocytes in the blood increases. This makes more neutrophils and monocytes available to the inflamed tissue area. Vasodilatation of arterioles around the injured area leads to more blood flows to the injured site. Due to increased blood flow, the injured area becomes red and warm. Redness and heat are the first two signs of inflammation in the injured area.

- <u>Increase the stickiness of endothelium</u>: The chemical mediators alter the inside surface of the capillary endothelium causing them more sticky, consequently, more neutrophils and monocytes going to stick to the capillary walls in the inflamed area, a process called **margination** of neutrophils and monocytes.

- <u>Increased vascular permeability</u>: These chemical mediators cause the endothelial cells of the capillaries and small venules to separate easily, allowing openings large enough for plasma proteins to leave the circulation, as well as neutrophils and monocytes to pass by rapid **diapedesis** into the tissue spaces. Diapedesis is the passage of white blood cells through normal unruptured wall of a blood vessel by changing in shape through gaps between endothelial cells of the vessel walls into the tissue spaces.

The escape of fluid, proteins, and blood cells from the vascular system into the interstitial tissue or body cavities is known as **exudation**. An exudate is an inflammatory extravascular fluid (not necessarily due to infection) that has a high protein concentration, cellular debris, and a specific gravity above 1.020. It is due to significant alteration in the normal permeability of small blood vessels in the area of injury. **Pus**, a purulent exudate, is an inflammatory exudate due to infection rich in leukocytes (mostly neutrophils), the debris of dead cells and, in many cases, microbes.

- <u>Chemotaxis and phagocytosis</u>: The chemical mediators cause **chemotaxis** of neutrophils and monocytes to move toward the injured tissues. Because the number of monocytes in the blood is low, also the storage pool of monocytes in the bone marrow is much less than that of neutrophils, the buildup of macrophages in the inflamed tissue area is much slower than that of neutrophils, requiring several days to become effective. Furthermore, even after invading the inflamed tissue, monocytes are still immature cells requiring 8 hours or more to swell to much larger sizes and develop large quantities of lysosomes, only then acquiring the full capacity for phagocytosis. Thus, within several hours after tissue damage begins, the area becomes well supplied with neutrophils. Because the neutrophils are already mature cells, they are ready to begin immediately their phagocytic actions. However, in some circumstances eosinophils rather than neutrophils predominate in acute inflammation. This tends to occur with parasitic worms, against which neutrophils have little success, or with a response involving the antibody IgE.

Eosinophils release several proteins, which are often effective against parasites. Eosinophils also release several regulatory molecules that increase endothelial permeability. Note that eosinophils are also linked to certain types of allergies.

Killing/digestion of bacteria by macrophages is accomplished by several methods:
1. Reactive oxygen species
2. Nitric oxide production by iNOS (inducible Nitric Oxide Synthase)
3. Other proteins including lysozyme, lactoferrin, defensins
4. Hydrolytic enzymes

When neutrophils and macrophages engulf large numbers of bacteria and necrotic tissue, all neutrophils and many or most of macrophages eventually die. After several days a cavity is formed in the inflamed tissue containing dead neutrophils, dead macrophages, necrotic tissue and tissue fluid. Such a mixture is known as "**pus**". After the infection has been suppressed, dead cells and necrotic tissue in the pus gradually, autolyze over a period of days, and the end products are usually absorbed into the surrounding tissues until most of the evidence of tissue damage is gone.

NOTE: In contrast to exudate, a **transudate** is a fluid with low protein content and a specific gravity of less than 1.012. It is essentially an ultrafiltrate of blood plasma that results from osmotic or hydrostatic imbalance across the vessel wall without an increase in vascular permeability. **Edema** denotes an excess of fluid in the interstitial or serous cavities; it can be either an exudate or a transudate.

Therefore, WBCs and in particular, neutrophils and monocytes have the following general properties:
- Ability of margination
- Ability of diapedesis
- Ability of ameboid motion
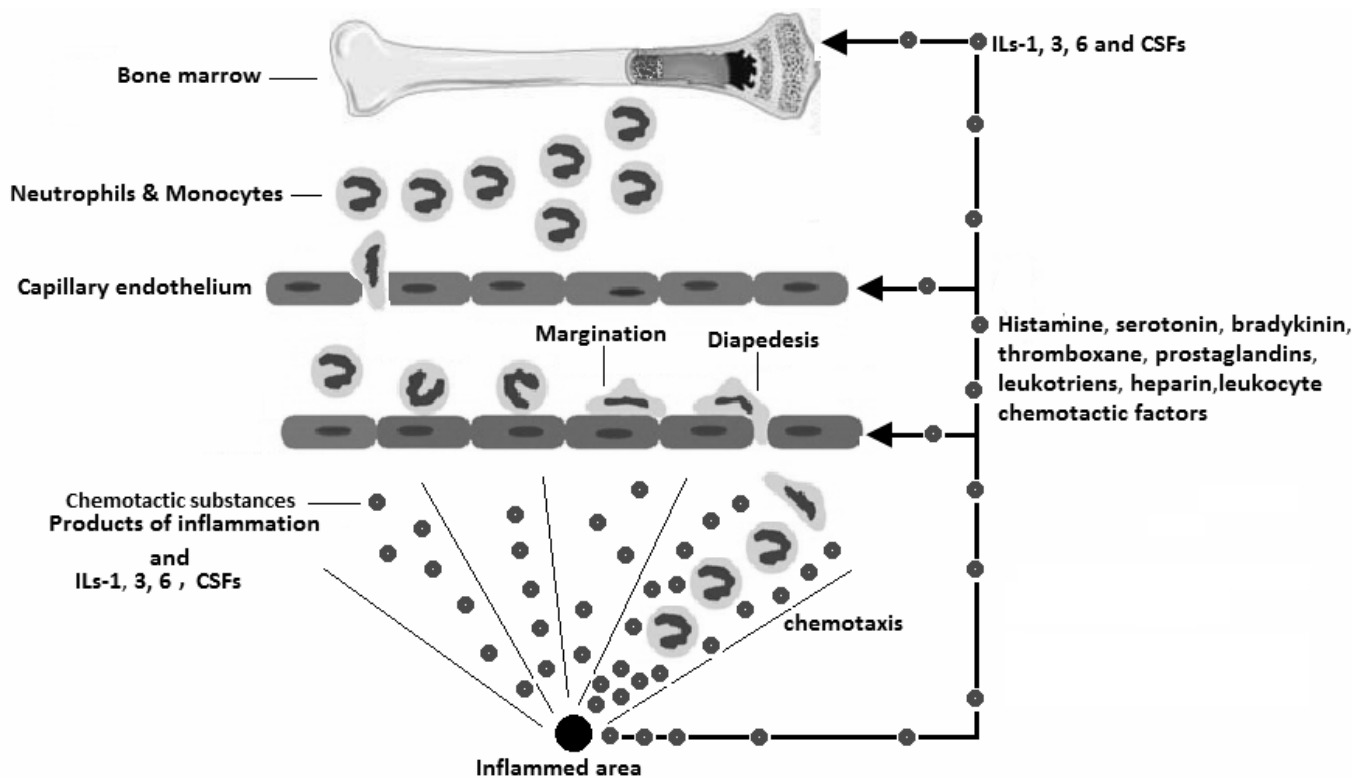- Move according to chemotaxis
- Ability of phagocytosis

Figure 10: macrophage and neutrophil response during inflammation.

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Adaptive immunity (specific immunity): Adaptive immune system is a specific immune system acts against specific organisms or particles, and becomes effective only after prior exposure to the invading agent. **Lymphocytes** are the key constituents of the immune system. In order to activate lymphocytes (1) Lymphocytes must be able to recognize the antigen and, (2) after recognition, the lymphocytes must increase in number to destroy the antigen.

The substance or particle that is capable of stimulating the immune system is called **"antigen" (Ag),** most antigens are conjugated proteins (like lipoproteins, glycoproteins and nucleoprotein). Antigens are of two types:
1. Autoantigens or self-antigens present on the body's own cells such as 'A' antigen and 'B' antigen in RBCs.
2. Foreign antigens or non-self-antigens that enter the body from outside.

The body has two types of adaptive immune defense systems; **humoral** and **cellular**. Both react to antigens.
• **Humoral immunity (B-cell immunity)** is immunity due to circulating antibodies (Abs) which are γ **globulins**. It is a major defense against bacterial infections.
• **Cellular or cell–mediated immunity (T-cell immunity)** is immunity due to formation of large numbers of activated lymphocytes that are specially designed to destroy the foreign agent. It constitutes a major defense against infections due to viruses, fungi and a few bacteria such as the tubercle bacillus. It is responsible for rejection of transplants of foreign tissue, and for delayed allergic reactions. It also helps defend against tumors.

Development of the adaptive Immune System:

➜ During fetal development and after birth, bone marrow lymphocyte precursors (stem cells) that have migrated to the **thymus** differentiate into lymphocytes responsible for cellular immunity (**T-lymphocytes**) (figure 11).

➜ Bone marrow lymphocyte precursors (stem cells) that have migrated to the **fetal liver** (during fetal development) or **bone marrow** itself (after birth) are transformed into lymphocytes responsible for humoral immunity (**B-lymphocytes**).

