MEDICAL CLUB

HEMATOPOIETIC &

LYMPHATIC SYSTEM

Physiology -HLS

‘The life so short, the craft so long to learn.”— Hippocrates

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THE BOOM 2

-In this course we begin discussing the blood cells and cells of the macrophage system and lymphatic system. We first present the functions of red blood cells (RBCs), which are the most abundant cells of the blood and are necessary for the delivery of oxygen to the tissues.

-BLOOD

- **INTRODUCTION :**

- Blood is a connective tissue in fluid form. It is considered as the 'fluid of life' because it carries oxygen from lungs to all parts of the body and carbon dioxide from all parts of the body to the lungs. It is known as 'fluid of growth' because it carries nutritive substances from the digestive system and hormones from endocrine gland to all the tissues. The blood is also called the 'fluid of health' because it protects the body against the diseases and gets rid of the waste products and unwanted substances by transporting them to the excretory organs like kidneys.

- **PROPERTIES OF BLOOD :**

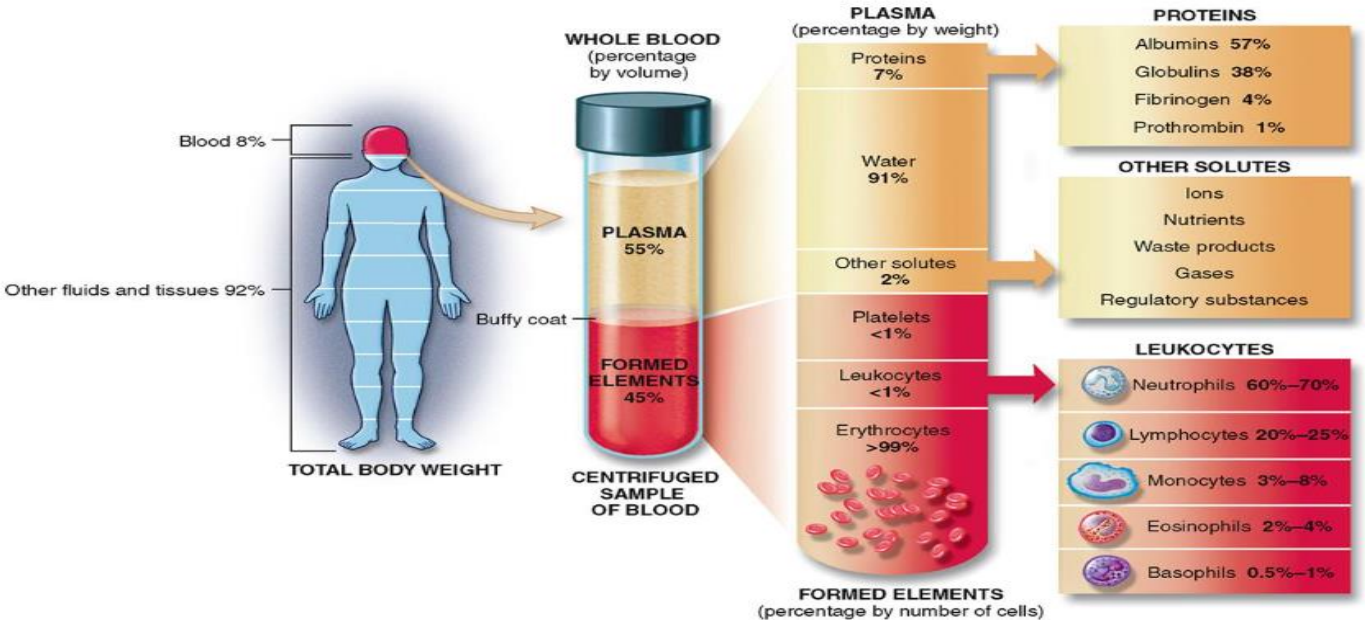
1) Color: Blood is red in color. Arterial blood is scarlet red because it contains more oxygen and venous blood is purple red because of more carbon dioxide.

2) Volume: Average volume of blood in a normal adult is 5 L. In a newborn baby, the volume is 450 ml. It increases during growth and reaches 5 L at the time of puberty. In females, it is slightly less and is about 4.5 L. It is about 8% of the body weight in a normal young healthy adult, weighing about 70 kg

3) Reaction and pH: Blood is slightly alkaline and its pH in normal conditions is 7.4

4) Viscosity: Blood is five times more viscous than water. It is mainly due to red blood cells and plasma proteins.

-**Composition of Blood :**



- Blood is composed of two parts:

A) Plasma: This is the fluid part of blood. It constitutes 55% of blood.

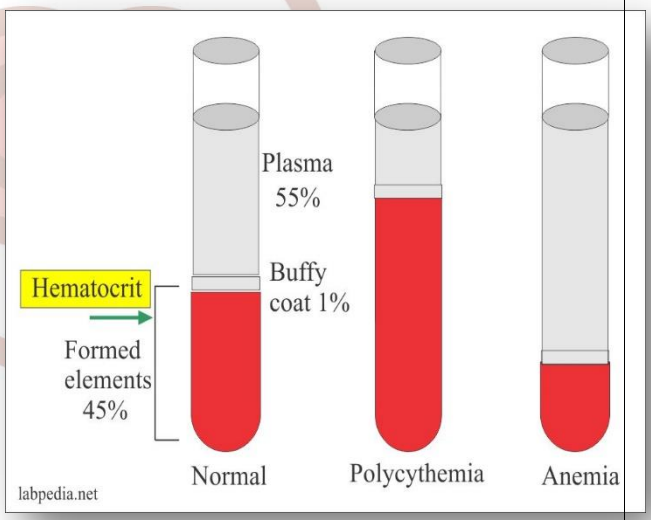
B) Cellular part: This part includes red blood corpuscles, white blood cells and platelets. It constitutes 45% of blood

-Hematocrit Value : (VIP IN PHYSIOLOGY LAB)

- is the volume percentage (vol%) of red blood cells (RBCs) in blood, measured as part of a blood test.The measurement depends on the number and size of red blood cells.It is normally 40.7–50.3% for males and 36.1–44.3% for females.

-It is a part of a person's complete blood count results,along with hemoglobin concentration, white blood cell count and platelet count.

- Because the purpose of red blood cells is to transfer oxygen from the lungs to body tissues, a blood sample's hematocrit—the red blood cell volume percentage—can become a point of reference of its capability of delivering oxygen. Hematocrit levels that are too high or too low can indicate a blood disorder, dehydration, or other medical conditions.



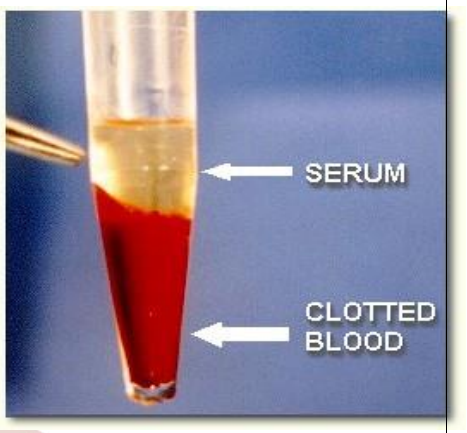
An abnormally low hematocrit may suggest anemia, a decrease in the total amount of red blood cells, while an abnormally high hematocrit is called polycythemia .

-PLASMA :

- Plasma is a straw-colored clear liquid part of blood. It contains 91% to 92% of water and 8% to 9% of solids.The solids are the organic and the inorganic substances

- SERUM :

- is the fluid and solute component of blood which does not play a role in clotting.It may be defined as blood plasma without the clotting factors, or as blood with all cells and clotting factors removed.



Serum includes all proteins not used in blood clotting;

all electrolytes, antibodies, antigens, hormones;

and any exogenoussubstances (e.g., drugs or microorganisms).

Serum does not contain white blood cells (leukocytes), red blood cells (erythrocytes), platelets, or clotting factors , SO → Serum = Plasma – Fibrinogen

- **FUNCTIONS OF BLOOD :**

→ TRANSPORT OF HORMONES AND ENZYMES :

Hormones which are secreted by ductless (endocrine) glands are released directly into the blood.

The blood transports these hormones to their target organs/tissues. Blood also transports enzymes.

→ DEFENSIVE FUNCTION :

Blood plays an important role in the defense of the body. The white blood cells are responsible for this function. Neutrophils and monocytes engulf the bacteria by phagocytosis. Lymphocytes are involved in development of immunity. Eosinophils are responsible for detoxification, disintegration and removal of foreign proteins

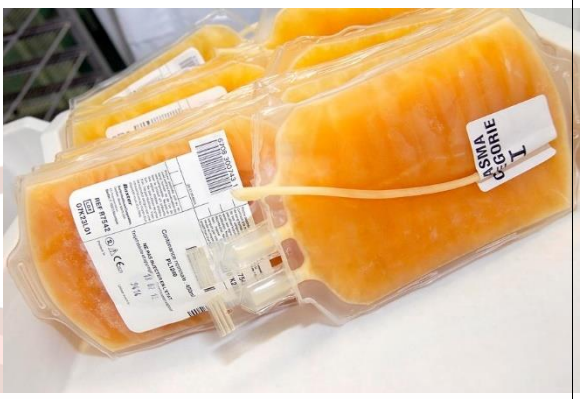
→ REGULATION OF BODY TEMPERATURE :

Because of the high specific heat of blood, it is responsible for maintaining the thermoregulatory mechanism in the body, i.e. the balance between heat loss and heat gain in the body

→HAEMOSTASIS (STOPPAGE OF BLEEDING) :

By the platelates and coagulation factors (discussed latter)

-Plasma Proteins :



-Plasma→ It is a yellow clear fluid.

• Its volume is about 3.5 L (5% of body weight).

• It clots on standing. The remnant is called serum.

-Composition of Plasma→

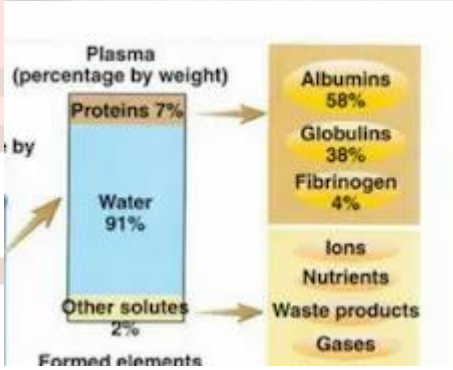
1-water 91%

2- Organic substances: **plasma protein** , lipids and

Other (Glucose)

3- Inorganic constituents : Na+ , Cl- and HCO-3

4-bloood gases : O2 , CO and N .

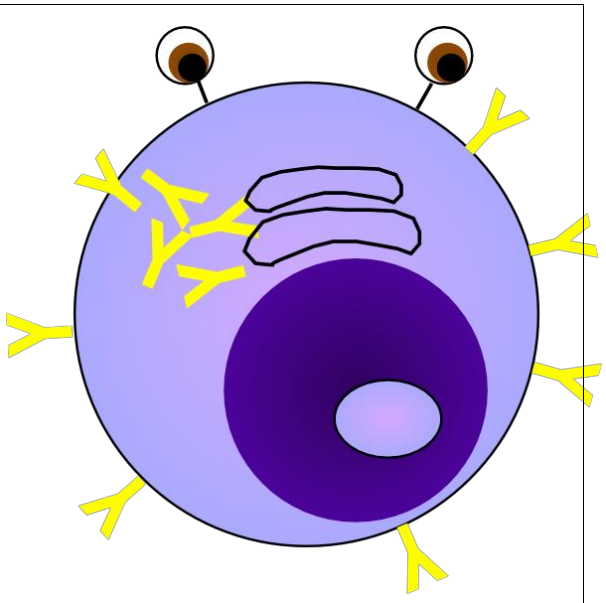


-PLASMA PROTEINS

- INTRODUCTION

- Plasma proteins are: 1. Serum albumin 2. Serum globulin 3. Fibrinogen.

Serum contains only albumin and globulin. Fibrinogen is absent in serum because, it is converted into fibrin during blood clotting. Because of this, the albumin and globulin are usually called serum albumin and serum globulin.



- NORMAL VALUES :

- Normal values of the plasma proteins are:

1) Total proteins : 7.3 g/dL (6.4 to 8.3 g/dL)

2) Serum albumin : 4.7 g/dL

3) Serum globulin : 2.3 g/dL

4) Fibrinogen : 0.3 g/dL

- ALBUMIN/GLOBULIN RATIO :

Albumin to Globulin (A/G) Ratio

Formula: $\text{A/G Ratio} = \frac{\text{Albumin Level}}{\text{(Total protein - Albumin Level)}}$

Normal Range: 1.5 - 2.5

- Ratio between plasma level of albumin and globulin is called albumin/globulin (A/G) ratio. It is an important indicator of some diseases involving liver or kidney. Normal A/G ratio is 2 : 1

- PROPERTIES OF PLASMA PROTEINS :

- MOLECULAR WEIGHT →

Type	Concentration (g/dl)	Molecular weight
1. Albumin	3.5-5	69,000
2. Globulin (α,β,γ)	2.5	90,000-156,000
3. Fibrinogen	0.4	340,000
4. Prothrombin	0.01	68,700

-Thus, the molecular weight of fibrinogen is greater than that of other two proteins

- Sites of Formation of Plasma Proteins ➔

→ 1. Albumin, fibrinogen and prothrombin: -

They are synthesized in the liver.

→2.Globulins: - 50% are synthesized in the liver.

- 50% (γ globulin) are synthesized in the plasma cells of the reticuloendothelial system (RES), which is a diffuse system of cells present in the liver, spleen, lymph nodes and bone marrow.

- **FUNCTIONS OF PLASMA PROTEINS** :

- Plasma proteins are very essential for the body. Following are the functions of plasma proteins →

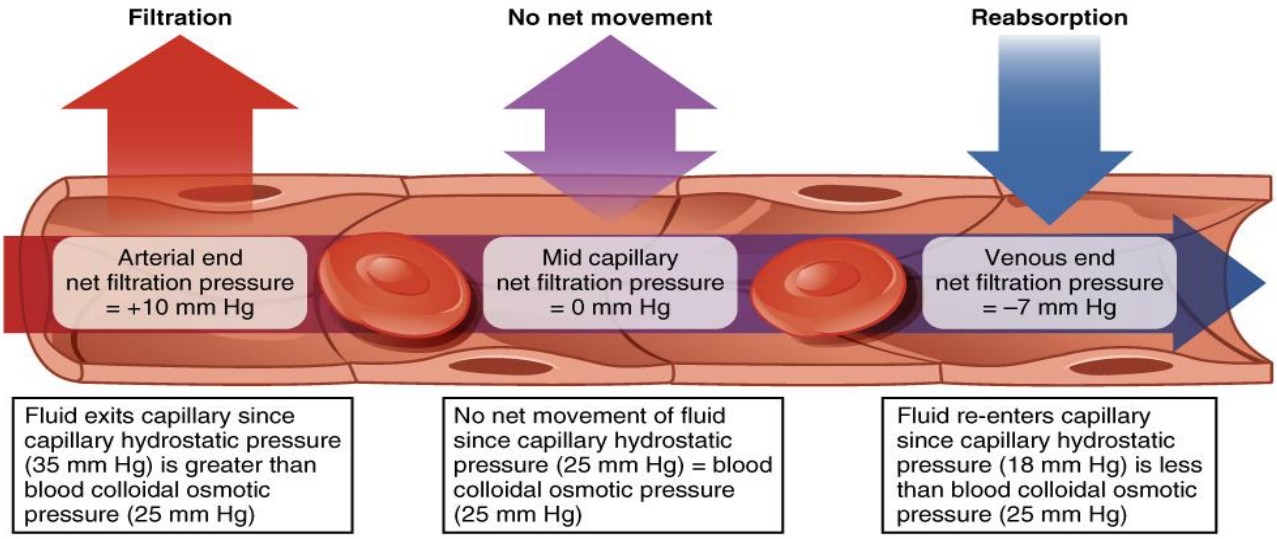
Functions of plasma proteins

Function	Example
Transport	**Thyroxine-binding globulin** (thyroid hormones) **Apolipoproteins** (cholesterol, triglyceride)
Humoral immunity	Immunoglobulins
Maintenance of oncotic pressure	All proteins, particularly albumin
Enzymes	Renin, coagulation factors, complement proteins
Protease inhibitors	α1-antitrypsin (acts on protease)
Buffering	All proteins

1)**Osmotic Function** :

The total osmotic pressure of plasma is about 5000 mmHg

Plasma proteins are responsible for the oncotic or osmotic pressure in the blood. Osmotic pressure exerted by proteins in the plasma is called colloidal osmotic (oncotic) pressure. Normally, it is about 25 mm Hg. Albumin plays a major role in exerting oncotic pressure



- The remaining pressure is caused by crystalloids, e.g. Na+ , Cl- , HCO3 - , and is called the crystalloid osmotic pressure

- Crystalloids are osmotically more powerful but less important than colloids, since they are present in the same concentration in the plasma and interstitial fluid (ISF). So their net osmotic effect is almost zero.

-Plasma proteins have a weaker osmotic effect, but they are more important, because they cannot diffuse through the capillary membrane. Therefore they are kept inside blood vessels and tend to draw water from ISF into capillaries.

-The colloidal osmotic pressure regulates blood volume by regulation of fluid exchange between ISF and blood.

2) **ROLE IN COAGULATION OF BLOOD**→ Fibrinogen is essential for the coagulation of blood

3) **ROLE IN DEFENSE MECHANISM OF BODY**→ Gamma globulins play an important role in the defense mechanism of the body by acting as antibodies (immune substances). These proteins are also called immunoglobulins. Antibodies react with antigens of various microorganisms, which cause diseases like diphtheria, typhoid, streptococcal infections, mumps, influenza, measles, hepatitis, rubella, polio myelitis, etc

4**) ROLE IN TRANSPORT MECHANISM**→ Plasma proteins are essential for the transport of various substances in the blood. Albumin, alpha globulin and beta globulin are responsible for the transport of the hormones, enzymes, etc. The

alpha and beta globulins play an important role in the transport of metals in the blood.

5**) ROLE IN REGULATION OF ACID-BASE BALANCE→**

- Plasma proteins, particularly the albumin, play an important role in regulating the acid base balance in the blood. This is because of the virtue of their buffering action. Plasma proteins are responsible for 15% of the buffering capacity of blood

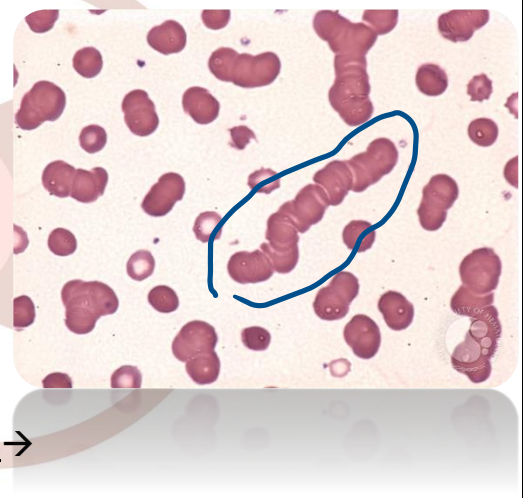
6**) ROLE IN VISCOSITY OF BLOOD→**

- Plasma proteins provide viscosity to the blood, which is important to maintain the blood pressure. Albumin provides maximum viscosity than the other plasma proteins

7**) ROLE IN ERYTHROCYTE SEDIMENTATION RATE→**

- Globulin and fibrinogen accelerate the tendency of rouleaux formation by the red blood cells.

Rouleaux formation is responsible for ESR, which is an important diagnostic and prognostic tool



8**) ROLE IN SUSPENSION STABILITY OF RED BLOOD CELLS→**

- During circulation, the red blood cells remain suspended uniformly in the blood. This property of the red blood cells is called the suspension stability. Globulin and fibrinogen help in the suspension stability of the red blood cells

9**) ROLE IN PRODUCTION OF TREPHONE SUBSTANCES→**

- Trephone substances are necessary for nourishment of tissue cells in culture. These substances are produced by leukocytes from the plasma proteins

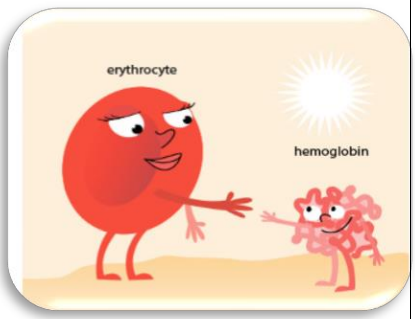
10**) ROLE AS RESERVE PROTEINS→**

- During fasting, inadequate food intake or inadequate protein intake, the plasma proteins are utilized by the body tissues as the last source of energy. Plasma proteins are split into amino acids by the tissue macrophages. Amino acids are taken back by blood and distributed throughout the body to form cellular protein molecules. Because of this, the plasma proteins are called the reserve proteins

-Table VIP→

Plasma Protein	Conditions when increases	Conditions when decreases
Total proteins	Hyperproteinemia: 1. Dehydration 2. Hemolysis 3. Acute infections like acute hepatitis and acute nephritis 4. Respiratory distress syndrome 5. Excess of glucocorticoids 6. Leukemia 7. Rheumatoid arthritis 8. Alcoholism	Hypoproteinemia: 1. Diarrhea 2. Hemorrhage 3. Burns 4. Pregnancy 5. Malnutrition 6. Prolonged starvation 7. Cirrhosis of liver 8. Chronic infections like chronic hepatitis or chronic nephritis
Albumin	1. Dehydration 2. Excess of glucocorticoids 3. Congestive cardiac failure	1. Malnutrition 2. Cirrhosis of liver 3. Burns 4. Hypothyroidism 5. Nephrosis 6. Excessive intake of water
Globulin	1. Cirrhosis of liver 2. Chronic infections 3. Nephrosis 4. Rheumatoid arthritis	1. Emphysema 2. Acute hemolytic anemia 3. Glomerulonephritis 4. Hypogammaglobulinemia
Fibrinogen	1. Acute infections 2. Rheumatoid arthritis 3. Glomerulonephritis 4. Myocardial infarction 5. Stroke 6. Trauma	1. Liver dysfunction 2. Use of anabolic steroids 3. Use of phenobarbital
A/G ratio	1. Hypothyroidism 2. Excess of glucocorticoids 3. Hypogammaglobulinemia 4. Intake of high carbohydrate or protein diet	1. Liver dysfunction 2. Nephrosis

RED BLOOD CORPUSCLES (RBCs)
ERYTHROCYTES



- INTRODUCTION :

- Red blood cells (RBCs) are the non-nucleated formed elements in the blood. Red blood cells are also known as erythrocytes (erythros = red). Red color of the red blood cell is due to the presence of the coloring pigment called hemoglobin.

RBCs play a vital role in transport of respiratory gases. RBCs are larger in number compared to the other two blood cells, namely white blood cells and platelets .

- NORMAL VALUE :

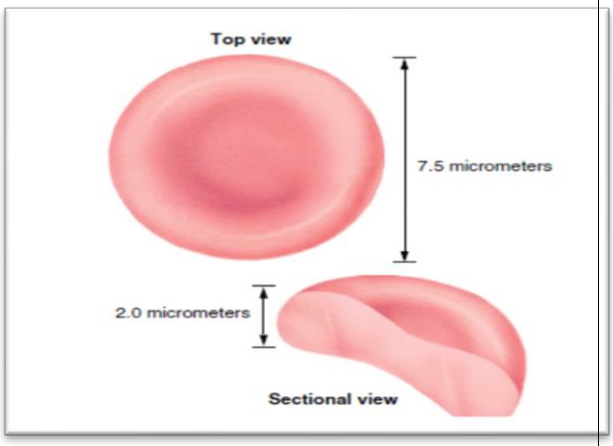
- RBC count ranges between 4 and 5.5 million/cu mm of blood.

In adult males, it is 5 million/cu mm and in adult females,

it is 4.5 million/cu mm

	Adult Man	Adult Woman
RBC Count (Millions/μL)	4.7-6.1	4.2-5.4
Hemoglobin (grams/dL)	14-18	12-16

- NORMAL SHAPE :



- Normally, the RBCs are disk shaped and biconcave (dumbbell shaped). Central portion is thinner and periphery is thicker. The biconcave contour of RBCs has some mechanical and functional advantages.

- Their volume is 90 μm3

- Advantages of Biconcave Shape of RBCs→

1) Biconcave shape helps in equal and rapid diffusion of oxygen and other substances into the interior of the cell .

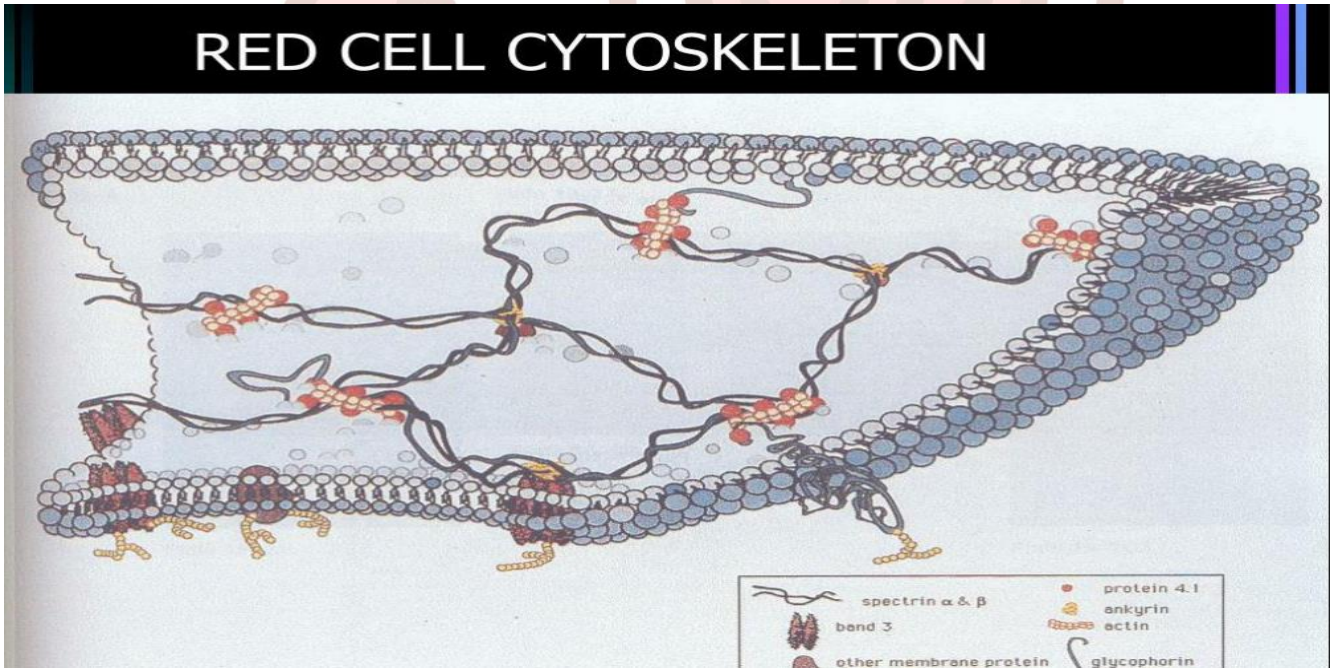
2) Large surface area is provided for absorption or removal of different substances.

3) Minimal tension is offered on the membrane when the volume of cell alters

4) Because of biconcave shape, while passing through minute capillaries, RBCs squeeze through the capillaries very easily without getting damage

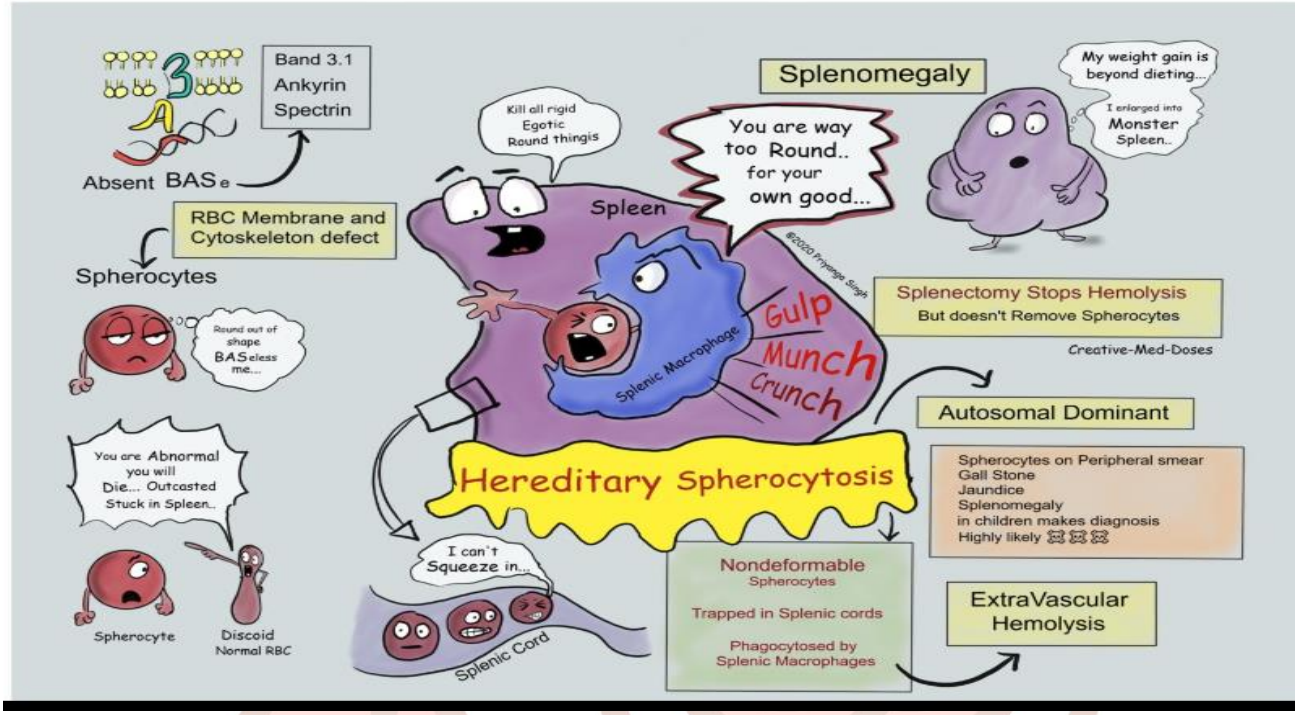
-NORMAL STRUCTURE :

- Red blood cells are nonnucleated. Only mammal, which has nucleated RBC is camel. Because of the absence of nucleus in human RBC, the DNA is also absent. Other organelles such as mitochondria and Golgi apparatus also are absent in RBC. Because of absence of mitochondria, the energy is produced from glycolytic process. Red cell does not have insulin receptor and so the glucose uptake by this cell is not controlled by insulin.



- RBC has a special type of cytoskeleton, which is made up of actin and spectrin. Both the proteins are anchored to transmembrane proteins by means of another protein called ankyrin. Absence of spectrin results in hereditary spherocytosis. In this condition, the cell is deformed, losses its biconcave shape and becomes

globular (spherocytic). The spherocyte is very fragile and easily ruptured (hemolyzed) in hypotonic solutions.



- LIFESPAN OF RED BLOOD CELLS :

- When RBCs are delivered from the bone marrow into the circulatory system, they normally circulate an average of 120 days before being destroyed.

- the metabolic systems of old RBCs become progressively less active and the cells become more and more fragile, presumably because their life processes wear out. Once the RBC membrane becomes fragile, the cell ruptures during passage through some tight spot of the circulation. Many of the RBCs self-destruct in the spleen, where they squeeze through the red pulp of the spleen .

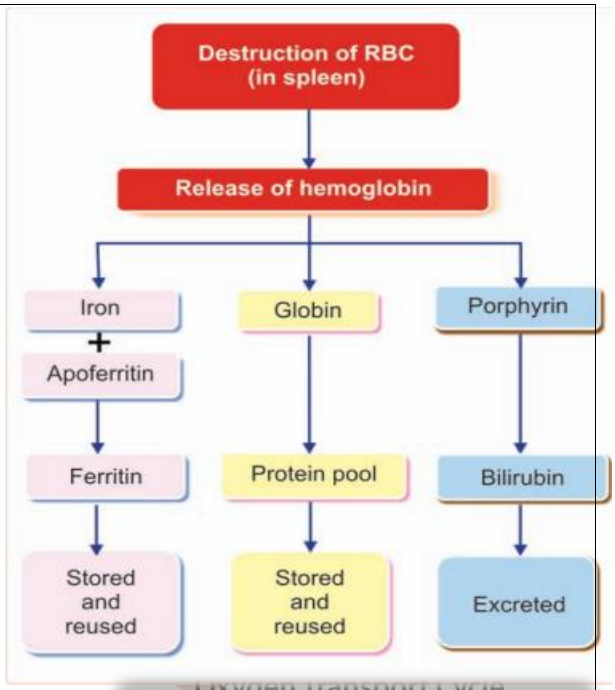
- FATE OF RED BLOOD CELLS :

- Destroyed RBCs are fragmented and hemoglobin is released from the fragmented parts. Hemoglobin is immediately phagocytized by macrophages of the body, particularly the macrophages present in liver (Kupffer cells), spleen and bone marrow.