After residence in the thymus or bone marrow, many of the T- and B-lymphocytes migrate to the lymph nodes and general circulation. Lymphocytes enter the bloodstream for the most part via the lymphatics. At any given time, only about 2% of the body lymphocytes are in the peripheral blood. Most of the rest are in the lymphoid organs of lymph nodes, spleen, bone marrow and GI tract.

Figure 11: Development of the adaptive Immune

From stem cells' differentiation, many million different T- and B-lymphocytes are formed. Each B- or T-lymphocyte has receptors on the surface for binding with a particular antigen. Therefore, each responds to only one specific type of antigen.

**The B cells** differentiate into **plasma cells** and **memory B cells**.

The T cells differentiate into four varieties (figure 12A):

   1. **Helper T cells** (also called **CD4 cells** because of the presence of molecules called CD4 on their surface), which are the most numerous of the T cells, "help" in the functions of the humoral and cellular immune systems. They serve as the major regulator of virtually all immune functions. They do this by releasing cytokines (small proteins that are important in cell signaling). They are released by cells and affect the behavior of other cells that act on other cells of the immune system as well as on bone marrow cells. Among the important cytokines secreted by the helper T cells are the following: **ILs**, **GM-CSF**, and **interferon**. It is the helper T cells that are inactivated or destroyed by the acquired immunodeficiency syndrome (AIDS) virus, with eventual loss of immune function and death from a variety of infections or cancer.

   2. **Suppressor T cells** are also involved in regulation of humoral and cellular immunity. They are capable of suppressing the functions of both humoral and cellular immunity, keeping them from causing excessive immune reactions that might be severely damaging to the body.

   3. **Cytotoxic T cells** (are also called CD8 cells because of the presence of molecules called **CD8** on their surface), also called **killer cells**, are direct attack cells capable of killing microorganisms and destroying transplanted and other foreign cells.

   4. **Memory T cells:** Memory B and memory T cells are cells that have been exposed to an Ag and are readily converted to effector cells by a later encounter with the same Ag. Unlike other lymphocytes, they persist in the body for months or even years.
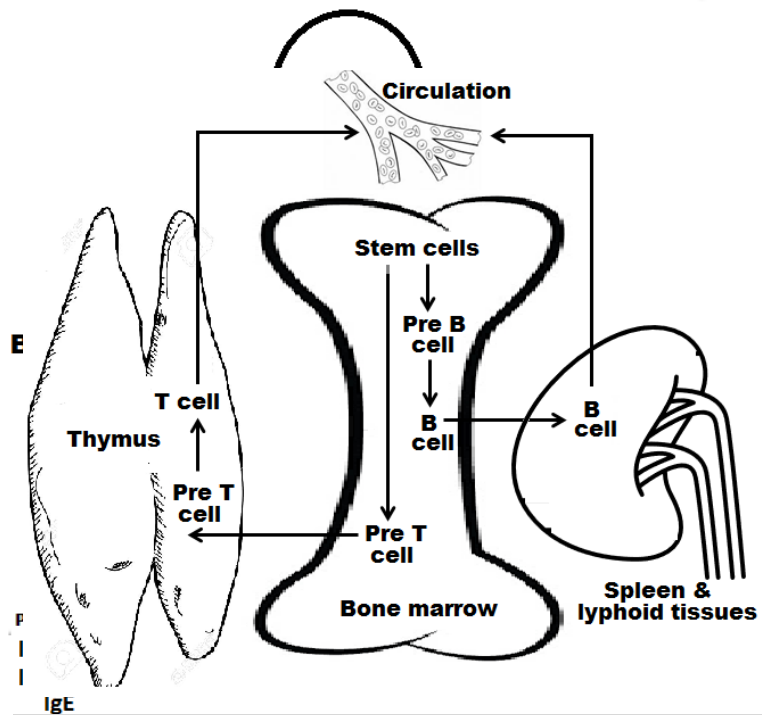
Cellular immunity: Cellular immunity is mediated by **T lymphocytes**. It starts developing when antigenic materials in the invading microbial or non-microbial organisms enter the body and are phagocytized and digested by **Antigen-presenting cells (APC)** (figure 12B) with consequent releasing the organism antigenic materials from invading organisms. Antigen-presenting cells are the special type of cells in the body, which are responsible for the release of antigenic materials from invading organisms and later present these materials to the helper T cells.

Antigen-presenting cells are of three types:
- **Macrophages** (are the major antigen-presenting cells),
- **Dendritic cells** (Ag-trapping cells in the spleen, lymph nodes, and skin), and
- **B lymphocytes** (least efficient antigen presenting cells).

These antigenic peptide products (from phagocytized invading organism) move towards the surface of the antigen-presenting cells and bind with **human leukocyte antigen (HLA)**. HLA is a cell surface protein. Then the antigen-presenting cells present the Ag to the helper T cells. This activates the helper T cells through series of events. Each T cell is designed to be activated only by one type of antigen. It is capable of developing immunity against that antigen only. This property is called the **specificity of T cells.** Helper T cell then proliferates and the proliferated cells enter the circulation for activation of cytotoxic T cells (resulting in development of cellular immunity) and activate the B cells, resulting in the development of humoral immunity. B-lymphocytes can be activated also directly by APC. Then the activated cytotoxic T cells (that are activated by helper T cells) circulate through blood, lymph and lymphatic tissues and destroy the invading organisms that have the Ag, which activated them by attacking them directly, and destroy cells. This killing power of the cytotoxic T cell can extend to involve cancer cells, transplanted cells, or any other cells, which are foreign bodies or even body's own tissues that are affected by the foreign bodies, particularly the viruses.

Suppressor T cells, develop more slowly than cytotoxic T cells help terminate the immune response by suppressing the functions of cytotoxic and helper T cells.

Memory T cells: Some of the T cells activated by an antigen do not enter the circulation but remain in lymphoid tissue. These T cells are called memory T cells. In later periods, the memory cells migrate to various lymphoid tissues throughout the body. When the body is exposed to the same Ag for the second time, the memory cells identify the Ag and immediately activate the other T cells. So, the invading organism is destroyed very quickly and more powerfully.

Humoral immunity: Humoral immunity is mediated by **B-lymphocytes**. The humoral immunity is the major defense mechanism against the bacterial infection. The sequence of activation of B-lymphocytes is similar to what described in the events of activation of Helper T cells, except that it involves B-lymphocytes rather than cytotoxic T-lymphocytes. The proliferated B cells are transformed into two types of cells: **Plasma cells**, and **memory cells**. Both of the recognize the same antigen that activated them. Each plasma & memory cells are designed to be activated only by one type of antigen. It is capable of developing immunity against that antigen only. This property is called the **specificity of plasma cells**, and **memory cells.** In lymphoid tissues, the
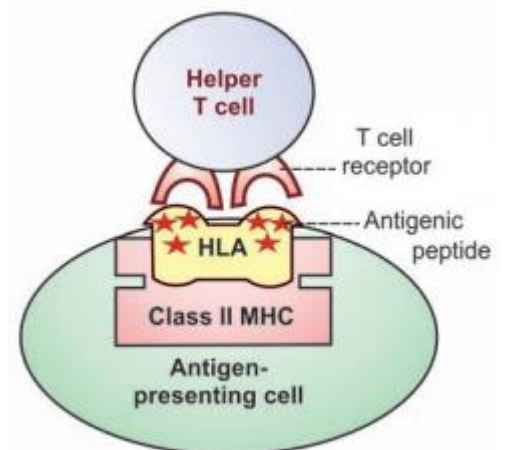

Figure 12B: Antigen presentation. The antigen-presenting cells present their class II MHC molecules together with antigen-bound HLA to the helper T cells.

lymphocytes, which produce a specific antibody, are called **the clone of lymphocytes.** The plasma cells secrete large quantities of Abs, which are specific to the Ag, into the general circulation. The Abs circulates in the globulin fraction of the plasma and are called **immunoglobulins (Igs)**. The memory B cells circulate through the body and populate the lymphoid tissue, but remain immunologically dormant until activated once again by a new quantity of the same Ag. Subsequent exposure to the same Ag will then cause a much more rapid and much more potent Ab response because there are many more memory cells than there were original B-lymphocytes of the same Ag specificity. This phenomenon forms the basic principle of vaccination against the infections.

Five general types or classes of Igs are produced by lymphocyte-plasma cell system: **IgG**, **IgA**, **IgM**, **IgD**, and **IgE**. The basic component of each is a symmetric unit containing four polypeptide chains; two identical long chains (**heavy chains**) and two identical short chains (**light chains**) (figure 3.18). Most Ig molecules are consisted of this basic unit (IgG, IgD, and IgE). However, some Igs are consisted of two or three units (some of the IgA molecules) or of five units (the IgM molecules). Each heavy and light chain has a **variable portion** (in which the amino acid sequence is variable) and the remainder of each chain is the **constant portion** called **Fc region**. The Fc region is the tail region of an antibody that interacts with cell surface receptors and some proteins of the "complement system". This property allows antibodies to activate the immune system. The amino acids present in this region are similar in number and sequence in all the antibodies of each type. The variable region is smaller compared to constant region. Amino acids occupying this region are different in number and sequence in each antibody.

        The variable portion is different for each specific type of Ab. The variable portion attaches specifically to a particular type of Ag. Each basic Ig unit has two Ag binding sites (figure 13).

1. The **IgG** (<u>one basic unit</u>) constitutes about 75% of the Abs of the normal person and is the <u>**only** class of Ab that can pass the human placental barrier</u>. It is the <u>main Ab produced during secondary response</u>, which occurs after a second exposure to the same Ag. It protects against microbial infections by binding, recruiting, activating neutrophils, NK cells, monocytes.

2. The **IgD** (<u>one basic unit</u>)**:** IgD is present mainly on the surface of B cells, acting as a <u>receptor for Ag</u> (for Ag recognition by B cells).

3. The **IgM** (<u>five basic units</u>): A large share of Abs produced <u>during the primary response</u> that occurs on first exposure to the Ag are of this type. The IgM molecule has 10 Ag binding sites. It is potent activator of complement and excellent cytotoxin

4. The **IgA** (<u>two or three basic units</u>) is the major Ab found in secretions of **respiratory**, **gastrointestinal,** and **genitourinary tracts**, where it can bind foreign Ags and impair their entry into the body. It is the second most abundant Ig in the serum. It is synthesized by mucosal epithelial cells and can be found in secretions (**tears**, **saliva**, **colostrum**, **mucous**), It can activates complement by the Alternate Pathway.

5. The **IgE** (<u>one basic unit</u>) are found in trace amounts in plasma. IgE is secreted in increased amounts in patients with <u>allergic conditions</u> and <u>parasitic infections</u>. It is lowest serum Ig concentration and is able to activate and degranulate mast cells

Antibodies protect the body from invading organisms in two ways:

1. by direct actions: Antibodies directly inactivate the invading organism by any one of the following methods:

- **Agglutination:** In this, the foreign bodies like RBCs or bacteria with antigens on their surfaces are held together in a clump by the antibodies.
- **Precipitation:** In this, the soluble antigens like tetanus toxin are converted into insoluble forms and then precipitated.
- **Neutralization:** During this, the antibodies cover the toxic sites of antigenic products.
- **Lysis:** It is done by the most potent antibodies. These antibodies rupture the cell membrane of

the organisms and then destroy them.

2. by activation of complement system: <u>Only IgG and IgM can activate this system</u>. Complement system is a system of plasma enzymes, which are identified by numbers from C1 to C9. Including the three subunits of C1 (C1q C1r C1s), there are 11 enzymes in total. Normally, these enzymes are in inactive form and are activated in two ways:

A. Classical pathway, and

B. Alternate pathway

➔**Classical pathway** In this the C1 binds with the antibodies already attached to its specific antigen, such as a bacterial cell wall and triggers a series of events in which other enzymes are activated in sequence. These enzymes or the byproducts formed during these events produce the following activities:

- **Opsonization:** By which complement proteins, antibodies, and plasma protein called opsonin form a coat around pathogenic organisms so that neutrophil and macrophages identify them easily and phagocytize them.
- **Agglutination:** Clumping of foreign bodies like RBCs or bacteria.
- **Neutralization:** Covering the toxic sites of antigenic products.
- **Lysis:** Destruction of bacteria by rupturing the cell membrane.
- **Chemotaxis:** Attraction of leukocytes to the site of antigen-antibody reaction.
- **Activation of mast cells and basophils**, which liberate <u>histamine</u>: Histamine dilates the blood vessels and increases capillary permeability. So, plasma proteins from blood enter the tissues and inactivate the antigenic products.

➔**Alternate pathway:** Complementary system is also activated by another way, which is called alternate pathway. It is due to a protein in circulation called **factor I**. It binds with polysaccharides present in the cell membrane of the invading organisms. This binding activates C3 and C5, which ultimately attack the antigenic products of invading organism.

Vaccination: It is the process of inducing immunity against specific diseases. It is done by exposing the body to an Ag (the organism or its toxic products which are made harmless and non-pathogenic, but their Ags are still intact) so that humoral or cellular immune response occurs. If subsequent exposure occurs to the same Ag (the live organism or its toxic products), the response will be more powerful and Abs or activated T cells will react with the Ag and protect the body.
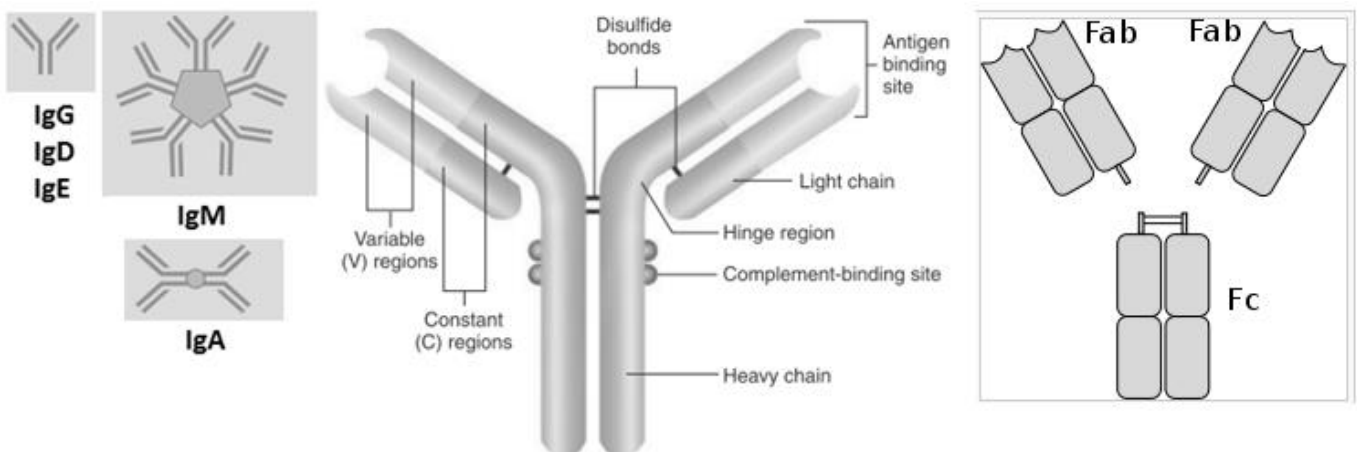

Figure 13: Structure and types of antibodies.

Blood types (blood groups): The membranes of human RBCs contain a variety of Ags (integral membrane glycoproteins or glycolipids), at least 30 commonly occurring and hundreds of other rare Ags. Most of them are weak and therefore, of importance principally for studying the inheritance of genes. Two groups of Ags are the most important and are more likely to cause blood transfusion reactions; the ABO system of Ags and the Rh system of Ags.

The ABO system: According to ABO system of Ags (also called **agglutinogens)**, bloods are grouped into 4 major types: **O**, **A**, **B**, and **AB**, depending on the presence or absence of **Ags A** and **B** on the RBCs. When only type (A) Ag is present, the blood is **type A**, when only type (B) Ag is present, the blood is **type B**, when both (A) and (B) Ags are present, the blood is **type AB**, and when neither (A nor B) Ag is present, the blood group is **type O**.

   A and B Ags are found in many tissues in addition to blood, these include salivary glands, saliva, pancreas, kidney, liver, lungs, testes, semen, and amniotic fluid.

   Antibodies against red cell agglutinogens are called **agglutinins**. When type A agglutinogen is not present in a person's red cells, **anti-A** agglutinins develop in the plasma. In addition, when type B agglutinogen is not present in the red blood cells, **anti-B** agglutinins develop in the plasma. Thus, group A blood contains anti–B Abs, group B blood contains anti-A Abs, group O blood contains both, and group AB blood contains neither (table 3.2). The percentages of blood groups differ from one region of the world to another and among ethnic groups because people tend to marry within their locality and ethnic group and perpetuate statistical variations particular to that group.

Table 3.2: Blood groups with their genotypes and their constituent agglutinogens and agglutinins.			
Genotypes	**Blood types**	**Agglutinogens**	**Agglutinins**
OO	O	—	Anti-A and Anti-B
OA or AA	A	A	Anti-B
OB or BB	B	B	Anti-A
AB	AB	A and B	—

   Immediately after birth, the quantity of agglutinins in the plasma is almost zero. Two to eight months after birth, the baby begins to form agglutinins. A maximum level is reached usually at 8-10 years of age and this gradually declines throughout the remaining years of life. **Small amounts of group A and B Ags enter the body in the food, in bacteria, and in other ways,** and these substances **initiate the development of the anti-A or anti-B agglutinins** in the infant. That is why these agglutinins are produced in individuals who do not have the respective agglutinogens in their red blood cells.The agglutinins are γ **globulins** like other Abs, and are produced by the same cells that produce Abs to any other Ags. **Most of them are IgM and IgG immunoglobulin molecules.**

   When recipient and donor bloods are mismatched, so that anti-A or anti-B agglutinins are mixed with RBCs containing A or B agglutinogens, respectively, transfusion reaction occurs. Agglutinins have two Ag binding sites (IgG) or 10 Ag binding sites (IgM), so a single agglutinin can attach to two or more different red cells at the same time, thereby causing the cells to adhere to each other. This causes the cells to clump together which is the process of **agglutination**. Then these clumps plug small blood vessels throughout the circulatory system. In few hours to few days either physical distortion of the cells or attack by phagocytic WBCs destroys the agglutinated cells, releasing Hb into the plasma, this means that hemolysis of RBCs occur. Sometimes, after the agglutinins attach to the RBCs, immediate hemolysis of RBCs occurs in the circulating blood **(immediate intravascular hemolysis)**. This is far less common than agglutination followed by delayed hemolysis, described above.