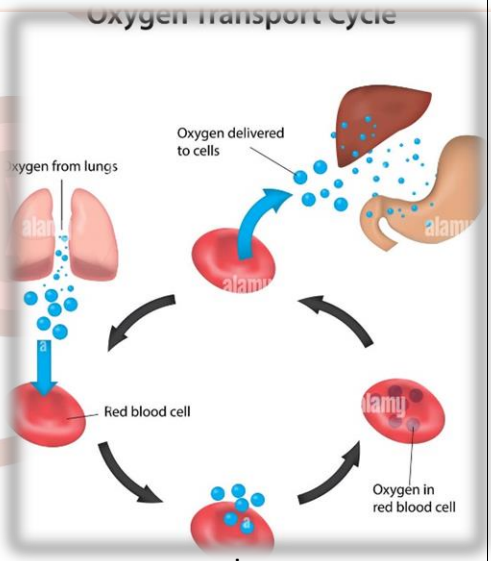
- Hemoglobin is degraded into iron, globin, and porphyrin

- the macrophages release iron from the hemoglobin and pass it back into the blood, to be carried by transferrin either to the bone marrow for the production

of new RBCs or to the liver and other tissues for storage in the form of ferritin. the porphyrin portion of the hemoglobin molecule is converted by the macrophages, through a series of stages, into the bile pigment bilirubin, which is released into the blood and later removed from the body by secretion through the liver into the bile.



- FUNCTIONS OF RED BLOOD CELLS :



1) Transport of Oxygen from the Lungs to the Tissues → Hemoglobin in RBC combines with oxygen to form oxyhemoglobin. About 97% of oxygen is transported in blood in the form of oxyhemoglobin

2) Transport of Carbon Dioxide from the Tissues to the Lungs , RBCs contain a large amount of the carbonic anhydrase. This enzyme is necessary for the formation of bicarbonate from water and carbon dioxide Thus, it helps to transport carbon dioxide in the form of bicarbonate from tissues to lungs. About 63% of carbon dioxide is transported in this form.

3) Buffering Action in Blood→ Hemoglobin functions as a good buffer. By this action, it regulates the hydrogen ion concentration and thereby plays a role in the maintenance of acidbase balance

4) In Blood Group Determination → RBCs carry the blood group antigens like A antigen, B antigen and Rh factor. This helps in determination of blood group and enables to prevent reactions due to incompatible blood transfusion

- VARIATIONS IN NUMBER OF RED BLOOD CELLS :

1) PHYSIOLOGICAL VARIATIONS

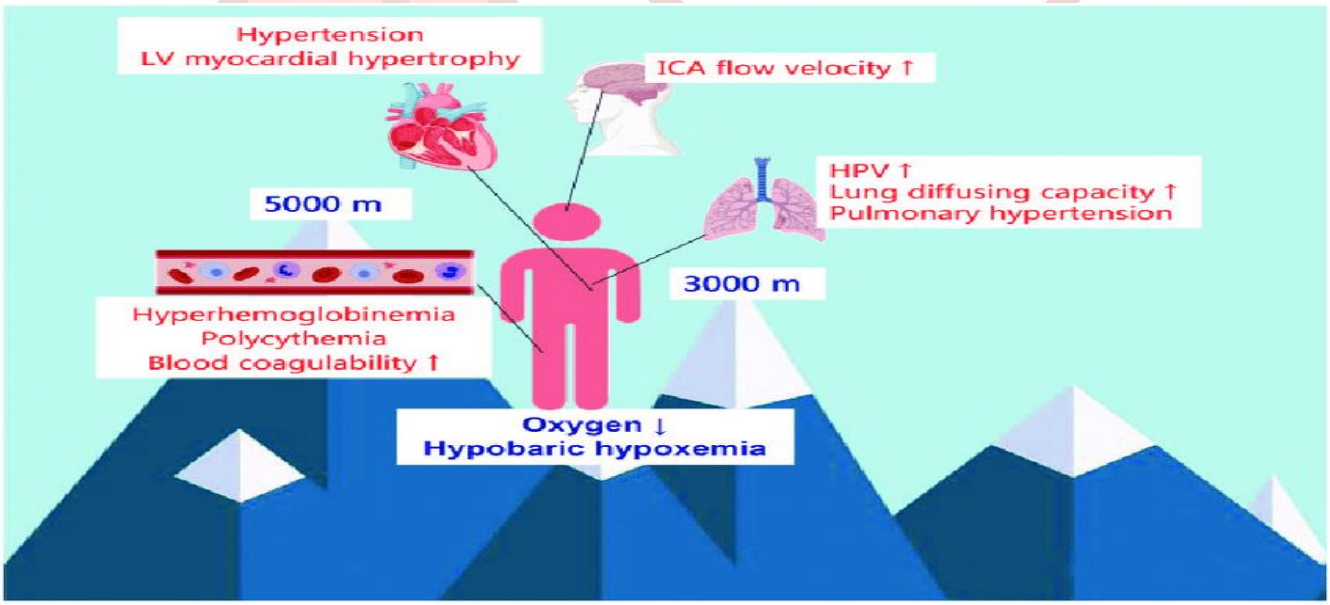
2) PATHOLOGICAL VARIATIONS

-PHYSIOLOGICAL →

A-AGE : At birth, the RBC count is 8 to 10 million/cu mm of blood. The count decreases within 10 days after birth due to destruction of RBCs causing physiological jaundice in some newborn babies. However, in infants and growing children, the cell count is more than the value in adults .

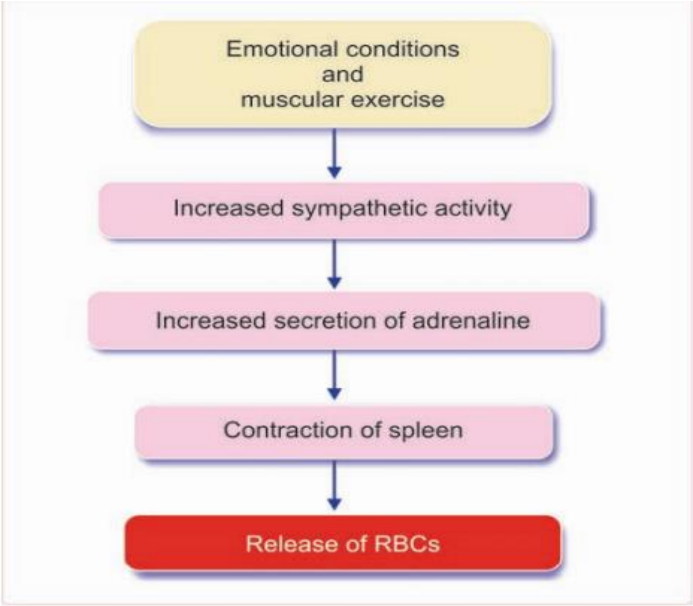
B-SEX : Before puberty and after menopause in females the RBC count is similar to that in males. During reproductive period of females, the count is less than that of males (4.5 million/cu mm)

C- High altitude: Inhabitants of mountains (above 10,000 feet from mean sea level) have an increased RBC count of more than 7 million/cu mm. It is due to hypoxia in high altitude. Hypoxia stimulates the kidney to secrete a hormone called erythropoietin. The erythropoietin in turn stimulates the bone marrow to produce more RBCs



D- Muscular exercise : There is a temporary increase in RBC count after exercise. It is because of mild hypoxia and contraction of spleen. Spleen stores RBCs Hypoxia increases the sympathetic activity resulting in secretion of adrenaline

from adrenal medulla. Adrenaline contracts spleen and RBCs are released into blood .



E- Emotional conditions : RBC count increases during emotional conditions such as anxiety. It is because of increase in the sympathetic activity as in the case of muscular exercise.

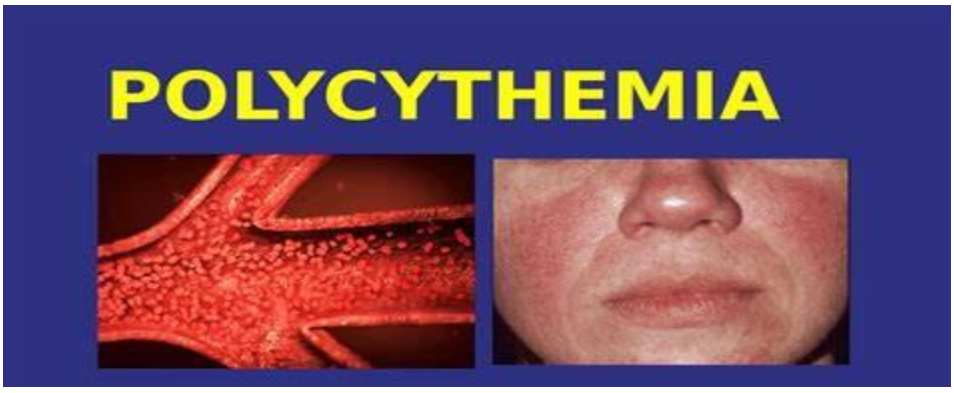
F- Increased environmental temperature : Increase in atmospheric temperature increases RBC count. Generally increased temperature increases all the activities in the body including production of RBCs

G- After meals : There is a slight increase in the RBC count after taking meals. It is because of need for more oxygen for metabolic activities

NOTE→ in sleep , Pregnancy and High barometric pressures : the RBC counts will decrease

- PATHOLOGICAL VARIATIONS →

A- Pathological Polycythemia :



Pathological polycythemia is the abnormal increase in the RBC count. Red cell count increases above 7 million/ cu mm of the blood. Polycythemia is of two types, **the primary polycythemia** and **secondary polycythemia**.

- **Primary Polycythemia – Polycythemia Vera** →It is a disease characterized by persistent increase in RBC count above 14 million/cu mm of blood. This is always associated with increased white blood cell count above 24,000/cu mm of blood. Polycythemia vera occurs in myeloproliferative disorders like malignancy of red bone marrow, So not only does the hematocrit increase, but the total blood volume also increases, sometimes to almost twice normal. As a result, the entire vascular system becomes intensely engorged. Also, many blood capillaries become plugged by the viscous blood; the viscosity of the blood in polycythemia vera sometimes increases from the normal of 3 times the viscosity of water to 10 times that of water

- **Secondary Polycythemia**→ Whenever the tissues become hypoxic because of too little oxygen in the breathed air, such as at high altitudes, or because of failure of oxygen delivery to the tissues, such as in cardiac failure, the blood-forming organs automatically produce large quantities of extra RBCs. This condition is called secondary polycythemia, and the RBC count commonly rises to 6 to 7 million/mm3 , about 30 percent above normal. A common type of secondary polycythemia, called physiological polycythemia, occurs in natives who live at altitudes of 14,000 to 17,000 feet, where the atmospheric oxygen is very low. The blood count is generally 6 to 7 million/mm3; this blood count allows these people to perform reasonably high levels of continuous work even in a rarefied atmosphere .

- Anemia :

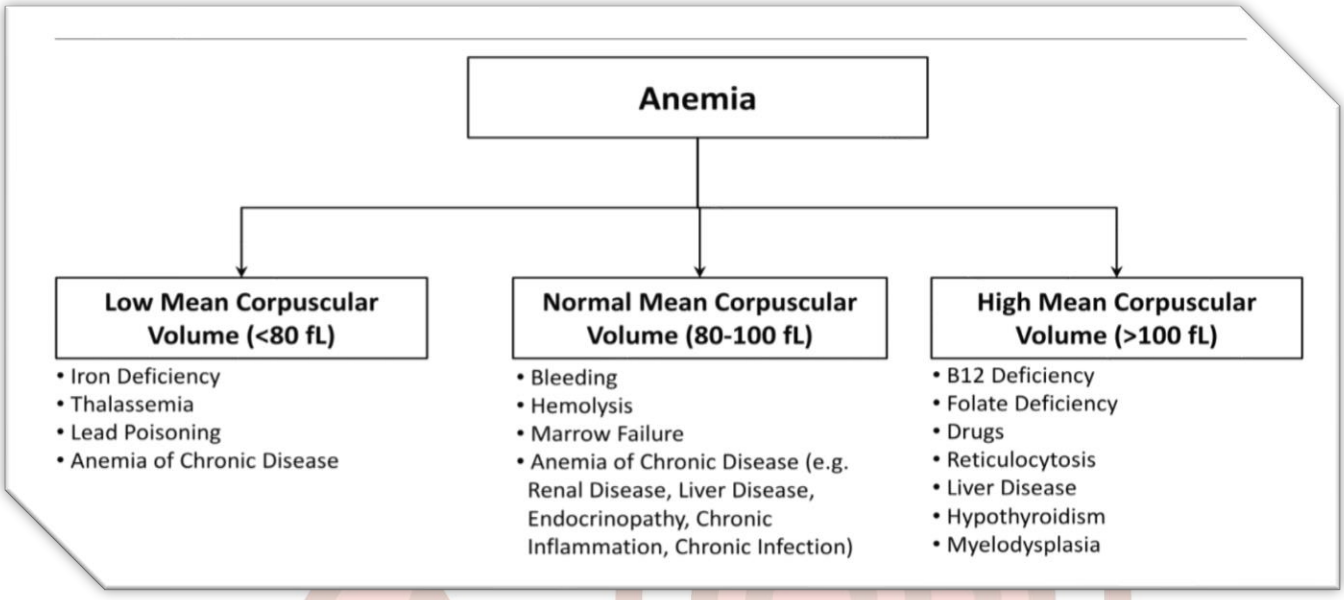
- Anemia means deficiency of hemoglobin in the blood, which can be caused by either too few RBCs or too little hemoglobin in the cells.

- Under physiological conditions, the size of RBCs in venous blood is slightly larger than those in arterial blood. In pathological conditions, the variations in size of RBCs are:

1-Microcytes (smaller cells)

2- Microcytes (smaller cells)

3- normocytic (Normal cells)



A)MICROCYTES→ Microcytes are present in: Iron-deficiency anemia ,Prolonged forced breathing and Increased osmotic pressure in blood

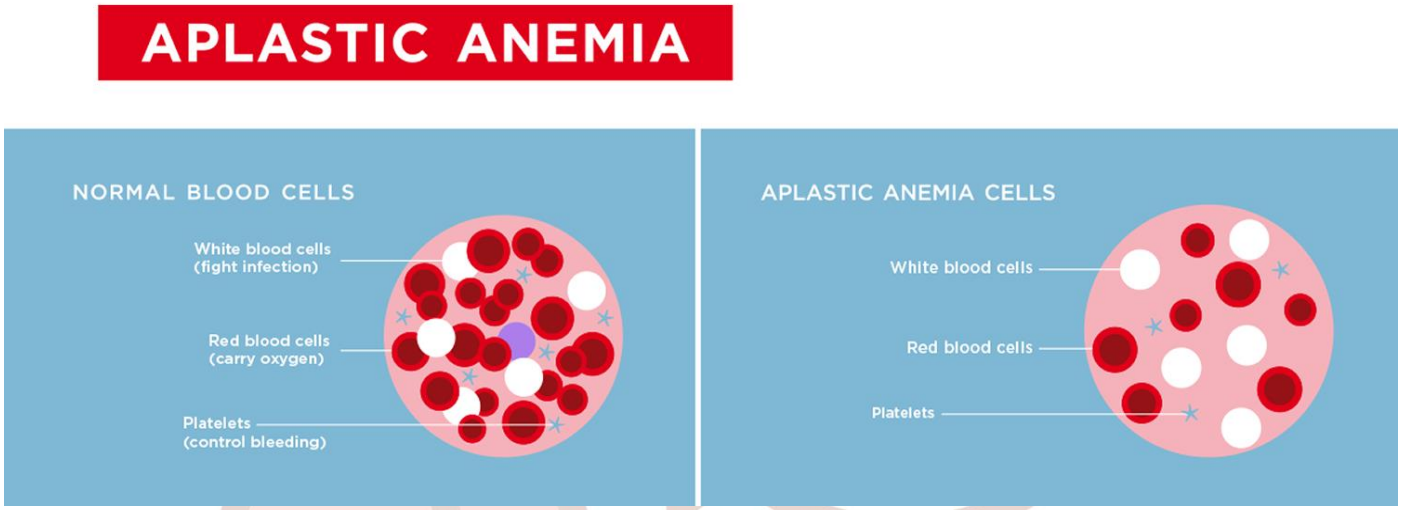
B) MACROCYTES →Macrocytes are present in: Megaloblastic anemia , Decreased osmotic pressure in blood.