Genetic determination of the agglutinogens: A and B Ags are inherited as mendelian **dominants**. Two genes (one on each of two paired chromosomes) determine ABO blood groups. The genes may be any one of three types; A, B, or O, but only one type on each chromosome. Type O gene is either functionless or almost functionless, so that it causes no significant type O agglutinogen on the cells. On the other hand, type A and type B genes do cause strong agglutinogens on the cells (agglutinogens A and B respectively). There are six possible combinations of genes (genotypes), and each person is one of the six genotypes (Table 3.2).

The Rh system: The Rh factor named for the rhesus monkey because it was first studied using the blood of this animal, is a system composed of many Ags. Unlike the ABO Ags, the system has not been detected in tissues other than RBCs. There are 6 common types of Rh Ags, these are **C, D, E, c, d, e**. The most common and the most antigenic is the **D Ag**. Anyone who has agglutinogen D is said to be **Rh positive**, whereas a person who does not have agglutinogen D is said to be **Rh negative**, and forms the anti-D agglutinin when injected with D-positive cells. About 85% of all white people are Rh (+) and 15% are Rh (−). In routine blood typing, the Rh typing serum used is anti-D serum.

Unlike the Abs of the ABO system, which develops spontaneously**, anti-D Abs do not develop without exposure of a D-negative individual to D-positive red cells.** This exposure occurs by:
1. Transfusion of Rh positive blood to Rh negative recipient.
2. Entrance of Rh positive fetal blood into the maternal circulation of an Rh negative mother.

Transfusion of Rh-positive blood to Rh-negative recipient: If Rh positive blood is transfused into Rh-negative person for the first time, the anti-Rh agglutinins will develop slowly and the maximum concentration of agglutinins occurs about 2-4 months later, so there will be no immediate reaction. But in some persons, the immune response occurs to a much greater extent, and anti-Rh Abs develop in sufficient quantities during the next 2-4 weeks and cause agglutination of the transfused Rh positive cells that are still in the blood, these cells are then hemolyzed by phagocytosis. So a delayed transfusion reaction occurs which is usually mild. However, on subsequent transfusion of Rh-positive blood into the same person, who is now sensitized or immunized against the Rh factor, the transfusion reaction is greatly enhanced and can be severe.

Erythroblastosis fetalis: when an Rh negative mother carries an Rh positive fetus (the Rh positive Ag has been inherited from the Rh positive father), and when small amounts of fetal blood enter the maternal circulation at the time of delivery, sensitization of the mother can occur and anti-Rh Abs are formed in the mother after delivery (figure 14A).
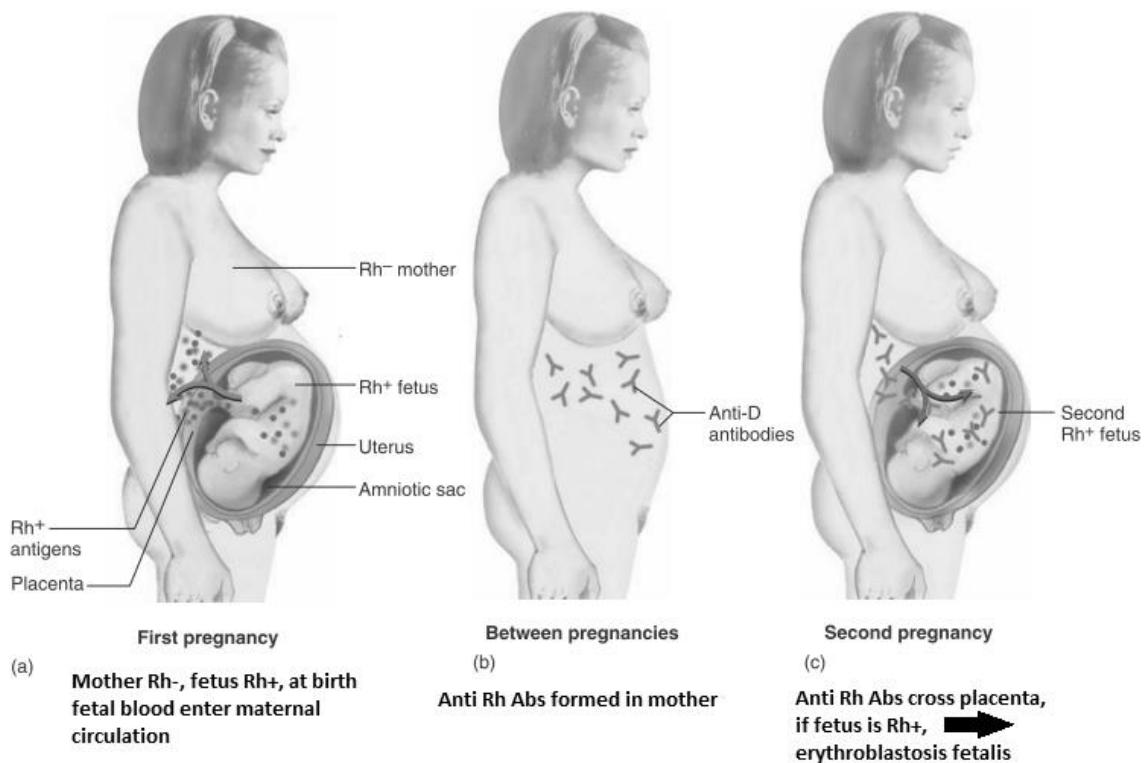

Figure 14A: Erythroblastosis fetalis.

During the next pregnancy, the mother's agglutinins cross the placenta to the fetus. In addition, there are some cases of fetal-maternal hemorrhage during pregnancy in which some fetal blood enter the maternal circulation, and sensitization can occur during pregnancy. In any case, when anti-Rh agglutinins cross the placenta to the fetus and if the fetus is Rh positive, agglutination of fetal RBCs occur and the agglutinated RBCs are then hemolyzed releasing Hb, which will be converted to bilirubin and cause jaundice, and various forms of **hemolytic disease of the newborn (erythroblastosis fetalis)** occur. In severe cases death of the fetus or the newborn may occur. Since sensitization of Rh-negative mother by carrying an Rh-positive fetus generally occurs at birth, the first child is usually normal if the mother has not been previously sensitized by transfusion of Rh-positive blood. However the incidence of erythroblastosis fetalis rises progressively with subsequent pregnancies because the mother who is already sensitized by the first Rh-positive baby will develop Abs rapidly and high concentrations of Abs will be present upon subsequent pregnancies with Rh positive fetuses. It is possible to prevent sensitization from occurring the first time by administering a single dose of **anti-D immunoglobulin** within 72 hours of delivery of the Rh-positive baby. This does not harm the mother, and will destroy the baby's cells that have leaked into the mother's circulation and prevent Ab formation by the mother. Not all hemolytic disease of the newborn is due to Rh incompatibility, however. About 2% of cases result from incompatibility of ABO and other blood types.

Transfusion Reactions: In some conditions a patient may need blood transfusion. Transfusion reactions occur when blood is transfused into a recipient with an incompatible blood type i.e. **the recipient has agglutinins against the donor RBCs**, **so the donor's RBCs are agglutinated**. It is very rare that the donor's agglutinins cause agglutination of the recipient's cells, because **the plasma of the donor**

becomes diluted by all the plasma of the recipient, decreasing the titer of the agglutinins to a level too low to cause agglutination. On the other hand, the infused blood (donor blood) does not dilute the agglutinins in the recipient's plasma to a major extent. Therefore, the recipient's agglutinins can still agglutinate   the donor's cells.

As explained earlier, all transfusion reactions eventually cause either immediate hemolysis or later hemolysis resulting from phagocytosis of agglutinated cells. Because of hemolysis, Hb is released from RBCs into the plasma. The severity of the resulting transfusion reaction may vary from an asymptomatic minor rise in the plasma bilirubin, to severe jaundice and renal tubular damage (caused in some way by the products liberated from hemolyzed cells), to renal failure and death.

Cross-matching test: Before giving a blood transfusion, it is necessary to **determine the blood type** of the recipient and the blood type of the donor blood, so that the bloods can be appropriately matched, then cross-matching test is done. In cross-matching test, the donor's RBCs are mixed with recipient's plasma on a slide and checked for agglutination. If agglutination occurs it means that the donor blood is incompatible with the recipient blood and blood transfusion cannot occur.

Persons with AB group have been called **universal recipients** because they have no circulating agglutinins and can be given blood of any type without developing transfusion reaction due to ABO incompatibility (figure 14B). Persons with O group have been called **universal donors**, because they lack A and B Ags, and type O blood can be given to anyone without producing a transfusion reaction due to ABO incompatibility. However, this does not mean that blood should ever be transfused without being cross-matched, since the possibility of reactions or sensitization due to incompatibilities in systems (subgroups) other than ABO system always exists. You can follow the directions of arrows shown in figure 14B for Blood transfusion policy and blood compatibility, **But Never in Reverse**.
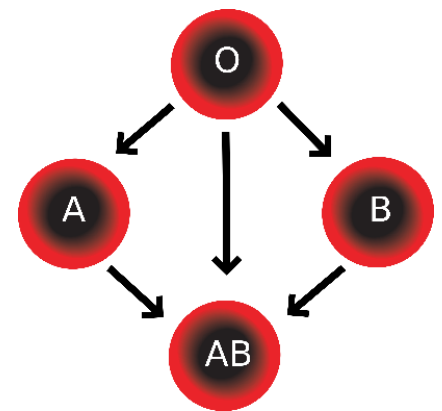

Figure 14B: Blood transfusion policy and blood compatibility.