C) Normocytic→ Acute Blood Loss (Hemorrhagic Anemia) , Bone Marrow Depression (Aplastic Anemia) and Excessive Breakdown of RBCs (Hemolytic Anemia)

-Now will talk about of this causes :

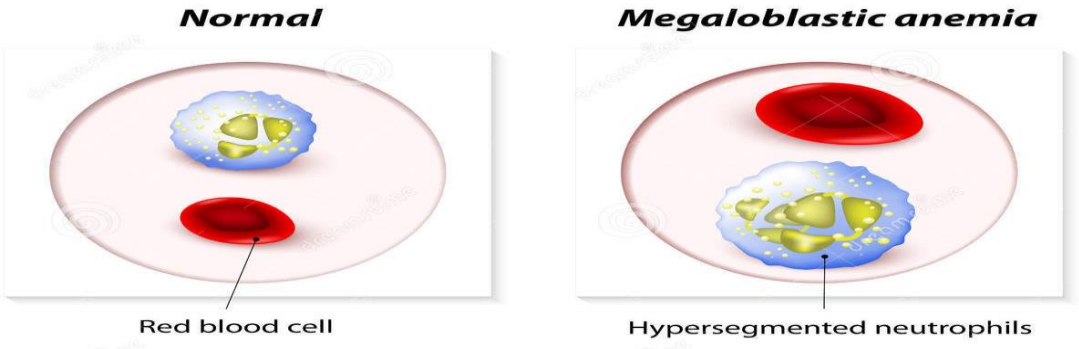
Blood Loss Anemia. After rapid hemorrhage, the body replaces the fluid portion of the plasma in 1 to 3 days, but this response results in a low concentration of RBCs. If a second hemorrhage does not occur, RBC concentration usually returns to normal within 3 to 6 weeks. When chronic blood loss occurs, a person frequently cannot absorb enough iron from the intestines to form hemoglobin as rapidly as it is lost. RBCs that are much smaller than normal and have too little hemoglobin inside them are then produced, giving rise to microcytic, hypochromic anemia .

Aplastic Anemia Due to Bone Marrow Dysfunction. Bone marrow aplasia means lack of functioning bone marrow. For instance, exposure to high-dose radiation or chemotherapy for cancer treatment can damage stem cells of the bone marrow, followed in a few weeks by anemia. Likewise, high doses of certain toxic chemicals, such as insecticides or benzene in gasoline, may cause the same effect. In autoimmune disorders, such as lupus erythematosus, the immune system begins attacking healthy cells such as bone marrow stem cells, which may lead to aplastic anemia. In about half of aplastic anemia cases the cause is unknown, a condition called idiopathic aplastic anemia.



People with severe aplastic anemia usually die unless they are treated with blood transfusions—which can temporarily increase the numbers of RBCs—or by bone marrow transplantation .

Megaloblastic Anemia. Encompasses a heterogeneous group of anemias characterized by the presence of large red blood cell precursors called megaloblasts in the bone marrow. This condition is due to impaired DNA synthesis, which inhibits nuclear division. Cytoplasmic maturation, mainly dependent on RNA and protein synthesis, is less impaired , Deficiencies of vitamin B12 and folic acid are the leading causes of megaloblastic anemia.



Hemolytic Anemia . Different abnormalities of the RBCs, many of which are hereditarily acquired, make the cells fragile, so they rupture easily as they go through the capillaries, especially through the spleen. Even though the number of RBCs formed may be normal, or even much greater than normal in some hemolytic diseases, the life span of the fragile RBC is so short that the cells are destroyed faster than they can be formed, and serious anemia results.

-Erythropoiesis

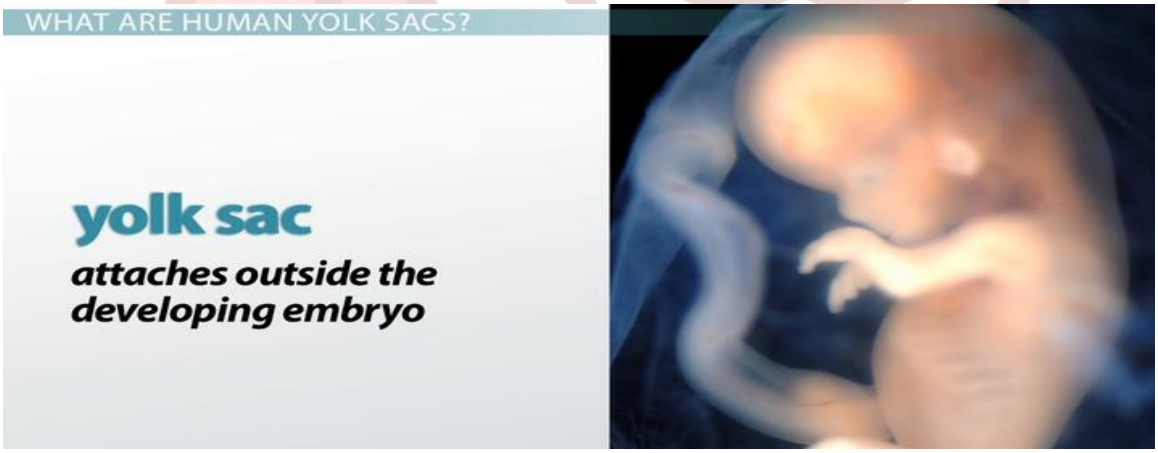
- **DEFINITION :**

Is the process which produces red blood cells (erythrocytes), which is the development from erythropoietic stem cell to mature red blood cell.

- **SITE OF ERYTHROPOIESIS :**

A) IN FETAL LIFE→ In fetal life, the erythropoiesis occurs in three stages :

1) **Mesoblastic Stage**→ During the first two months of intrauterine life, the RBCs are produced from mesenchyme of yolk sac



2) **Hepatic Stage**→ From third month of intrauterine life, liver is the main organ that produces RBCs. Spleen and lymphoid organs are also involved in erythropoiesis.

3) **Myeloid Stage**→ During the last three months of intrauterine life, the RBCs are produced from red bone marrow and liver.

-IN NEWBORN BABIES, CHILDREN AND ADULTS :

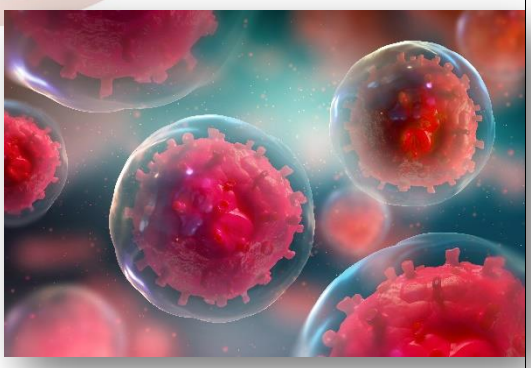
In newborn babies, growing children and adults, RBCs are produced only from the red bone marrow

- Up to the age of 20 years: RBCs are produced from red bone marrow of all bones (long bones and all the flat bones)

- After the age of 20 years: RBCs are produced from membranous bones like vertebra, sternum, ribs, scapula, iliac bones and skull bones and from the ends of long bones. After 20 years of age, the shaft of the long bones becomes yellow bone marrow because of fat deposition and looses the erythropoietic function.

- In adults, liver and spleen may produce the blood cells if the bone marrow is destroyed or fibrosed. Collectively bone marrow is almost equal to liver in size and weight. It is also as active as liver. Though bone marrow is the site of production of all blood cells, comparatively 75% of the bone marrow is involved in the production of leukocytes and only 25% is involved in the production of erythrocytes. But still, the leukocytes are less in number than the erythrocytes, the ratio being 1:500. This is mainly because of the lifespan of these cells. Lifespan of erythrocytes is 120 days whereas the lifespan of leukocytes is very short ranging from one to ten days. So the leukocytes need larger production than erythrocytes to maintain the required number .

- PROCESS OF ERYTHROPOIESIS :

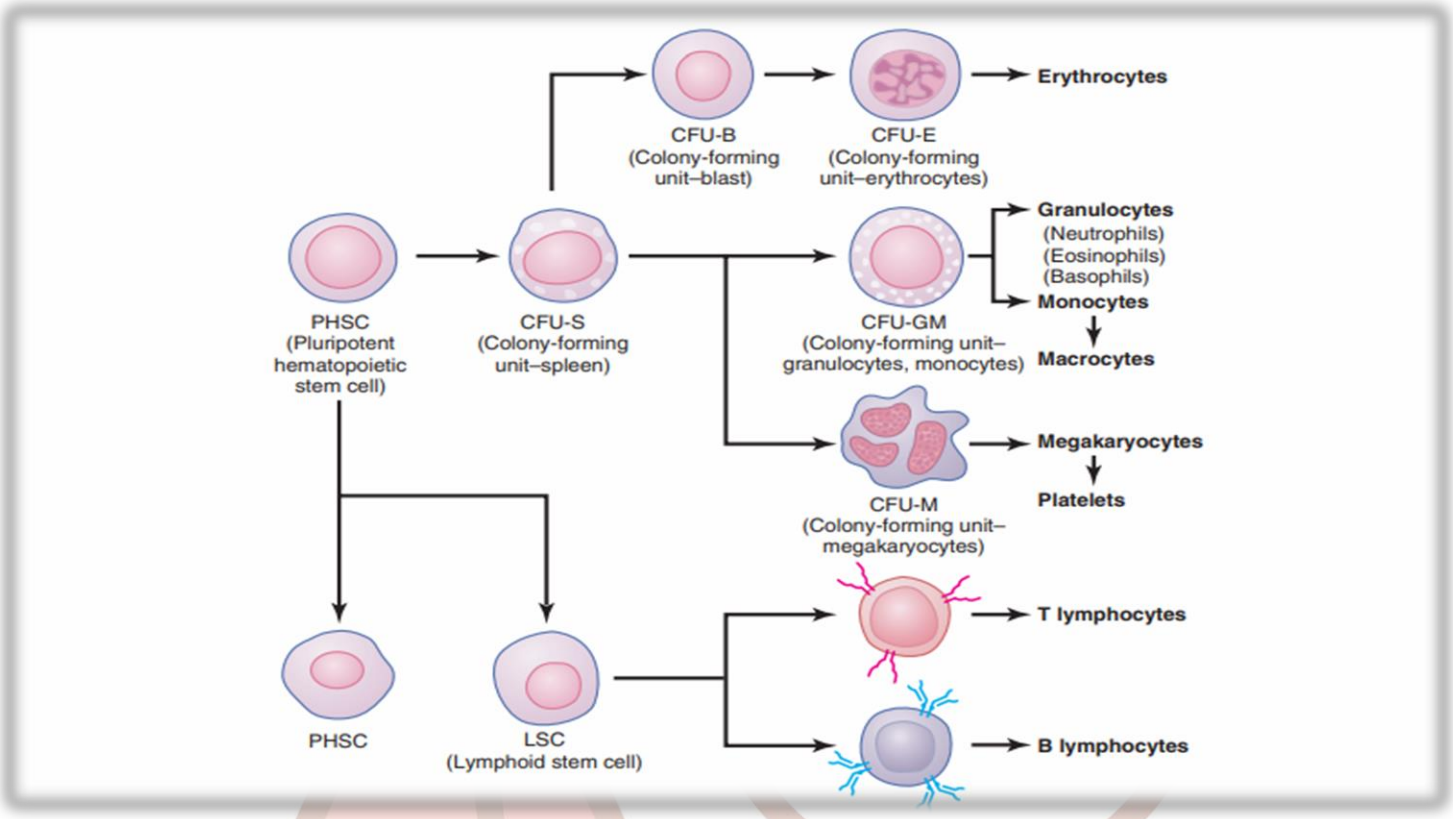


- STEM CELLS (Stem cells are the primary cells capable of self-renewal and differentiating into specialized cells)

Hemopoietic stem cells are the primitive cells in the bone marrow, which give rise to the blood cells .

Hemopoietic stem cells in the bone marrow are called uncommitted **pluripotent hemopoietic stem** cells (PHSC). PHSC is defined as a cell that can give rise to all types of blood cells. In early stages, the PHSC are not designed to form a particular type of blood cell. And it is also not possible to determine the blood cell to be developed from these cells: hence, the name uncommitted PHSC. In adults, only a few number of these cells are present. But the best source of these cells is the umbilical cord blood , When the cells are designed to form a particular type of blood cell, the uncommitted PHSCs are

called committed PHSCs. Committed PHSC is defined as a cell, which is restricted to give rise to one group of blood cells.



- Committed PHSCs are of two types:

1. Lymphoid stem cells (LSC) which give rise to lymphocytes and natural killer (NK) cells

2. Colony forming blastocytes, which give rise to myeloid cells. Myeloid cells are the blood cells other than lymphocytes. When grown in cultures, these cells form colonies hence the name colony forming blastocytes.

- Different units of colony forming cells are:

1)Colony forming unit-erythrocytes (CFU-E) – Cells of this unit develop into erythrocytes

2) Colony forming unit-granulocytes/monocytes (CFU-GM) – These cells give rise to granulocytes (neutrophils, basophils and eosinophils) and monocytes

3) Colony forming unit-megakaryocytes (CFU-M) – Platelets are developed from these cells.

- FACTORS NECESSARY FOR ERYTHROPOIESIS :

Development and maturation of erythrocytes require variety of factors, which are classified into three categories:

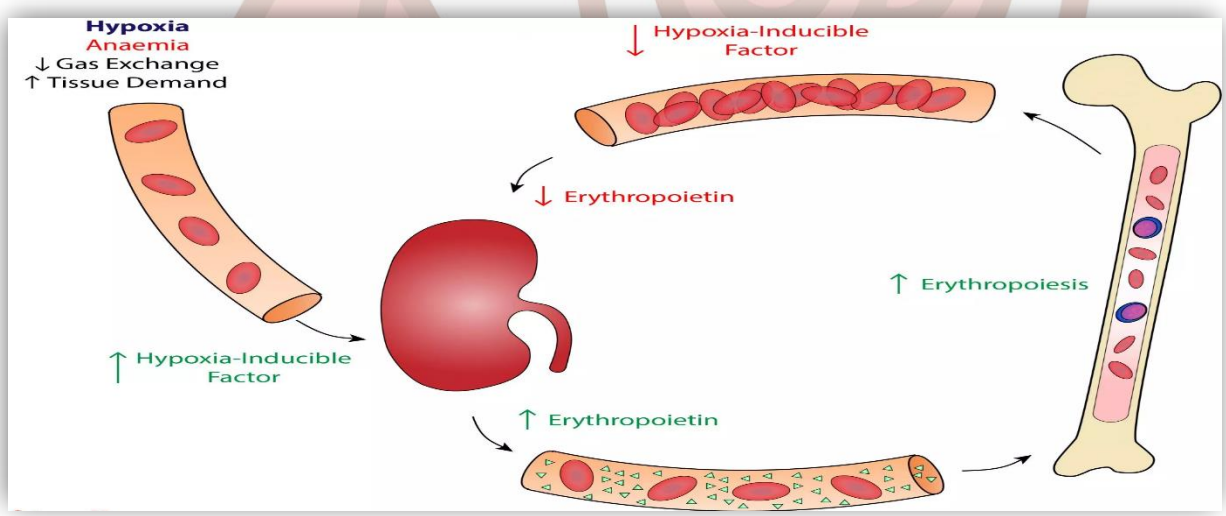
1. General factors

2. Maturation factors

3. Factors necessary for hemoglobin formation

1.General Factors : i. Erythropoietin ii. Thyroxine iii. Hemopoietic growth factors iv. Vitamins

i. **Erythropoietin** → Most important general factor for erythropoiesis is the hormone (Glycoprotein) called erythropoietin. It is also called hemopoietin or erythrocyte stimulating factor , Major quantity of erythropoietin is secreted by peritubular capillaries of kidney. A small quantity is also secreted from liver and brain , Hypoxia is the stimulant for the secretion of erythropoietin .



Actions of erythropoietin : Erythropoietin causes formation and release of new RBCs into circulation. After secretion, it takes 4 to 5 days to show the action. Erythropoietin promotes the following processes:

a. Production of proerythroblasts from CFU-E of the bone marrow

b. Development of proerythroblasts into matured RBCs through the several stages – early normoblast, intermediate normoblast, late normoblast and reticulocyte

c. Release of matured erythrocytes into blood. Even some reticulocytes (immature erythrocytes) are released along with matured RBCs. Blood level of erythropoietin increases in anemia .

ii. **Thyroxine→** Being a general metabolic hormone, thyroxine accelerates the process of erythropoiesis at many levels. So, hyperthyroidism and polycythemia are common.

iii. **Hemopoietic Growth Factors→** Hemopoietic growth factors or growth inducers are the interleukins and stem cell factor (steel factor). Generally, these factors induce the proliferation of PHSCs. Interleukins (IL) are glycoproteins, which belong to the cytokines family .