Platelets (thrombocytes): Platelets are the smallest of the cellular elements of blood. They are non-nucleated, granulated bodies, 2-4 micrometers in diameter. Their normal concentration in blood ranges from 150,000 to 400,000 per microliter. They are formed in the bone marrow from giant cells, the megakaryocytes that fragment into platelets, which are extruded into the circulation (figure 3.1). The platelet production is regulated by multiple factors of cytokines. Their life span is 7-10 days, and then they are eliminated from the circulation mainly by the tissue macrophage system. When a platelet encounters a break in the endothelium, it encounters molecules that trigger its activation. One such molecule is **collagen**, which is characteristically found almost everywhere except inside a blood vessel. In addition, **thromboxane A2**, **ADP**, **serotonin**, and **thrombin, von Willebrand factor** and **fibrinogen** are other factors that trigger the same activation. Activation of platelets is mediated through increase in cytosolic Ca^{2+} concentration. Their cytoplasm contains actin, myosin, glycogen, lysosomes, and two types of granules:

- **Dense granules** contain non-protein substances such as ADP, ATP, and serotonin.
- α-granules contain protein substances such as clotting factors and platelet-derived growth factor which stimulates wound healing. In addition, the platelet membrane contains large amounts of phospholipids that play several activating roles at multiple points in the blood clotting process.

Besides other functions, platelets most important functions are in hemostasis and blood coagulation

- They secrete clotting factor (factor XII), which promote blood clotting.
- They secrete vasoconstrictors, which cause vascular spasms in broken vessels.
- They form temporary platelet plugs to stop bleeding.

Hemostasis: When a small blood vessel is transected or injured, a spontaneous and natural process occurs to arrest bleeding; this process is called "hemostasis". It involves a series of events that leads to clot formation (**thrombosis**) and prevention of further blood loss. These include:

- **Contraction of the injured vessel,**
- **Formation of platelet plug at the site of injury,**
- **Activation of blood coagulation,**
- **Clot retraction.**

As a normal secondary response in hemostasis, activation of the fibrinolytic system, which gradually dissolves away the fibrin clot as tissue repair, is taking place.

1. Contraction of the vessel wall (vasoconstriction): This reduces the flow of blood from the vessel rupture. Most of vasoconstriction results probably from direct effect of injury upon vascular smooth muscle cells. Vasoconstrictor substances (**Endothelin**) released from the endothelial cells, and **neural reflexes** initiated by local pain receptors contribute to this vasoconstriction. The spasm mechanism becomes more and more efficient as the amount of tissue damage increases, and is most effective in the smaller blood vessels. The spasm response is valuable because a strongly constricted artery can significantly reduce blood loss for 20–30 minutes, allowing time for the next two steps, platelet plug formation and blood clotting, to occur.
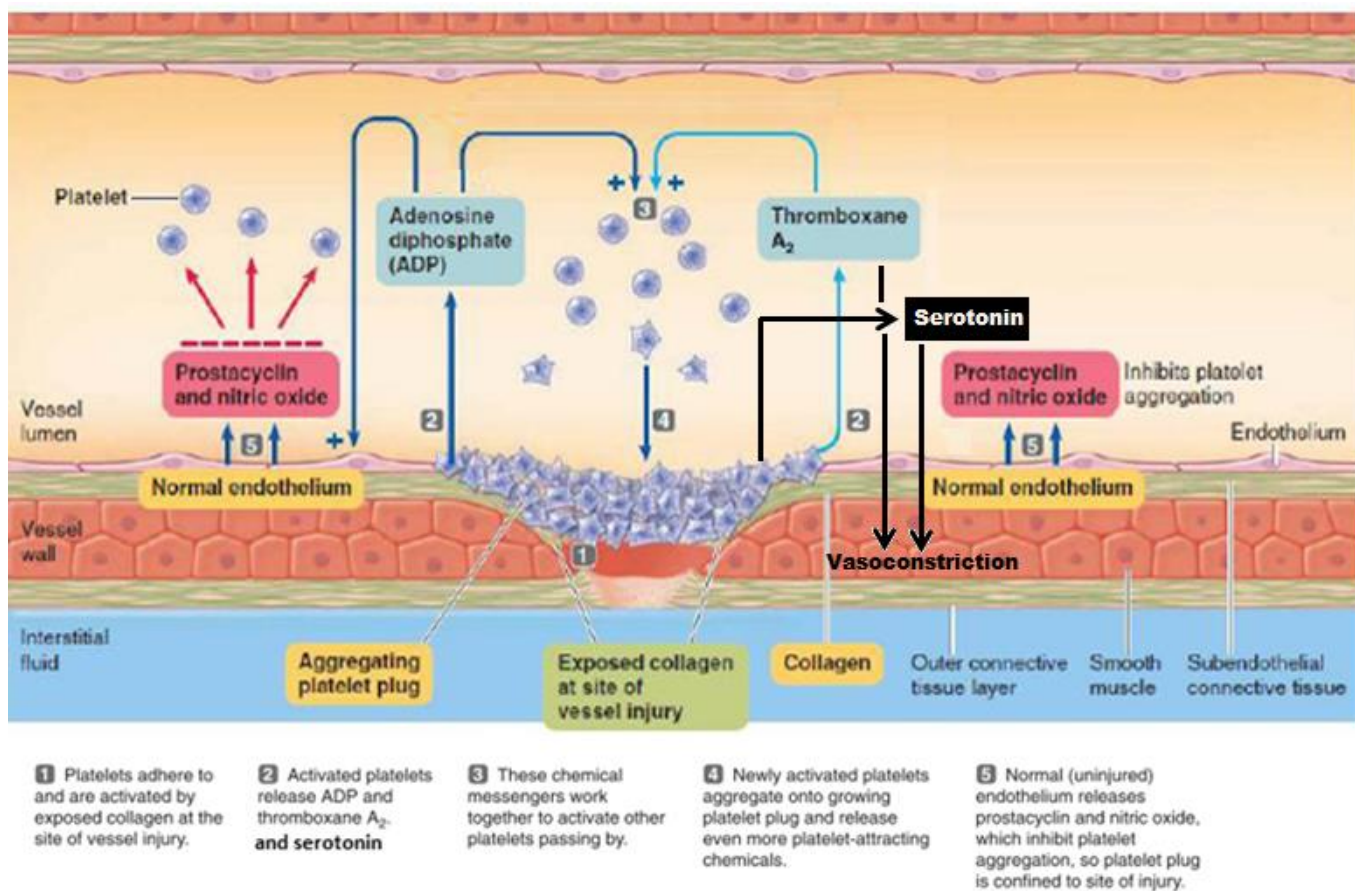

Figure 15: Mechanism of Formation of a platelet plug

2. Formation of a platelet plug: Platelets will not adhere to the endothelium of undamaged blood vessels. This is because intact endothelial cells are normally **very smooth** and release **nitric oxide** and a

prostaglandin called **prostacyclin** (also called **PGI2**). Both chemicals prevent platelet aggregation in undamaged tissue and restrict aggregation to the site of injury. The endothelium produces nitric oxide in response to ADP from activated platelets. This nitric oxide works to keep the vessel open (vasodilation), which decreases the probability of clotting.

When a vessel is broken, however, subendothelial collagen fibers are exposed to the blood. Platelets bind directly to collagen with collagen-specific surface receptors. This adhesion is strengthened further by plasma glycoprotein **von Willebrand factor (vWF) and** forms additional links between the platelets' vWF-specific surface receptors and the collagen fibrils. vWF is released to the plasma from the endothelium and from α-granules of platelets. Upon platelets binding to collagen (directly or indirectly through vWF) , platelets become active. They begin to swell, become sticky (stickiness causes circulating platelets to adhere to the platelets already attached to the collagen), and start to put out long spiny pseudopods that adhere to the vessel and to other platelets; the pseudopods then contract and draw the walls of the vessel together. The mass of platelets thus formed, called a platelet plug, may reduce or stop minor bleeding.

In addition, the activated platelets release **[A] serotonin**, **[B] thromboxane A$_2$**, and **[C] ADP** (figure 15).

- thromboxane A$_2$ and Serotonin ➔ enhance vasoconstriction.
- Thromboxane A$_2$ and ADP ➔ activate other nearby platelets and increase their stickiness and this causes circulating platelets to adhere to the platelets already attached to the collagen, so platelets will aggregate (platelets stick to each other) and form platelet plug at the site of the injury.
- ADP enhances the endothelium to release NO to prevent platelet aggregation in undamaged tissue and restrict platelets aggregation to the site of injury.