Interleukins involved in erythropoiesis:

a. Interleukin-3 (IL-3) secreted by T-cells

b. Interleukin-6 (IL-6) secreted by T-cells, endothelial cells and macrophages

c. Interleukin-11 (IL-11) secreted by osteoblast

iv. **Vitamins→** Some vitamins are also necessary for the process of erythropoiesis. Deficiency of these vitamins cause anemia associated with other disorders

a. Vitamin B: Its deficiency causes anemia and pellagra (disease characterized by skin lesions, diarrhea, weakness, nervousness and dementia).

b. Vitamin C: Its deficiency causes anemia and scurvy (ancient disease characterized by impaired collagen synthesis resulting in rough skin, bleeding gum, loosening of teeth, poor wound healing, bone pain, lethargy and emotional changes).

c. Vitamin D: Its deficiency causes anemia and rickets.

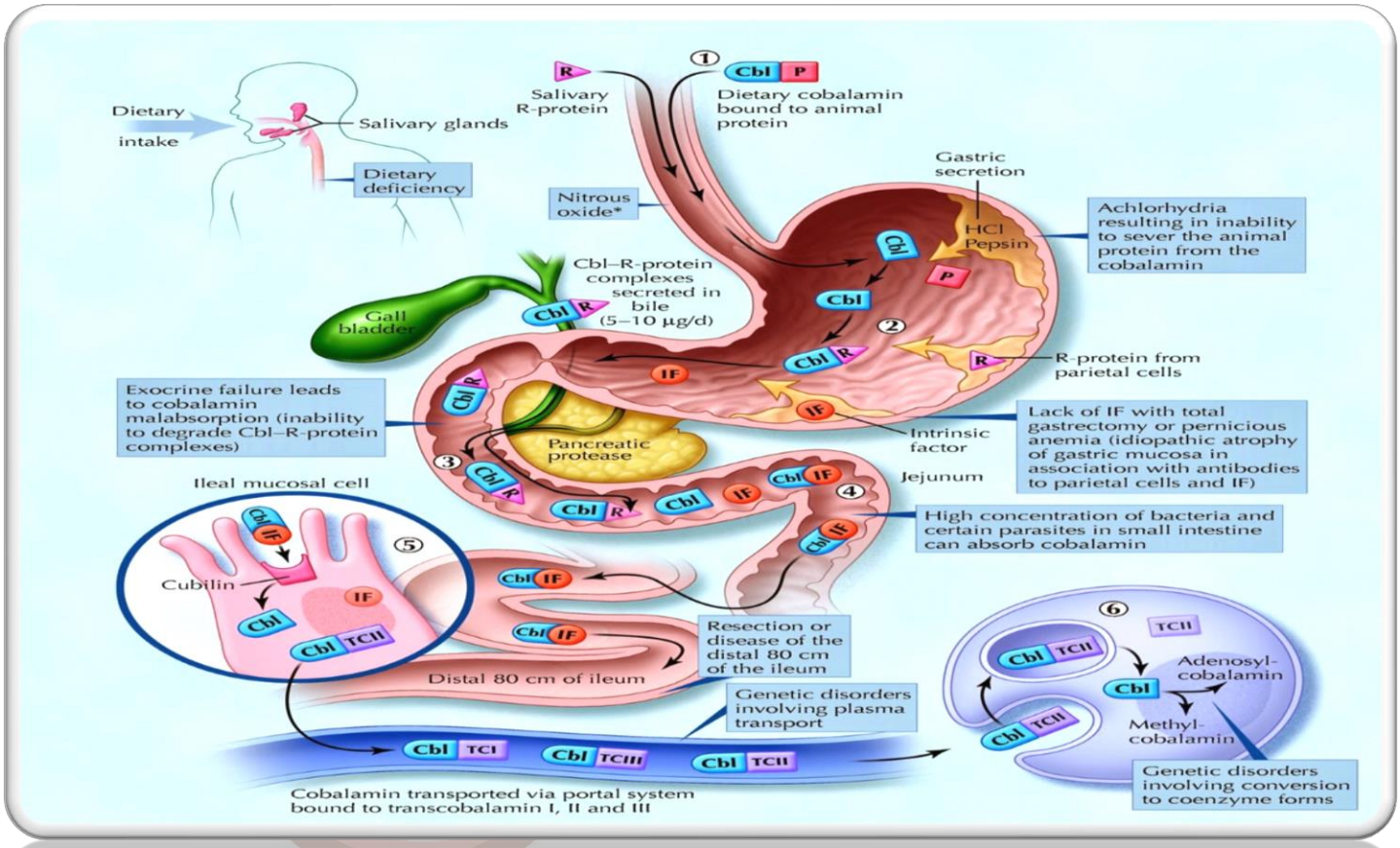
d. Vitamin E: Its deficiency leads to anemia and malnutrition.

-MATURATION FACTORS:

Vitamin B12, intrinsic factor and folic acid are necessary for the maturation of RBCs

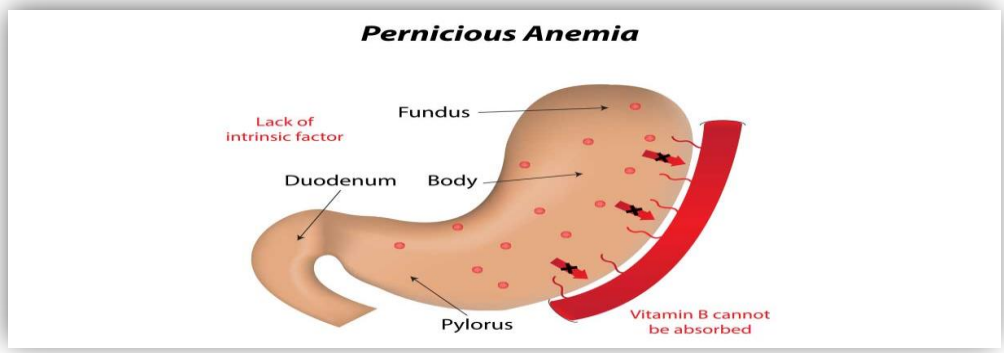
1.**Vitamin B12 (Cyanocobalamin)** :

Source→ Vitamin B12 is called extrinsic factor since it is obtained mostly from diet. Its absorption from intestine requires the presence of intrinsic factor of Castle. Vitamin B12 is stored mostly in liver and in small quantity in muscle. When necessary, it is transported to the bone marrow to promote maturation of RBCs. It is also produced in the large intestine by the intestinal flora.



Action : Vitamin B12 is essential for synthesis of DNA in RBCs. Its deficiency leads to failure in maturation of the cell and reduction in the cell division. Also, the cells are larger with fragile and weak cell membrane resulting in macrocytic anemia

Deficiency of vitamin B12 causes pernicious anemia. So, vitamin B12 is called antipernicious factor .



- **2. Intrinsic Factor of Castle** :

intrinsic factor of castle is produced in gastric mucosa by the parietal cells of the gastric glands. It is essential.

for the absorption of vitamin B12 from the intestine. In the absence of intrinsic factor, vitamin B12 is not absorbed from the intestine. This leads to pernicious anemia. Deficiency of intrinsic factor occurs in:

1-Severe gastritis

2-Ulcer

3-Gatrectomy.(surgical removal of a part or the whole of the stomach.)

Hematinic principle : Hematinic principle is the principle thought to be produced by the action of intrinsic factor on extrinsic factor. It is also called or antianemia principle. It is a maturation factor.

-So, in Summary→

-Causes of vitamin B12 deficiency:

a. Absence of intrinsic factor due to atrophy of the gastric mucosa. The anemia, which develops due to absence of intrinsic factor, is known as pernicious anemia.
b. Liver diseases: as they result in defective storage of the vitamin.

c. Disease or surgical resection of terminal ileum.

d. Very rarely there is deficient vitamin B12 in the diet.

- Effect of vitamin B12 deficiency:

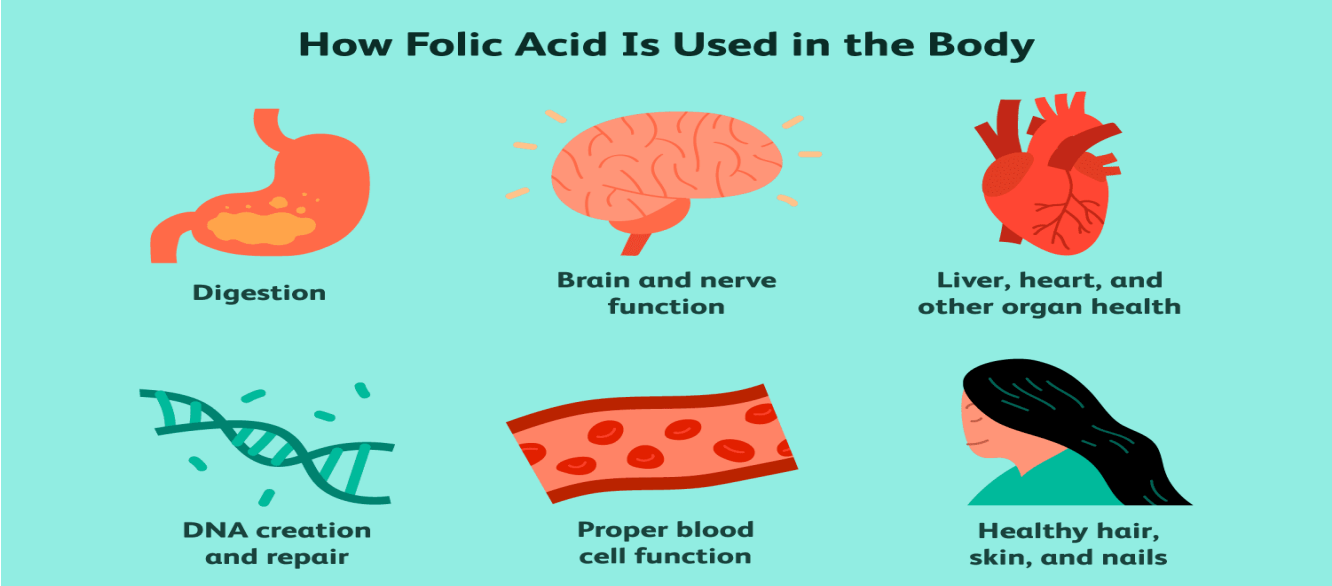
a. Failure of nuclear maturation and division of erythroblasts in bone marrow. Therefore, erythroblasts **increase in size** and develop into megaloblasts and megalocytes. They are larger in size, contain a larger amount of hemoglobin and have a shorter life span than erythrocytes. Therefore, the anemia, which develops due to vitamin B12 deficiency, is also called megaloblastic or Macrocytic anemia.

b. Neurological symptoms: Since vitamin B12 is essential for the metabolism of **the myelin sheath of nerves**, its deficiency causes neurological manifestations.

-Treatment of vitamin B12 deficiency: Vitamin B12 must be given by injection for life

-**3. Folic Acid**:

-Folic acid is also essential for maturation. It is required for the synthesis of DNA. In the absence of folic acid, the synthesis **of DNA decreases causing failure of maturation**. This leads to anemia in which the cells are larger and appear in megaloblastic (proerythroblastic) stage. And anemia due to folic acid deficiency is called megaloblastic anemia.



-Causes of folic acid deficiency:

a. Dietary deficiency of folic acid

b. GIT diseases interfere with folic acid absorption.

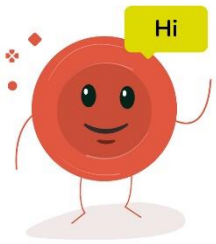
c. Cytotoxic drugs (antifolates) used in treatment of cancer.

-Hemoglobin and Iron Metabolism

- INTRODUCTION :

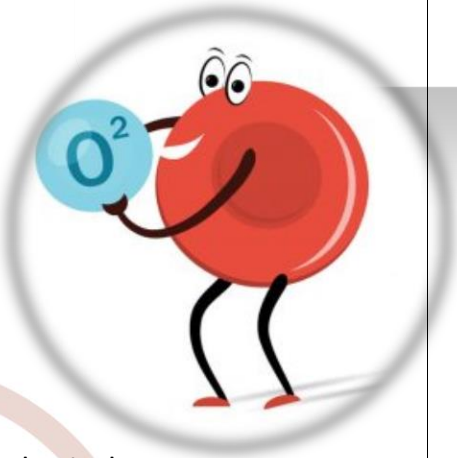
Hemoglobin (Hb) is the iron containing coloring matter of red blood cell (RBC).

It is a chromoprotein forming 95% of dry weight of RBC and 30% to 34% of wet weight. Function of hemoglobin is to carry the respiratory gases, oxygen and carbon dioxide. It also acts as a buffer



- NORMAL HEMOGLOBIN CONTENT:

Average hemoglobin (Hb) content in **blood is 14 to 16 g/dL**. However, varies depending upon the age and sex of the individual.



- FUNCTIONS OF HEMOGLOBIN:

TRANSPORT OF RESPIRATORY GASES→

Main function of hemoglobin is the transport of respiratory.

gases:

1. Oxygen from the lungs to tissues.

2. Carbon dioxide from tissues to lungs.

-Transport of Oxygen→ When oxygen binds with hemoglobin, a physical process called oxygenation occurs, resulting in the formation of oxyhemoglobin. The iron remains in ferrous state in this compound. Oxyhemoglobin is an unstable compound, and the combination is reversible, i.e. when more oxygen is available, it combines with hemoglobin and whenever oxygen is required, hemoglobin can release oxygen readily. When oxygen is released from oxyhemoglobin, it is called reduced hemoglobin or ferrohemoglobin

- When carbon dioxide binds with hemoglobin, carbhemoglobin is formed. It is also an unstable compound, and the combination is reversible, i.e. the carbon dioxide can be released from this compound. The affinity of hemoglobin for carbon dioxide is 20 times more than that for oxygen.

BUFFER ACTION→

Hemoglobin acts as a buffer and plays an important role in acid base balance

-STRUCTURE OF HEMOGLOBIN:

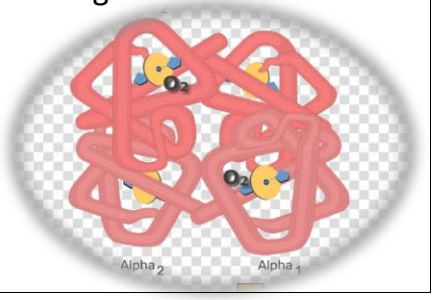
Hemoglobin is a conjugated protein. It consists of a protein combined

with an iron containing pigment. The protein part is globin and the

iron containing pigment is heme. Heme also forms a part of the

structure of myoglobin (oxygenbinding pigment in muscles) and neuroglobin

(oxygenbinding pigment in brain).



IRON→ Normally, it is present in ferrous (Fe2+) form. It is in unstable or loose form. In some abnormal conditions, the iron is converted into ferric (Fe3+) state, which is a stable form .

-Functions of iron: Iron is important for the formation of hemoglobin and myoglobin. Iron is also necessary for the formation of other substances like cytochrome, cytochrome oxidase, peroxidase and catalase.

- NORMAL VALUE AND DISTRIBUTION OF IRON IN THE BODY: Total quantity of iron in the **body is about 4 g**. Ap proximate distribution of iron in the body is as follows:

70 % in hemoglobin

3 % in myoglobin

1 % in oxidative enzymes

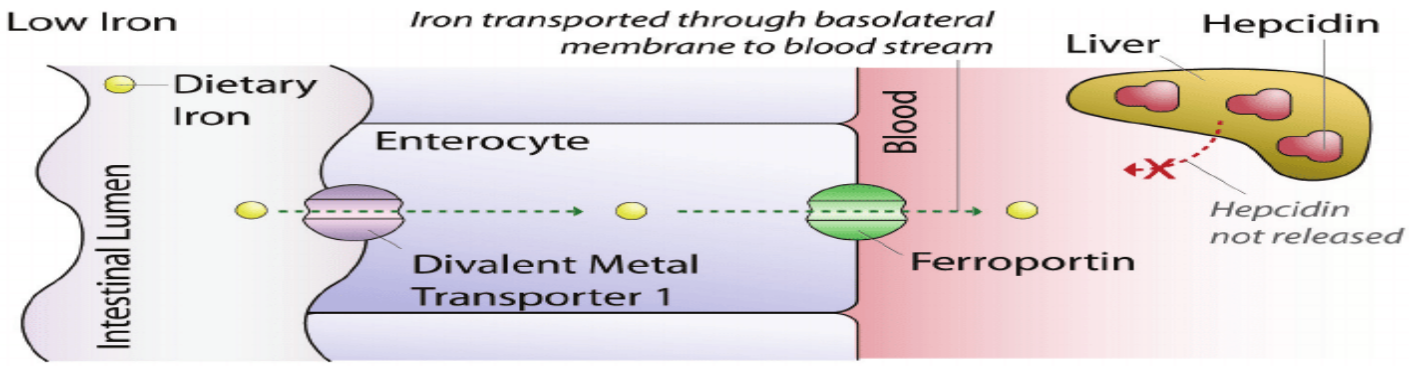
26 % stored in liver and spleen .