3. Blood coagulation: Platelet plug is converted into the definitive clot by formation of fibrin. The clotting mechanism responsible for the formation of fibrin involves a cascade of reactions in which inactive enzymes are activated, and the activated enzymes in turn activate other inactive enzymes. **Ca^{2+}**, **platelet phospholipids**, high molecular weight **kininogen**, and **kallikrein** are chemical substances necessary at different stages of coagulation cascade. Blood coagulation reactions are shown in figure 16, but for the sake of simplification, the sites of actions of Ca^{2+}, platelet phospholipids, kininogen, and kallikrein were not shown. Most of the various clotting factors are designated by Roman numerals (table 3.4).

Number	Name	Origin	Function
Table 3.4: System for naming blood-clotting factors.			
I	Fibrinogen	Liver	Precursor of fibrin
II	Prothrombin	Liver	Precursor of thrombin
III	Tissue thromboplastin	Perivascular tissue	Activates factor VII
V	Proaccelerin	Liver	Activates factor VII; combines with factor X to form prothrombin activator
VII	Proconvertin	Liver	Activates factor X in extrinsic pathway
VIII	Antihemophiliac factor A	Liver	Activates factor X in intrinsic pathway
IX	Antihemophiliac factor B	Liver	Activates factor VIII
X	Thrombokinase, or Stuart prower factor	Liver	Combines with factor V to form prothrombin activator
XI	Antihemophiliac factor C	Liver	Activates factor IX
XII	Hageman factor	Liver, platelets	Activates factor XI and plasmin; converts prekallikrein to kallikrein
XIII	Fibrin-stabilizing factor	Platelets, plasma	Cross-links fibrin filaments to make fibrin polymer and stabilize clot
PF1	Platelet factor 1	Platelet	Platelets Same role as factor V; also accelerates platelet activation
PF2	Platelet factor 2	Platelet	Accelerates thrombin formation
PF3	Platelet factor 3	Platelet	Platelets Aids in activation of factor VIII and prothrombin activator
PF4	Platelet factor 4	Platelet	Binds heparin during clotting to inhibit its anticoagulant effect
Vitamin k dependent clotting factors are: factors II (prothrombin), VII, IX and X, & proteins C, S and Z			

Coagulation mechanisms (figure 16): Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium lining the vessel. Exposure of blood to the space under the endothelium initiates three processes:

- Changes in platelets due to exposed subendothelial collagen and then degranulation and releasing clotting factor and provide membrane phospholipids, as described before.
- Contact of the blood with subendothelial negative changed collagen initiates intrinsic mechanism of coagulation,
- The entrance of **tissue factor** (**TF, tissue thromboplastin, factor III)** from the damaged cells to the blood, initiates extrinsic mechanism of coagulation.

1. Extrinsic mechanism results from the entry into the blood of **tissue factor** from the damaged cells, they are not found normally in the circulation (they are extrinsic to the circulation). Factor III combines with factor VII to form a complex, which then activates factor X. In addition, it catalyzes the activation of factor IX, which can then help activate even more factor X by way of the intrinsic pathway.

2. Intrinsic mechanism begins when blood encounters **negatively charged surfaces** such as **collagen** underlying the endothelium in the blood vessels or in vitro when blood encounters **glass or**

Figure 16: Extrinsic and intrinsic coagulation cascade reactions.

activated platelets. The contact activation pathway (intrinsic mechanism) begins by interaction of collagen fibers, high-molecular-weight kininogen (HMWK), prekallikrein, and factor XII (Hageman factor) which ultimately leads to activation of factor XII. Activated factor XII (XIIa) then activates factor XI. Activated factor XI (XIa) activates factor IX. Activated factor IX (IXa) forms a complex with active factor VIII. The complex of IXa and activated factor VIII (VIIIa) activate factor X.
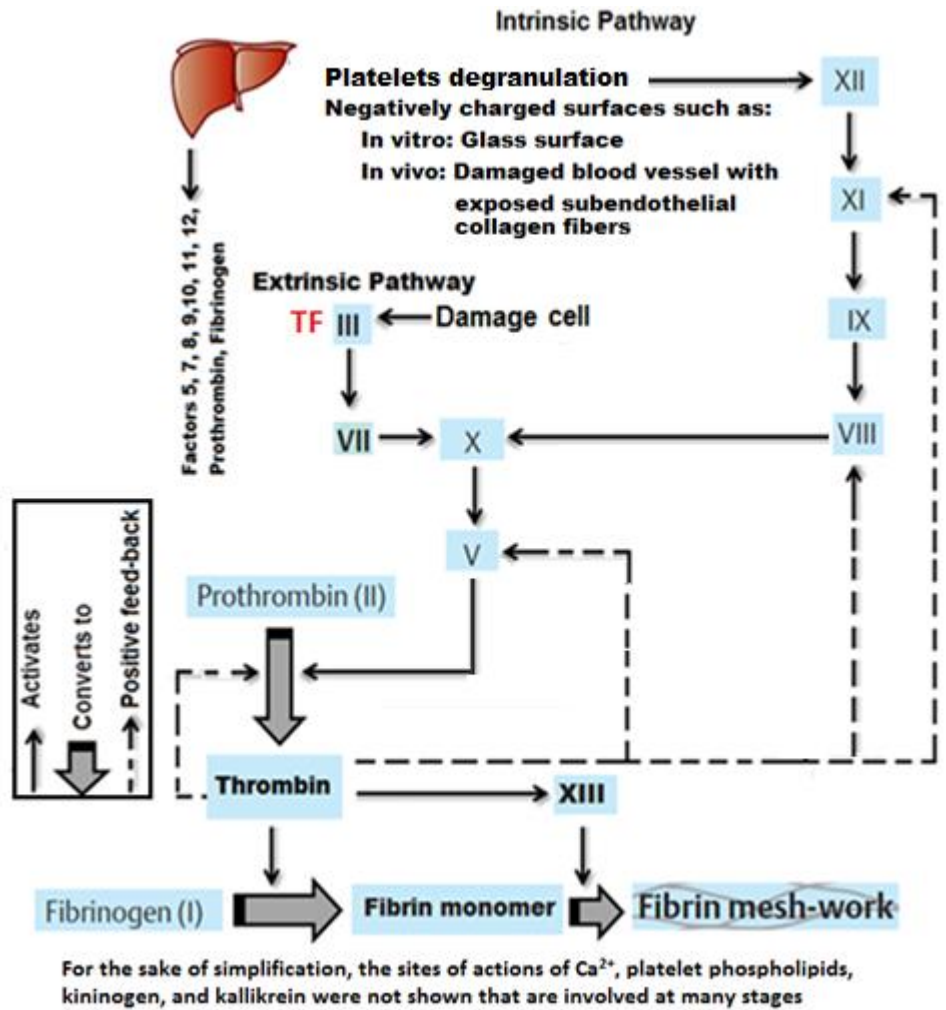
After activation of factor X either by extrinsic or intrinsic mechanisms, both pathways proceed by a common pathway: Activated factor X (Xa) catalyzes the conversion of **prothrombin** to **thrombin**. Thrombin catalyzes the conversion of the soluble plasma protein **fibrinogen** to insoluble **fibrin**. The fibrin is initially a loose mesh of interlacing strands; it is converted by the formation of covalent cross-linkages to a dense, tight aggregate. This reaction is catalyzed by active factor XIII (the fibrin-stabilizing

factor). <u>Factor XIII is present normally in plasma in small amounts but it is also released from the platelets entrapped in the clot and is activated by thrombin</u>.

Ca^{2+}, platelet phospholipids, high molecular weight kininogen, and kallikrein are necessary at intrinsic, extrinsic, and common pathways of coagulation cascade.

The blood clot is composed of fibrin network entrapping RBCs, WBCs, platelets, and plasma. Fibrin fibers adhere to damaged surface of blood vessels therefore blood clots become adherent to any vascular opening and thereby prevent blood loss.

The intrinsic and extrinsic pathways usually work together and are interconnected in many ways, but there are significant differences between them.

The intrinsic pathway	The extrinsic pathway
Called intrinsic because the factor needed for triggering clotting (factor XII) is present within (intrinsic to) the blood.	Called extrinsic because the factor needed for triggering clotting is not present within (extrinsic to) the blood (tissue thromboplastin or factor III).
Triggered by negatively charged surfaces such as activated platelets, subendothelial collagen, or glass (this is why this pathway can initiate clotting in a test tube.)	Triggered by exposing blood to a factor found in tissues underneath the damaged endothelium. This factor is called tissue factor (TF) or factor III.
Slower because it has many intermediate steps	Faster because it bypasses several steps of the intrinsic pathway. In severe tissue trauma, it can form a clot in 15 seconds

IMPORTANT NOTES TO REMEMBER
- Both intrinsic pathway and extrinsic pathway occur within ruptured blood vessel. Under physiological conditions, however, the two pathways are not parallel but <u>are actually activated sequentially</u>, with thrombin serving as the link between them. There are also several points at which the two pathways interact.
- Coagulation usually <u>starts with the extrinsic pathway</u>. <u>As soon as you make a little Xa, the extrinsic pathway is turned off. The small amount of thrombin that has been generated during the action of the extrinsic system goes up and turns on the intrinsic pathway, which finishes out the job of making the rest of the fibrin.</u>

Anticoagulant drugs like **warfarin** (coumarin derivatives)**,** which is given orally inhibits the action of vitamin K, so the plasma levels of **prothrombin (factor II)**, and **factors VII**, **IX**, and **X** begin to fall due to decreased liver formation of these factors, because vitamin k is required for their synthesis.