- Iron absorption, transport and storage: -

Absorption of Iron from the Intestinal Tract→ Iron absorption occurs in the

upper part of the small intestine by an active process, mostly by the following mechanism.

the liver secretes moderate amounts **of apotransferrin into the bile**, which flows through the bile duct into the duodenum. Here, the apotransferrin binds with free iron and also with certain iron compounds, such as hemoglobin and myoglobin from meat, two of the most important sources of iron in the diet. this combination is called transferrin. It, in turn, is attracted to and binds with receptors in the membranes of the intestinal epithelial cells. then, by pinocytosis, the transferrin molecule, carrying its iron store, is absorbed into the epithelial cells and later released into the blood capillaries beneath these cells in the form of **plasma transferrin.**



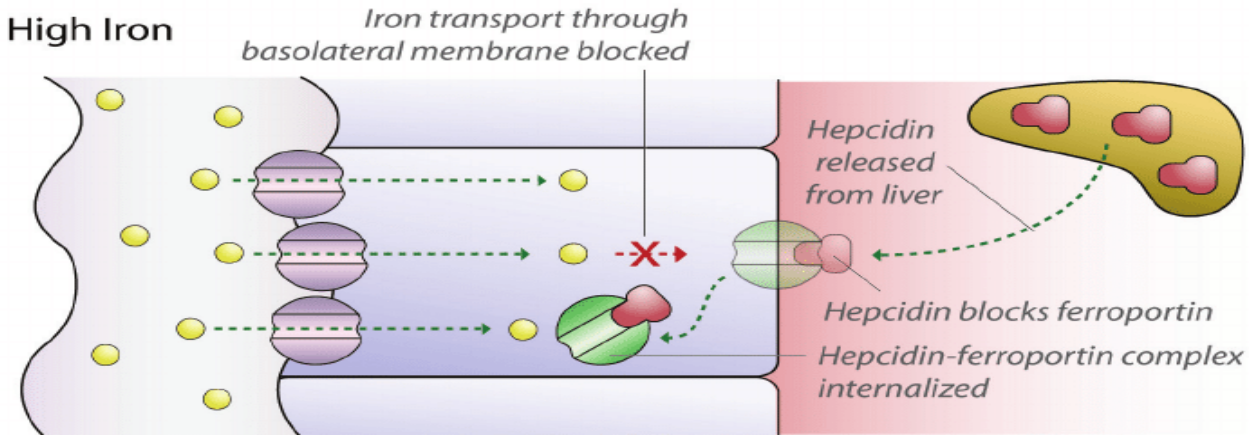
- Iron absorption from the intestines is extremely slow, at a maximum rate of only a few milligrams per day. this slow rate of absorption means that even when tremendous quantities of iron are present in the food, only small proportions can be absorbed .

- Iron is present mostly in ferric (Fe3+) form**. It is converted into ferrous form (Fe2+) which is absorbed into the blood.** *Hydrochloric acid from gastric juice* makes the ferrous iron soluble so that it could be converted into ferric iron by the enzyme ferric reductase from enterocytes. From enterocytes, ferric iron is transported into blood by a ferroportin. In the blood**, ferric iron is converted into ferrous iron and transported.**

- Regulation of iron absorption: When the body has become saturated with iron so that essentially all apoferritin in the iron storage areas is already combined with iron, the rate of additional iron absorption from the intestinal tract becomes greatly decreased **by decreases the amount of DMT1 on enterocytes** . Conversely, when the iron stores have become depleted, the rate of absorption can accelerate probably five or more times normal. thus, total body iron is regulated mainly by altering the rate of absorption.

Role of Hepcidin: - Hepcidin is a 25-amino acid hormone secreted by the liver. Hepcidin is a major regulator of iron intestinal absorption and iron release by macrophages , **Hepcidin binds to iron export protein ferroportin present in duodenal enterocytes**, macrophages, and liver cells. Such binding is followed by degradation of ferroportin molecules. This leads to:

1- Inhibition of intestinal absorption of iron
2- inhibition of release of recycled iron from macrophages
3- Inhibition of release of iron from liver and other store sites

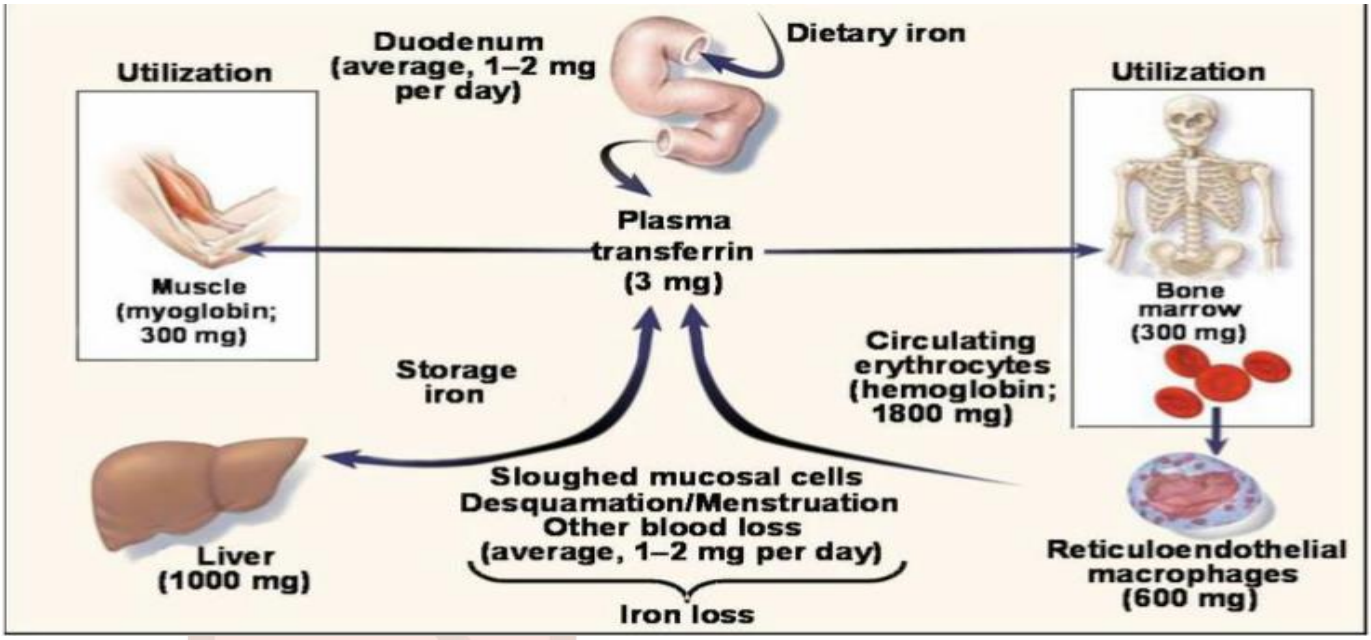


- Factors affecting hepcidin secretion:

- **Hypoxia and erythropoietin hormone decreases hepcidin secretion**. In this way, iron absorption and release are increased to supply the increased demand by accelerated erythropoiesis for iron .

- Iron loading increases hepcidin secretion by the liver

- Inflammation increases hepcidin secretion. This explains why anemia is a common complication of many inflammatory diseases.



- STORAGE OF IRON: Iron is stored in large quantities in reticuloendothelial cells and liver hepatocytes. In other cells also it is stored in small quantities. In the cytoplasm of the cell, iron is stored as ferritin in large amount. Small quantity of iron is also stored as hemosiderin.

- DAILY LOSS OF IRON:

In males, about 1 mg of iron is excreted everyday through feces. In females, the amount of iron loss is very much high. This is because of the menstruation .

- Hemosiderosis :

It is due to excess hemosiderin deposits in tissues in conditions of iron overload. These excess deposits may lead to pigmentation of the skin, pancreatic damage **leading to diabetes (bronze diabetes),** liver cirrhosis and high incidence of hepatic carcinoma. These diseases are typically diseases in which chronic blood

loss requires frequent blood transfusions, such as sickle cell anemia and thalassemia, though beta thalassemia minor has been associated with hemosiderin deposits in the liver in those with non-alcoholic fatty liver disease independent of any transfusion

- Effect of iron deficiency: Iron deficiency anemia Caused by →

i**. Decreased iron intake in diet** which may occur: In infants fed milk only, as milk is poor in iron and In females during pregnancy and lactation

ii. **Failure of iron absorption** which may be due to: Partial gastrectomy (insufficient HCl secretion) , Diseases of upper small intestine , Vitamin C deficiency and too much Phytic acid, oxalates and phosphates in diet .

iii. **Chronic blood loss It results in iron deficiency**, as the iron stores are insufficient and dietary iron cannot compensate for the amount of iron lost. It occurs in:

- Excessive bleeding during menstruation in females.

-Bleeding peptic ulcer and piles.

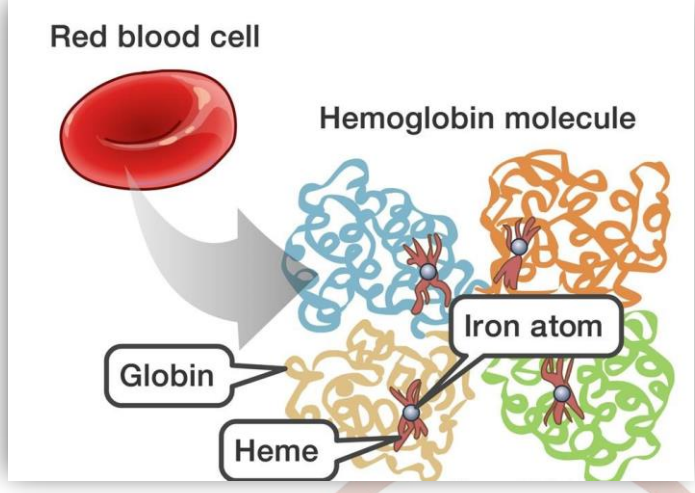
- Parasitic infestation.

-FACTORS NECESSARY FOR HEMOGLOBIN FORMATION:

- Various materials are essential for the formation of hemoglobin in the RBCs. Deficiency of these substances decreases the production of hemoglobin leading to anemia. Such factors are→

1. First class proteins and amino acids: Proteins of high biological value are essential for the formation of hemoglobin. Amino acids derived from these

proteins are required for the synthesis of protein part of hemoglobin, i.e. the globin .



2. Iron: Necessary for the formation of heme part of the hemoglobin.

3. Copper: Necessary for the absorption of iron from the gastrointestinal tract.

4. Cobalt and nickel: These metals are essential for the utilization of iron during hemoglobin formation

5. Vitamins: Vitamin C, riboflavin, nicotinic acid and pyridoxine are also essential for the formation of hemoglobin .

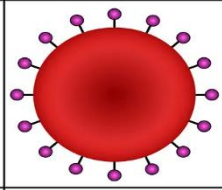
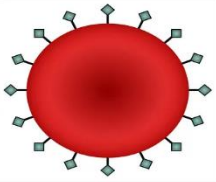
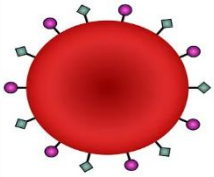
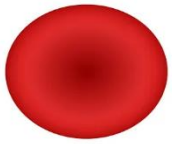
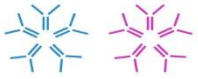
- Blood Groups

- INTRODUCTION:

When blood transfusions from one person to another were first attempted, immediate or delayed agglutination and hemolysis of the red blood cells (RBCs) often occurred, resulting in typical transfusion reactions that frequently led to death. Soon it was discovered that the bloods of different people have different antigenic and immune properties so that antibodies in the plasma of one blood type will react with antigens on the surfaces of the RBCs of another blood type. If proper precautions are taken, one can determine ahead of time whether the antibodies and antigens present in the donor and recipient bloods will cause a transfusion reaction.

- ABO BLOOD GROUPS:

Determination of ABO blood groups depends upon the immunological reaction between antigen and antibody. Landsteiner found two antigens on the surface of RBCs and named them as A antigen and B antigen. **These antigens are also called agglutinogens because of their capacity to cause agglutination of RBCs**. He noticed the corresponding antibodies or agglutinins in the plasma and named them anti-A or α-antibody and anti-B or β-antibody. However, a particular agglutinogen and the corresponding agglutinin cannot be present together. If present, it causes clumping of the blood. Based on this, Karl Landsteiner classified the blood groups. Later it became **the 'Landsteiner Law'** for grouping the blood.

ABO Blood Group System				
Group	A	B	AB	O
Red Blood Cell Type				
Antigens Present	 Antigen A	 Antigen B	 Antigen A & B	None
Antibodies Present	 Anti-B	 Anti-A	None	 Anti-A & Anti-B

Because each person has only two sets of chromosomes, only one of these alleles is present on each of the two chromosomes in any individual. However, the presence of three different alleles means that there are six possible combinations of alleles, as shown in: OO, OA, OB, AA, BB, and AB. these combinations of alleles are known as the genotypes, and each person is one of the six genotypes .

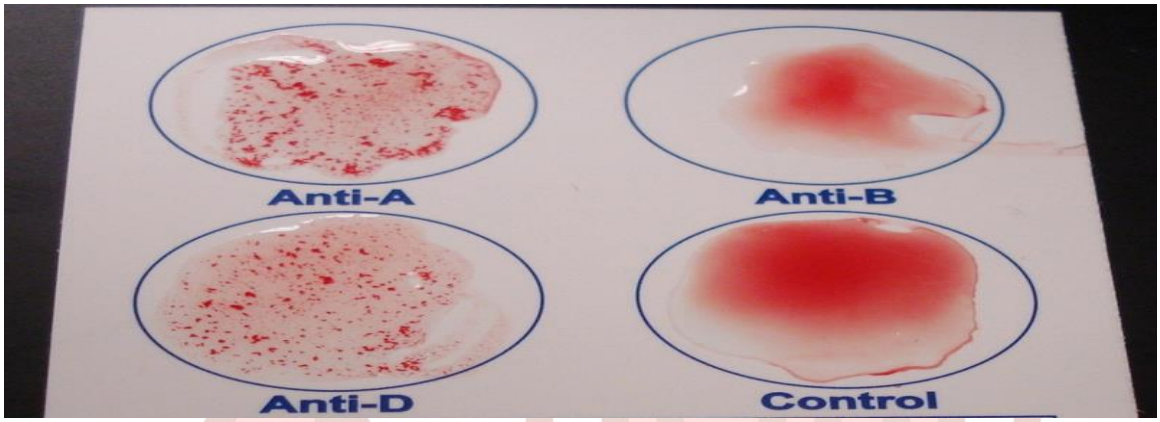
-Relative Frequencies of the Different Blood Types. the prevalence of the different blood types among one group of persons studied was approximately:

O	47%
A	41%
B	9%
AB	3%

-LANDSTEINER LAW:

Landsteiner law states that→ If a particular **agglutinogen** (antigen) is present in the RBCs, corresponding agglutinin (antibody) must be absent in the serum.

And If a particular agglutinogen is absent in the RBCs, the corresponding agglutinin must be present in the serum BUT in fact, it is not applicable to Rh factor.



- DETERMINATION OF ABO GROUP:

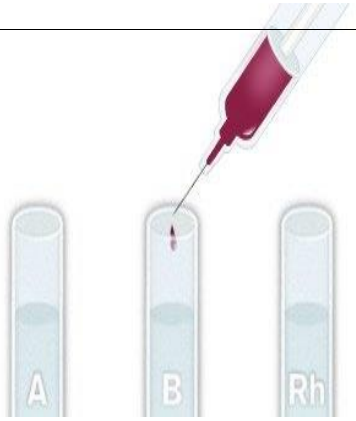
Determination of the ABO group is also called blood grouping, blood typing or blood matching.

Blood typing is done on the basis of agglutination. Agglutination means the collection of separate particles like RBCs into clumps or masses.

Agglutination occurs if an antigen is mixed with its corresponding antibody which is called isoagglutinin. Agglutination occurs when A antigen is mixed with anti-A or when B antigen is mixed with anti-B

Requisites for Blood Typing→ To determine the blood group of a person, a suspension of his RBC and testing antisera are required. Suspension of RBC is prepared by mixing blood drops with isotonic saline (0.9%). Test sera are:

1. Antiserum A, containing anti-A or α-antibody.
2. Antiserum B, containing anti-B or β-antibody

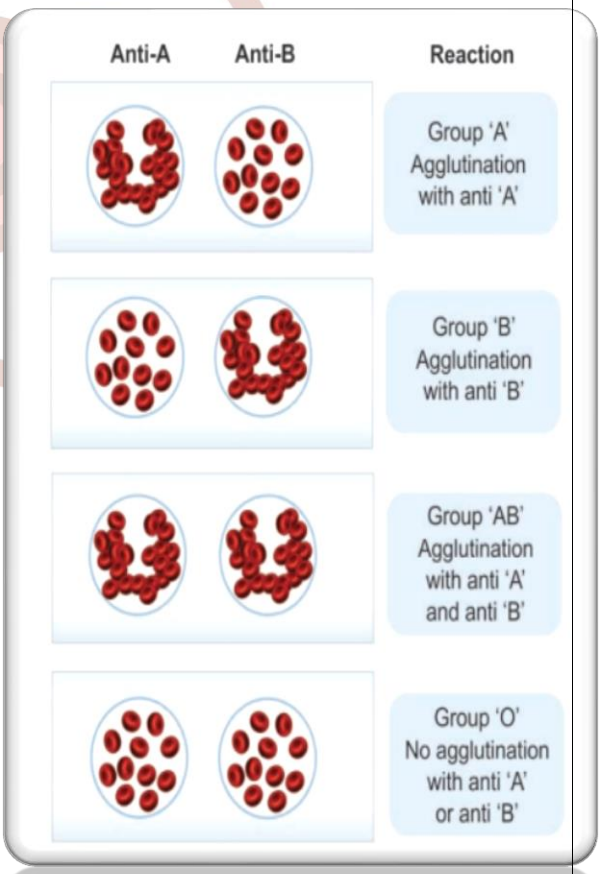


Procedure→ 1. One drop of antiserum A is placed on one end of a glass slide (or a tile) and one drop of antiserum B on the other end .

2. One drop of RBC suspension is mixed with each antiserum. The slide is slightly rocked for 2 minutes. The presence or absence of agglutination is observed by naked eyes and if necessary, it is confirmed by using microscope.

3. Presence of agglutination is confirmed by the presence of thick masses (clumping) of RBCs

4. Absence of agglutination is confirmed by clear mixture with dispersed RBCs



Results→ 1. If agglutination occurs with antiserum A: The antiserum A contains α-antibody. The agglutination occurs if the RBC contains A antigen. **So, the blood group is A**

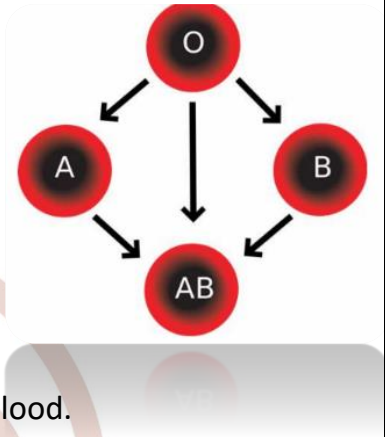
2. If agglutination occurs with antiserum B: The antiserum **B contains β-antibody**. The agglutination occurs if the RBC contains B antigen. So**, the blood group is B .**

3. If agglutination occurs with both antisera A and B: The RBC contains both A and B antigens to cause agglutination. And, **the blood group is AB.**

4. If agglutination does not occur either with antiserum A or antiserum B: The agglutination does not occur because RBC does not contain any antigen. **The blood group is O.**

- IMPORTANCE OF ABO GROUPS IN BLOOD TRANSFUSION:

During blood transfusion, only compatible blood must be used. The one who gives blood is called the **'donor'** and the one who receives the blood is called **'recipient'**. While transfusing the blood, the antigen of the donor and the antibody of the recipient are considered. The antibody of the donor and antigen of the recipient are ignored mostly.



Thus, RBC of 'O' group has no antigen and so agglutination does not occur with any other group of blood. So, 'O' group blood can be given to any blood group persons and the people with this blood group are called 'universal donors .

Plasma of AB group blood has no antibody.

This does not cause agglutination of RBC from any other group of blood.

People with **AB group can receive** blood from any blood group persons. So, people with this blood group are called 'universal recipients' .

NOTE→ For blood matching, RBC of the individual (recipient) and test sera are used. Cross-matching is done by mixing the serum of the recipient and the RBCs of donor. Cross-matching is always done before blood transfusion. If agglutination of RBCs from a donor occurs during cross-matching, the blood from that person is not used for transfusion.

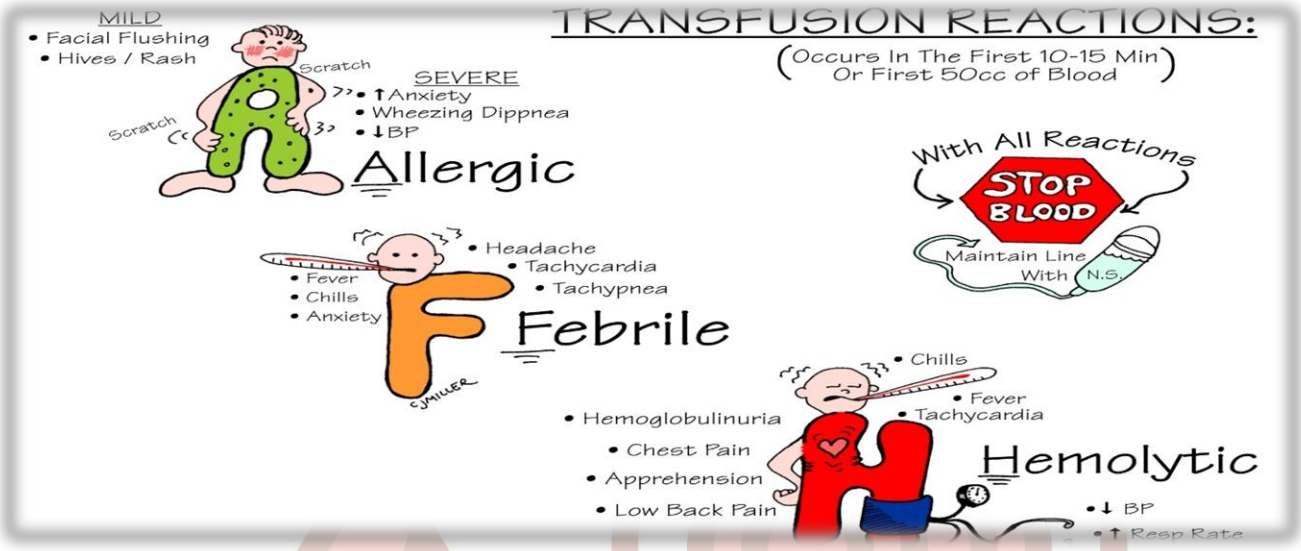
Matching = Recipient's RBC + Test sera.

Cross-matching = Recipient's serum + Donor's RBC.

- TRANSFUSION REACTIONS DUE TO ABO INCOMPATIBILITY:

Transfusion reactions are the adverse reactions in the body, which occur due to transfusion error that involves transfusion of incompatible (mismatched) blood. The reactions may be mild causing only fever and hives (skin disorder characterized by itching) or may be severe leading to renal failure, shock and death

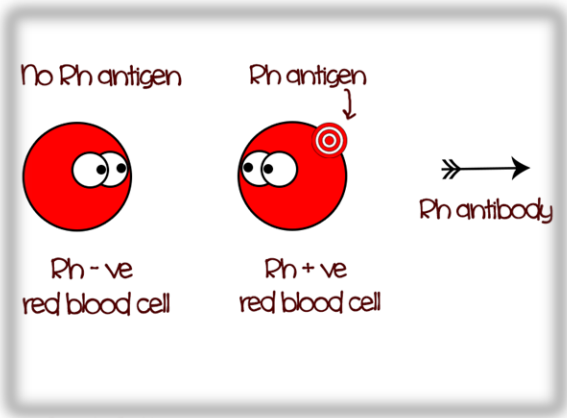
Cause for Transfusion Reactions→ Transfusion of incompatible blood produces hemolytic reactions. The recipient's antibodies (IgG or IgM) adhere to the donor RBCs, which are agglutinated and destroyed. Large amount of free hemoglobin is liberated into plasma. This leads to transfusion reactions.



-Rh FACTOR:

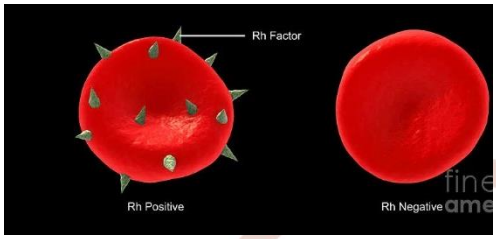
Along with the O-A-B blood type system, the Rh blood type system is also important when transfusing blood. the major difference between the O-A-B system and the Rh system is the following: In the O-A-B system, the plasma agglutinins responsible for causing transfusion reactions develop spontaneously, **whereas in the Rh system, spontaneous agglutinins almost never occur. Instead, the person must first be massively exposed to an Rh antigen**, such as by transfusion of blood containing the Rh antigen before enough agglutinins to cause a significant transfusion reaction will develop.

Rh factor is an antigen present in RBC. This antigen was discovered by Landsteiner and Wiener. It was first discovered in **Rhesus monkey** and hence the name 'Rh factor'. There are many Rh antigens but only the D antigen is more antigenic in human.



if Rh positive blood is transfused to a Rh-negative person anti-D is developed in that person. On the other hand, there is no risk of complications if the Rh positive person receives Rh negative blood.

-The persons having D antigen are called ‘Rh positive’ and those without D antigen are called ‘Rh negative’. Among Indian population, 85% of people are Rh positive and 15% are Rh negative. Percentage of Rh positive people is more among black people.



-INHERITANCE OF Rh ANTIGEN:

Rhesus factor is an inherited dominant factor. It may be homozygous Rhesus positive with DD or heterozygous Rhesus positive with Dd . Rhesus negative occurs only with complete absence of D

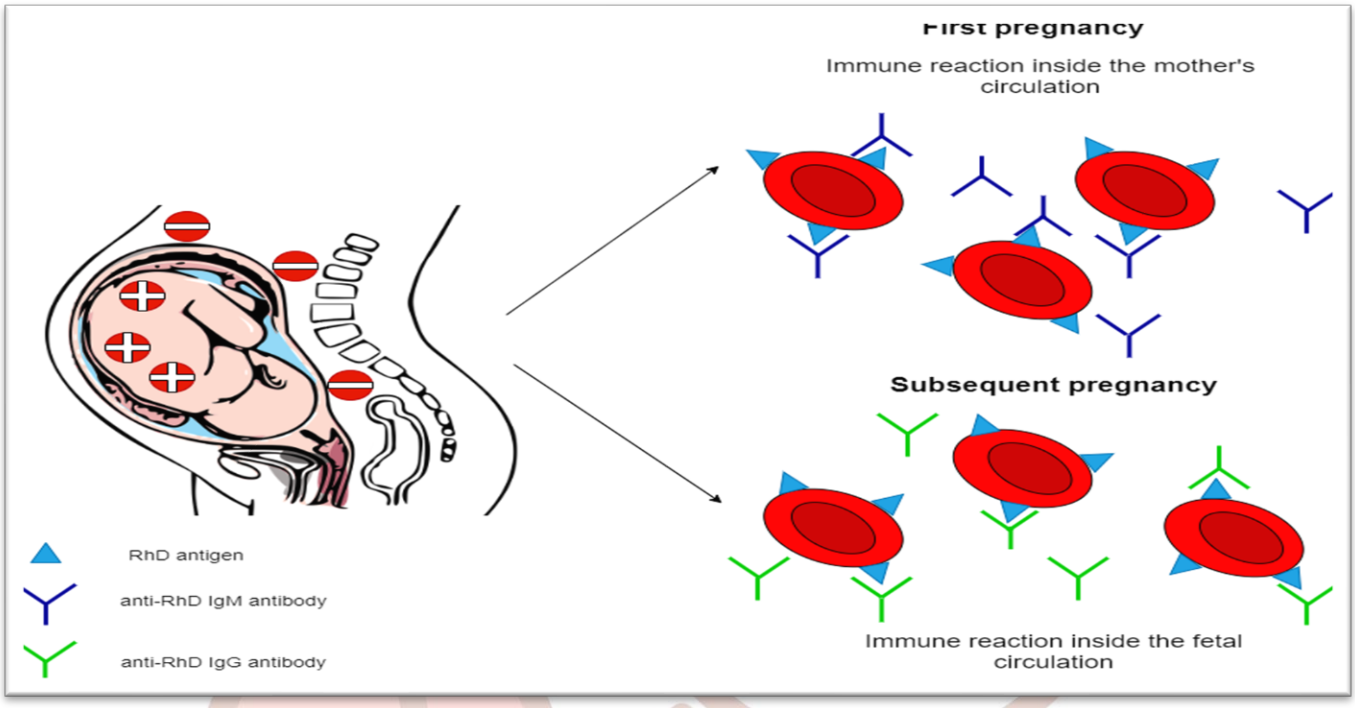
- Rh IMMUNE RESPONSE:

Formation of Anti-Rh Agglutinins. When RBCs containing Rh factor are injected into a person whose blood does not contain the Rh factor—that is, into an Rh-negative person—anti-Rh agglutinins develop slowly, reaching maximum concentration of agglutinins about 2 to 4 months later. "is immune response occurs to a much greater extent in some people than in others. With multiple exposures to the Rh factor, an Rh-negative person eventually becomes strongly “sensitized” to Rh factor.

- Importance of Rh factors:

1) Erythroblastosis Fetalis (“Hemolytic Disease of the Newborn”)→
Erythroblastosis fetalis is a disease of the fetus and newborn child characterized by agglutination and phagocytosis of the fetus’s RBCs. In most instances of erythroblastosis fetalis, the mother is Rh negative and the father is Rh positive. the baby has inherited the Rh-positive antigen from the father, and the mother develops anti-Rh agglutinins from exposure to the fetus’s Rh antigen. In turn, the mother’s agglutinins diffuse through the placenta into the fetus and cause RBC

- **Incidence of the Disease**→ An Rh-negative mother having her first Rh-positive child usually does not develop sufficient anti-Rh agglutinins to cause any harm. However, about 3 percent of second Rh-positive babies exhibit some signs of erythroblastosis fetalis; about 10 percent of third babies exhibit the disease; and the incidence rises progressively with subsequent pregnancies.

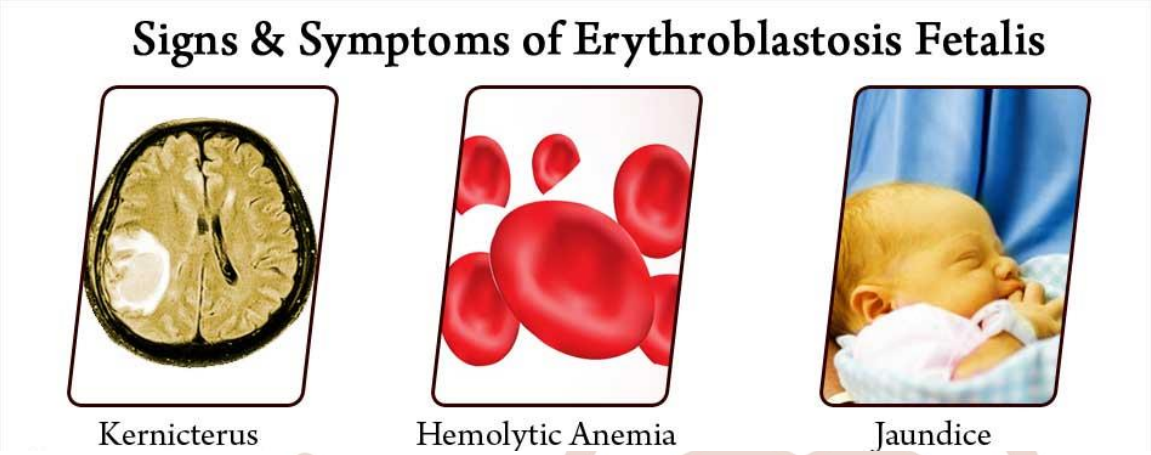


-Effect of the Mother's Antibodies on the Fetus→ After anti-Rh antibodies have formed in the mother, they diffuse slowly through the placental membrane into the fetus's blood. there they cause agglutination of the fetus's blood. the agglutinated RBCs subsequently hemolyze, releasing hemoglobin into the blood. the fetus's macrophages then convert the hemoglobin into bilirubin, which causes the baby's skin to become yellow (jaundiced). the antibodies can also attack and damage other cells of the body.

- Clinical Picture of Erythroblastosis→ the jaundiced, erythroblastosis newborn baby is usually anemic at birth, and the anti-Rh agglutinins from the mother usually circulate in the infant's blood for another 1 to 2 months after birth, destroying more and more RBCs.

the hematopoietic tissues of the infant attempt to replace the hemolyzed RBCs. the liver and spleen become greatly enlarged and produce RBCs in the same manner that they normally do during the middle of gestation. Because of the rapid production of RBCs, many early forms of RBCs, including many nucleated

blastic forms, are passed from the baby's bone marrow into the circulatory system, and it is because of the presence of these nucleated blastic RBCs that the disease is called erythroblastosis fetalis.