4.   Clot retraction: Within few minutes after a clot is formed, it begins to contract and usually expresses most of the fluid from the clot within 20-60 minutes. The fluid expressed is called **serum**. Serum differs from plasma in that it cannot clot because all its fibrinogen and most other clotting factors have been removed. **Platelets play essential role in clot retraction through the following mechanisms:**
A. They attached to the fibrin fibers in such a way that they actually bond different fibers together.
B. Platelets entrapped in the clot continue to release procoagulant substances (substances promoting coagulation), one of which is **factor XIII (fibrin stabilizing factor)** which cause more and more cross-linking bonds between the adjacent fibrin fibers.
C. Platelets themselves contribute directly to clot contraction by activating platelet contractile proteins (thrombosthenin, actin and myosin molecules) which cause strong contraction of platelet

spicules attached to the fibrin. This also helps compress the fibrin meshwork into a smaller mass. The contraction is activated or accelerated by thrombin and by calcium ions released from the calcium stores in the mitochondria, endoplasmic reticulum and Golgi apparatus of the platelets.

As the clot retracts, the edges of the broken blood vessel are pulled together. When the number of platelets in the circulating blood is low, there will be failure of clot retraction.

Platelets and **endothelial cells** secrete a mitotic stimulant named **platelet-derived growth factor (PDGF)**. PDGF stimulates fibroblasts and smooth muscle cells to multiply and repair the damaged blood vessel. Fibroblasts also invade the clot and produce fibrous connective tissue, which helps to strengthen and seal the vessel while the repairs take place.

Other actions of thrombin: In addition to those mentioned above thrombin also has a direct proteolytic positive feedback effect on prothrombin tending to convert this into still more thrombin. Thrombin also has positive feedback effect on activation of factors IX, X, XI, and XII (intrinsic pathway), and causes aggregation of platelets.

Fibrinolysis: Fibrinolysis is a process that occurs inside the body to break down thrombus (blood clots). The aims of fibrinolysis are:
  - Removing the clot that is formed by the coagulation process after the tissue repair is completed.
  - Fibrinolysis prevents blood clots from growing and extend beyond the site of ruptured vessels and becoming problematic

This is achieved by a process called fibrinolysis, the dissolution of a clot, through a small cascade of reactions. The main components of the fibrinolytic system are **plasminogen** and **plasmin**. Plasmin is produced in an inactive form, plasminogen, in the liver. Although plasminogen cannot cleave fibrin, it still has an affinity for it, and is incorporated into the clot when it is formed. Plasminogen activated by two ways (figure 17): Fibrinolysis starts at the same time of the formation of blood clot.

Figure 17: The fibrinolytic system (tPA = Tissue plasminogen activator).

A. Intrinsic activation (derived from plasma or blood cells); is achieved through plasma **kallikrein** which circulates in an inactive form (**prekallikrein**). On contact with activated factor XII, prekallikrein is converted to kallikrein. Kallikrein stimulates fibrinolysis by acting on plasminogen and converts it to plasmin. This means that once coagulation starts through intrinsic pathway, through activated factor XII, fibrinolysis starts simultaneously.

B. Extrinsic activation; includes **tissue plasminogen activator (t-PA,** synthesized mainly by endothelial cells) and **urokinase** (synthesized mainly by kidney cells). Normal plasma has a very low concentration of these activators, most of which bind with **plasminogen activator inhibitors**. This means that the endothelial cells that secrete tissue plasminogen activator, they secrete at the same time plasminogen activator inhibitors. This is to keep the fibrinolytic process in check and not to operate beyond the necessary limits, which otherwise the bleeding will resume once the clot is formed by dissoluting it.
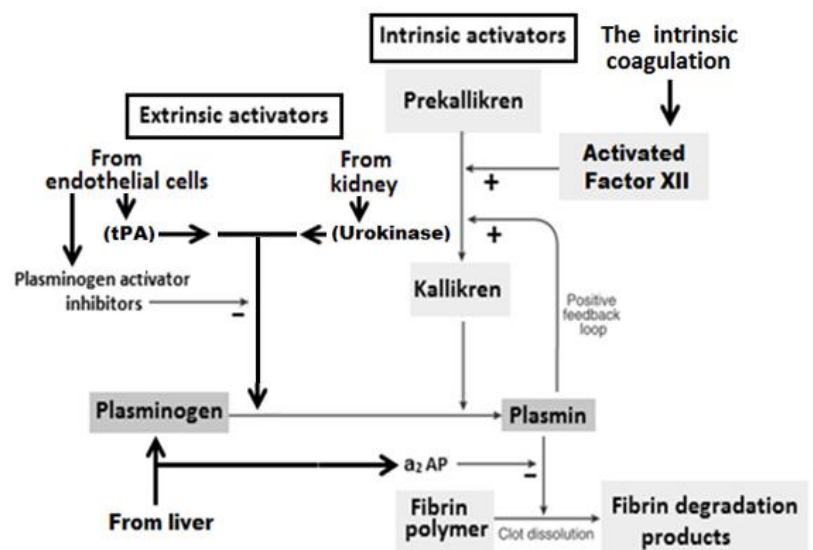
- When a clot is formed, a large amount of plasminogen is bound to fibrin,
- t-PA is released slowly from endothelial cells and is adsorbed on fibrin surface and activates the bind plasminogen
- Plasmin is generated at the clot surface, and begins to dissolve the fibrin clot with the production of **fibrin degradation products**.
- The fibrinolytic system removes clots from intravascular and extravascular sites.

An important function of the fibrinolytic system is to remove minute clots that form in tiny peripheral vessels which otherwise would become occluded.

The fibrinolysis is held in check by fibrinolysis inhibitors which otherwise the bleeding will resume once the clot is formed by dissoluting it. Inhibition of fibrinolysis is achieved by:

- A free plasmin may escape to the circulation, is inactivated by α_2AP. The serum **α_2-antiplasmin (α_2 AP)** which is the principal and most important physiologic inhibitor of plasmin (synthesized in the liver). This means that the liver produces plasminogen, and at the same time produces α_2AP to inactivate plasmin once its release becomes higher than that required for fibrinolysis.
- **Plasminogen activator inhibitors**, which inhibit t-PA and urokinase. They are synthesized by endothelial cells (and by placenta). They are present in the plasma in low concentrations.

Why clot does not formed in the normal vascular system? The anticlotting mechanisms:

The in vivo action of the clotting mechanism is balanced by limiting reactions that tend to prevent clotting in uninjured blood vessels and to break down any clots that do form, and prevent or limit excessive growth and extension of the clot in the injured vessel. These include:

1. Smooth and **prostacyclin-coated endothelium** prevents contact activation of the platelets

2. The thin layer of **glycocalyx**, on the surface of the endothelium that repels the clotting factors and platelets, thereby prevent activation of clotting.

3. All endothelial cells, except those in the cerebral microcirculation, produce **thrombomodulin** (a thrombin-binding protein) and express it on their surface. The functions of throbomodulin are:

- Binding to thrombin and consequently slows the clotting process by removing thrombin.
- The **thrombomodulin-thrombin complex**:
  - Inactivates factors **Va** (in the extrinsic pathway) and **VIIIa** (in the intrinsic pathway).
  - Inactivates plasminogen activator inhibitor (an inhibitor to t-PA) thereby, increasing the formation of plasmin.

So binding of thrombin with thrombomodulin prevents the extension of clots into blood vessels.

4. The balanced interaction between the platelet-aggregating effect of thromboxane A2 and platelet-antiaggregating effect of prostacyclin causes clots to form at the site when blood vessel is injured, but keeps the vessel lumen free of clot. Prostacyclin is produced by endothelial cells, and thromboxane A_2 by platelets from their common precursor, arachidonic acid (figure 19). Thromboxane A_2 promotes platelet aggregation and vasoconstriction. Prostacyclin inhibits platelet aggregation and promotes vasodilatation. Release of thromboxane A_2 by platelets at the immediate site of injury to a blood vessel promotes clot formation whereas prostacyclin formation by endothelial cells, bordering the injury site, keeps the clot localized and prevents extensive extension of the clot and maintains the patency of the rest of the vessel. **The thromboxane A_2-prostacyclin balance can be shifted toward prostacyclin by administration of low doses of aspirin**. Aspirin produces irreversible inhibition of cyclooxygenase, and this reduces production of both thromboxane A_2 and prostacyclin. However, endothelial cells produce new cyclooxygenase in a matter of hours whereas platelets cannot manufacture the enzyme and the level rises only as new platelets enter the circulation, which is a slow process. Therefore, administration of small amounts of aspirin for prolonged periods has been shown to be of value in preventing formation of clots in cerebral and myocardial vessels.

5. Antithrombin substances: These are:

- **Fibrin** acts as antithrombin, while a clot is forming, most of thrombin formed adsorbed to the fibrin meshwork. This prevents spread of thrombin into the remaining blood and therefore prevents excessive spread of the clot.

- **Antithrombin III:** It is a small protein molecule, produced by the liver, which inactivates several enzymes of the coagulation system. It inactivates several factors in the **intrinsic** and **extrinsic** pathways and to the great extent **thrombin**. The thrombin that does not adsorb to fibrin soon combines with antithrombin III in the plasma and becomes inactivated. In addition, **anticoagulant drugs** like **Heparin**, binds to antithrombin III increasing its effectiveness in inhibiting thrombin and factors IXa, Xa, XIa, and XIIa.

6. Thrombin dilution: By rapidly flowing blood. If heart is slowing as in shock, clot formation can occurs.
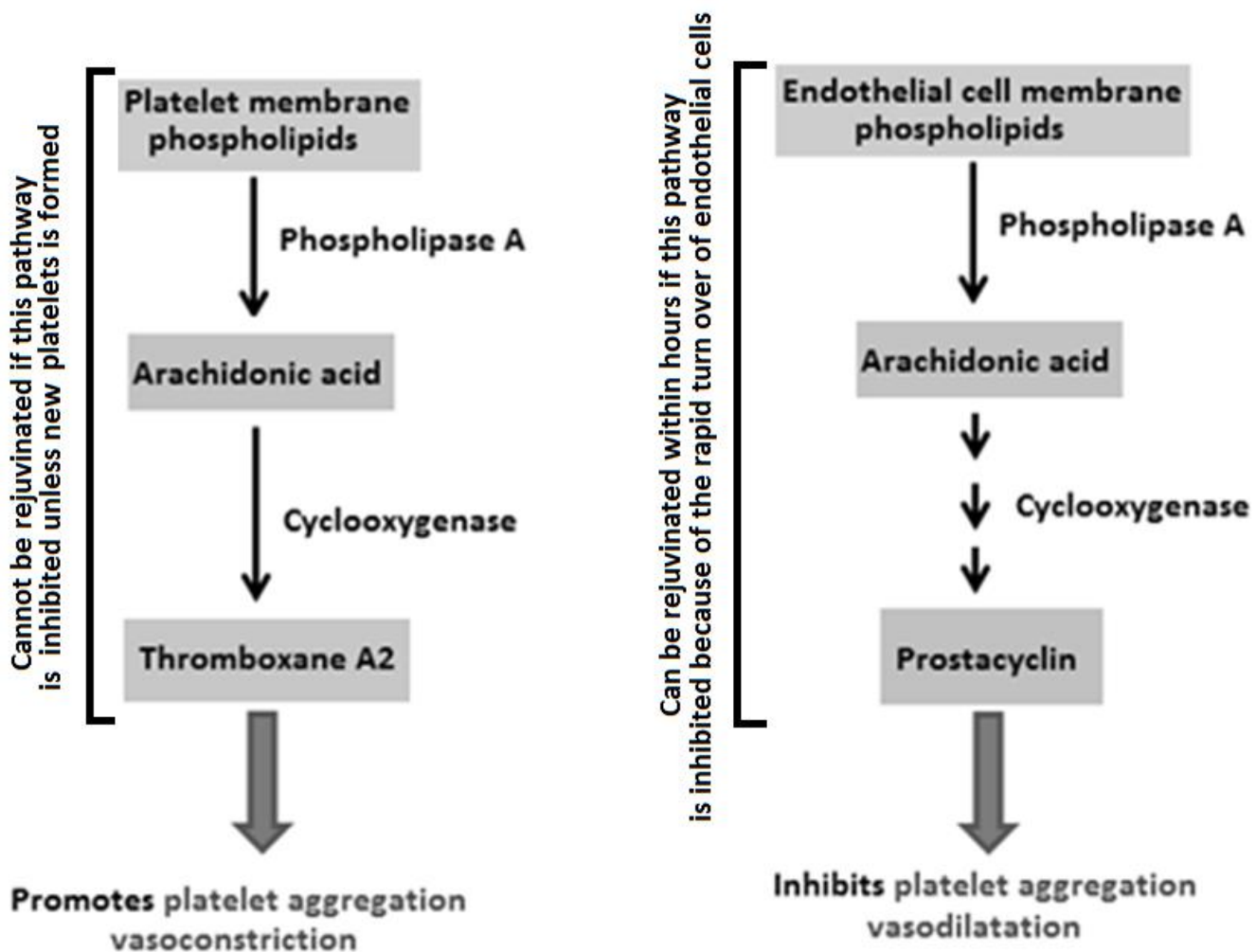
7. The fibrinolytic system.

Figure 19: The formation and the effect of thromboxane A2 and prostacyclin.

Abnormalities of Hemostasis:

- **Bleeding tendency:** Abnormal bleeding may occur in different conditions. Bleeding can be seen on the skin or mucous membranes as "**petechiae**", which are pin point purplish hemorrhagic spots, or as "**purpura**" which are extensive areas of red or dark purple discoloration. Bleeding can be in subcutaneous tissue in form of "**ecchymosis**" (or bruises) and **hematomas** (collection of blood). Bleeding also can occur in internal organs and from the body orifices. Bleeding tendency can occur due to vascular disorders, platelet disorders or disorders of blood coagulation.

Vascular disorders may be due to **damage of connective tissue** of vessel wall such as in **senility**, **hereditary disease** and **scurvy**. Alternatively, it may be due to **damage of endothelium** of blood vessel by for example some **infections** or **drugs**.

Platelet disorders: Normal platelet **number** and **function** is needed for normal hemostasis. Low platelet count is known as "**thrombocytopenia**" and could be due to many causes; such as decreased production of platelets, which occur with folate or B_{12} deficiency, radiation, and chemotherapy or marrow replacement by tumor. Alternatively, increased destruction of platelets by some drugs or in a certain condition known as "idiopathic thrombocytopenic purpura" specific Abs are formed and react against the platelets to destroy them.

Abnormal platelet function (**thrombocytopathy**) also causes bleeding tendency. Such as in **von Willebrand's disease**, which is an inherited disease, in which platelet count and structure are normal, but platelets, cannot adhere to vascular subendothelium because of deficiency of **von Willebrand factor**

(vWF). This factor is a protein synthesized by endothelial cells and platelets. Platelet adherence requires the presence of vWF because this factor forms bridges between platelets and subendothelial components allowing platelets to adhere to damaged vessel walls (figure 20). In addition, some *drugs* can also affect platelet function such as aspirin, which impairs aggregation of platelets, as explained earlier.

- **Disorders of blood coagulation** could be due to different causes such as:

Deficiency of clotting factors due to hereditary defects —as in **classic hemophilia** (factor VIII is deficient) and in **Christmas disease** (factor IX is deficient).

Deficiency of vitamin K which can cause decreased synthesis of **prothrombin** and factors **VII**, **IX** and **X** in liver, because vitamin K is necessary for formation of these factors in liver.

Liver diseases which cause defective production of coagulation factors (prothrombin, fibrinogen, and factors VII, IX, X... etc) because many or almost all clotting factors are formed by liver.
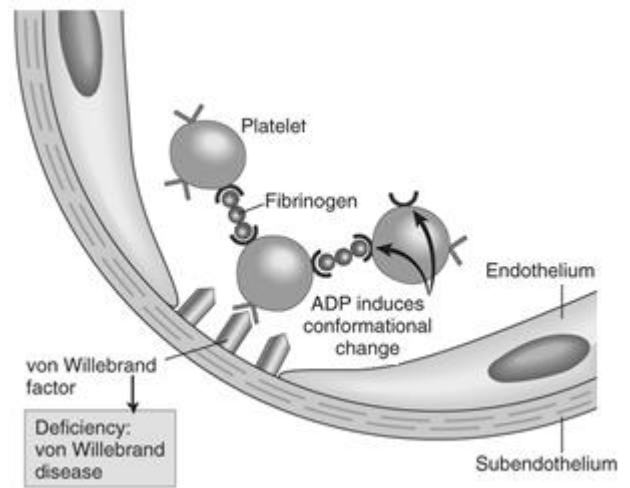

Figure 20: von Willebrand factor

Thromboembolic conditions: An abnormal clot that develops inside a blood vessel is called a "**thrombus**". Once a clot has developed, continued flow of blood past the clot is likely to break it away from its attachment or break off bits of the thrombus to flow along with the blood, such freely flowing clots are known as "**emboli**". Emboli do not stop flowing until they come to a narrow point in the circulatory system. Thus, emboli that originate in large arteries or in the left side of the heart eventually plug either smaller arteries or arterioles in the brain, kidneys, or elsewhere. Emboli that originate in the venous system and in the right side of the heart flow into the vessels of the lung to cause pulmonary arterial embolism**.**

The causes of thromboembolic conditions include:
1. **Roughened endothelial surface of a vessel**; as may be caused by atherosclerosis, is likely to initiate the clotting process.
2. **Slow blood flow:** Small amounts of thrombin continuously and spontaneously formed in the plasma, but at normal rates of blood flow the thrombin is diluted so quickly that a clot has little chance to form. If flow decreases, however, enough thrombin can accumulate to cause clotting. This can happen in circulatory shock, for example, when output from the heart is diminished and circulation slows down.
3. **Mutations in antithrombin III, and factor V.**

Anticoagulant drugs: To treat or prevent thromboembolic conditions, anticoagulants can be used clinically to delay coagulation process to a certain degree. These include:
1. **Heparin**.
2. Coumarin derivatives such as **warfarin.**
3. Aspirin.

Outside the body, in vitro clotting can be prevented by:
1. Heparin.

2. Removal of Ca^{2+} from the blood by addition of substances such as oxalates, which form insoluble salts with Ca^{2+}, or citrates, which bind Ca^{2+}.