Although **the severe anemia of erythroblastosis** fetalis is usually the cause of death, many children who barely survive the anemia exhibit permanent mental impairment or damage to motor areas of the brain because of precipitation of bilirubin in the neuronal cells, causing destruction of many, a condition called **kernicterus** (is the form of brain damage in infants caused by severe jaundice.)

- Treatment of Neonates with Erythroblastosis Fetalis→ One treatment for erythroblastosis fetalis is to replace the neonate's blood with Rh-negative blood. About 400 milliliters of Rh-negative blood are infused over a period of 1.5 or more hours while the neonate's own Rh-positive blood is being removed.

- Prevention of Erythroblastosis Fetalis→ Prevention involves giving Rh-negative mothers Rho(D) immune globulin at the following times:

- At 28 weeks gestation
- Within 72 hours of pregnancy termination
- After any episode of vaginal bleeding
- After amniocentesis or chorionic villus sampling

Manual removal of the placenta should be avoided because it may force fetal cells into maternal circulation.

Maternal sensitization and antibody production due to Rh incompatibility can be prevented by giving the woman Rho(D) immune globulin. This preparation contains high titers of anti-Rh antibodies, which neutralize Rh-positive fetal RBCs.

2**) TRANSFUSION REACTIONS RESULTING FROM MISMATCHED BLOOD TYPES**→
If an Rh negative person is transferred with Rh positive blood, he will produce agglutinins against Rh factor. If after sometime, this person is transfused again with Rh positive blood, agglutination occurs.

- IMPORTANCE OF KNOWING BLOOD GROUP:

Nowadays, knowledge of blood group is very essential medically, socially, and judicially. The importance of knowing blood group is:

1. Medically, it is important during blood transfusions and in tissue transplants.

2. Socially, one should know his or her own blood group and become a member of the Blood Donor's Club so that he or she can be approached for blood donation during emergency conditions.

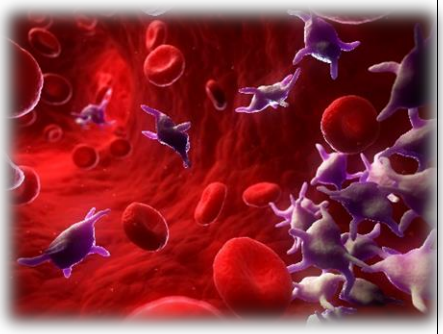
3. It general among the couples, knowledge of blood groups helps to prevent the complications due to Rh incompatibility and save the child from the disorders like erythroblastosis fetalis.

4. Judicially, it is helpful in medico-legal cases to sort out parental disputes.

-we Almost there !!!!!!!

- Platelets

- INTRODUCTION:

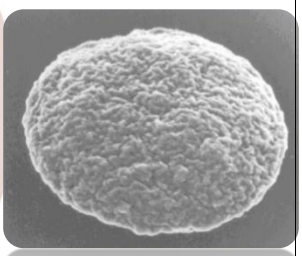


Platelets or thrombocytes are the formed elements of blood.

Platelets are small colorless, non-nucleated and moderately refractive bodies. These formed elements of blood are considered to be the fragments of cytoplasm.

- Shape of Platelets:

Normally, platelets are of several shapes, viz. spherical or rod-shaped and become oval or disk-shaped when inactivated. Sometimes, the platelets have dumbbell shape, comma shape, cigar shape or any other unusual shape. Inactivated platelets are without processes or filopodia and the activated platelets develop processes or filopodia.

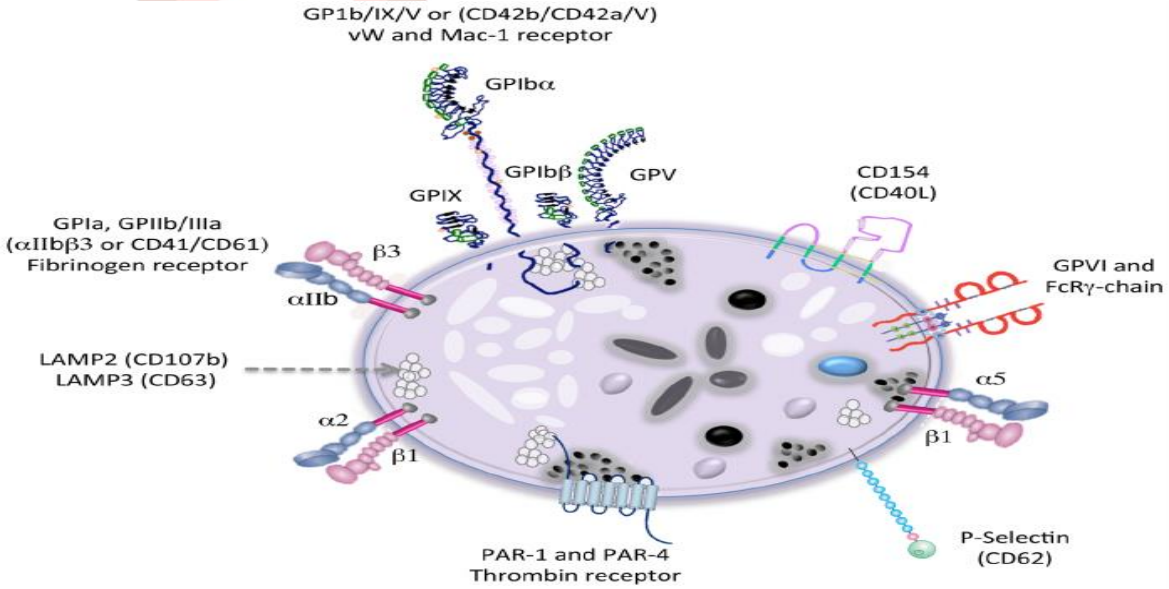


- STRUCTURE AND COMPOSITION:

Platelet is constituted by:

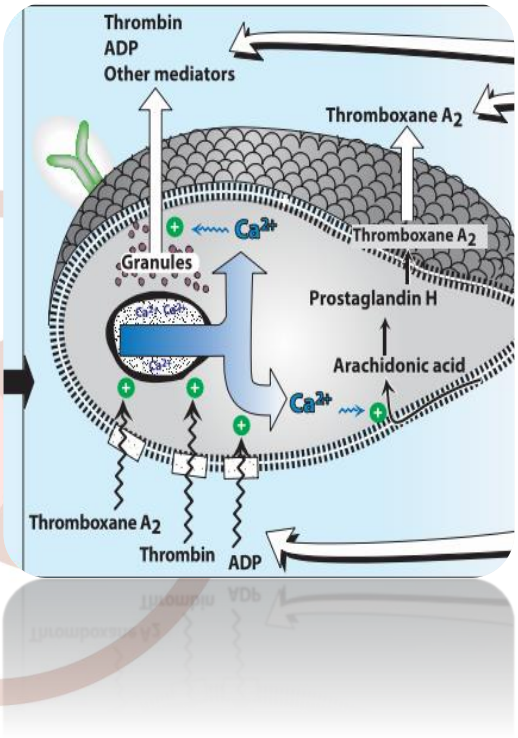
1.Cell membrane or surface membrane 2. Microtubules 3. Cytoplasm

-CELL MEMBRANE→ Cell membrane of platelet contains lipids in the form of phospholipids, cholesterol and glycolipids, carbohydrates as glycocalyx and glycoproteins and proteins. Of these substances, glycoproteins and phospholipids are functionally important.



-**Glycoproteins** prevent the adherence of platelets to normal endothelium, but accelerate the adherence of platelets to collagen and damaged endothelium in ruptured blood vessels. Glycoproteins also form the receptors for adenosine diphosphate (ADP) and thrombin.

- Phospholipids Phospholipids accelerate the clotting reactions. The phospholipids form the precursors of **thromboxane A2** and other **prostaglandin**-related substances



-**Microtubules** form a ring around cytoplasm below the cell membrane. Microtubules are made up of polymerized proteins called tubulin. These tubules provide structural support for the inactivated platelets to maintain the disklike shape.

- **Cytoplasm of platelets** **contains** the cellular organelles, Golgi apparatus, endoplasmic reticulum, mitochondria, microtubule, micro vessels, filaments and granules. Cytoplasm also <u>contains some chemical substances such as proteins, enzymes, hormonal substances</u>, etc

-Proteins→ 1)Contractile proteins:

i. Actin and myosin: Contractile proteins, which are responsible for contraction of platelets.

ii. Thrombosthenin: Third contractile protein, which is responsible for clot retraction.

2) **von Willebrand factor**: Responsible for adherence of platelets and regulation of plasma level of factor VIII.

3) **Fibrin-stabilizing factor**: A clotting factor

4) **Platelet-derived growth factor (PDGF)**: Responsible for repair of damaged blood vessels and wound healing. It is a potent mytogen (chemical agent that promotes mitosis) for smooth muscle fibers of blood vessels.

5) **Platelet-activating factor (PAF):** Causes aggregation of platelets during the injury of blood vessels, resulting in prevention of excess loss of blood.

-Enzymes→ 1. Adensosine triphosphatase (ATPase)

2. Enzymes necessary for synthesis of prostaglandins.

-Hormonal Substances→ 1. Adrenaline 2. 5-hydroxytryptamine (5-HT; serotonin) 3. Histamine

- Other Chemical Substances→ 1. Glycogen

2. Substances like blood group antigens

3. Inorganic substances such as calcium, copper, magnesium and iron.

- Platelet Granules→ Granules present in cytoplasm of platelets are of two types:
A. Alpha granules (contain: 1. Clotting factors – fibrinogen, V and XIII

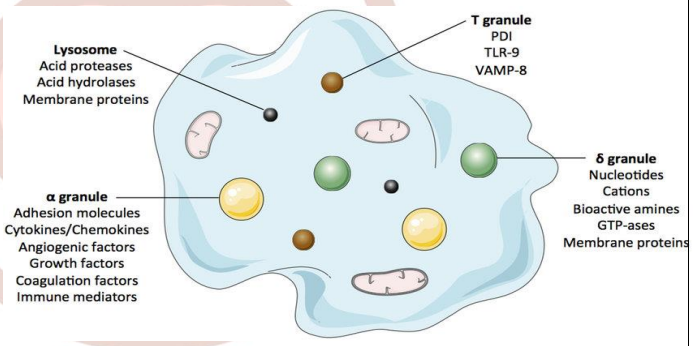
2. Platelet-derived growth factor

3. Vascular endothelial growth factor (VEGF)

4. Basic fibroblast growth factor (FGF)

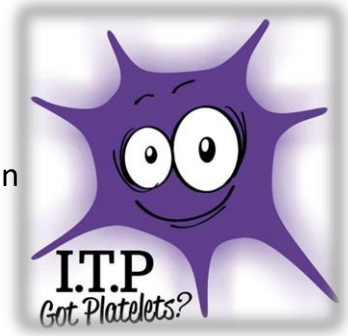
5. Endostatin

6. Thrombospondin



B. Dense granules (contain: 1. Nucleotides 2. Serotonin 3. Phospholipid

4. Calcium 5. Lysosomes.)



- NORMAL COUNT AND VARIATIONS:

Normal platelet count is 2,50,000/cu mm of blood. It ranges between

2,00,000 and 4,00,000/cu mm of blood

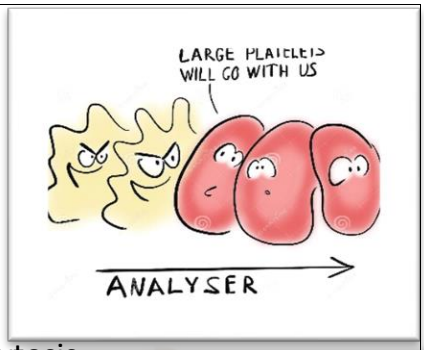
→PHYSIOLOGICAL VARIATIONS:

-Age: Platelets are less in infants

- Sex: There is no difference in the platelet count between males and females. In females, it is reduced during menstruation

- High altitude: Platelet count increases

- After meals: After taking food, the platelet count increases



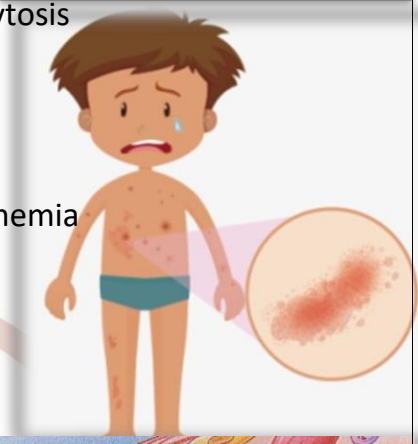
→PATHOLOGICAL VARIATIONS:

- 1. Thrombocytopenia (Decrease in platelet count is called thrombocytopenia. It leads to **thrombocytopenic purpura**)

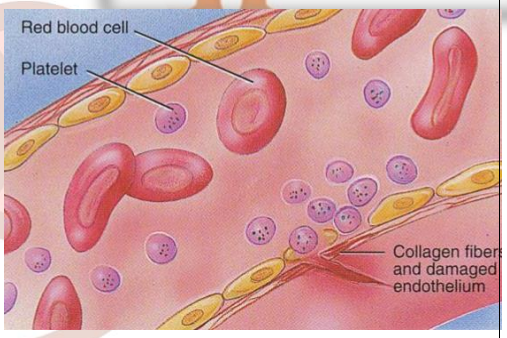
-2.Thrombocytosis (Increase in platelet count is called thrombocytosis Happen in ex, Allergic conditions , Hemorrhage, Bone fractures)

-3. Thrombocythemia (Thrombocythemia is the condition with persistent and abnormal increase in platelet count. Thrombocythemia occurs in the following conditions:

i.Carcinoma ii. Chronic leukemia iii. Hodgkin's disease)



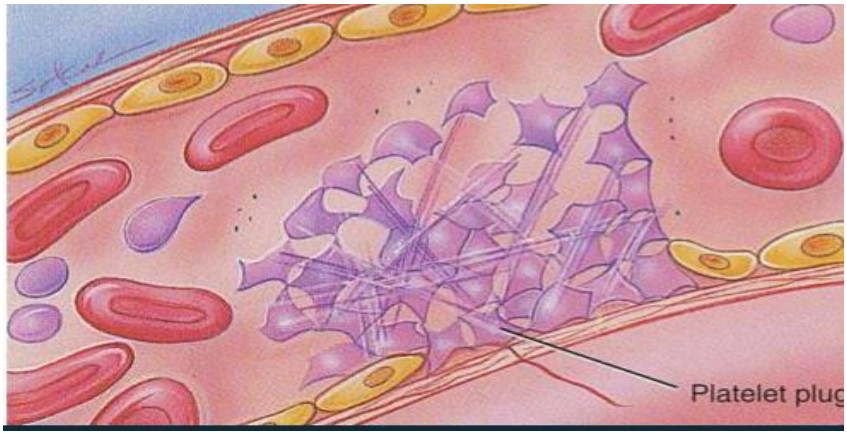
- PROPERTIES OF PLATELETS:



1. Adhesiveness→ Adhesiveness is the property of sticking to a rough surface. During injury of blood vessel, endothelium is damaged and the subendothelial collagen is exposed. While coming in contact with collagen, platelets are activated and adhere to collagen.

Adhesion of platelets involves interaction between von Willebrand factor secreted by damaged endothelium and a receptor protein called glycoprotein Ib situated on the surface of platelet membrane. Other factors which accelerate adhesiveness are collagen, thrombin, ADP, Thromboxane A2 , calcium ions, P-selectin and vitronectin .

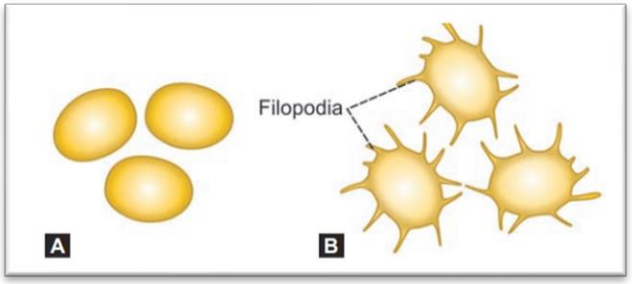
2.AGGREGATION (GROUPING OF PLATELETS)→ Aggregation is the grouping of platelets. Adhesion is followed by activation of more number of platelets by substances released from dense granules of platelets..



During activation, the platelets change their shape with elongation of long filamentous pseudopodia which are called processes or filopodia .

Filopodia help the platelets aggregate together.

Activation and aggregation of platelets is accelerated by ADP, thromboxane A2 and platelet-activating factor.

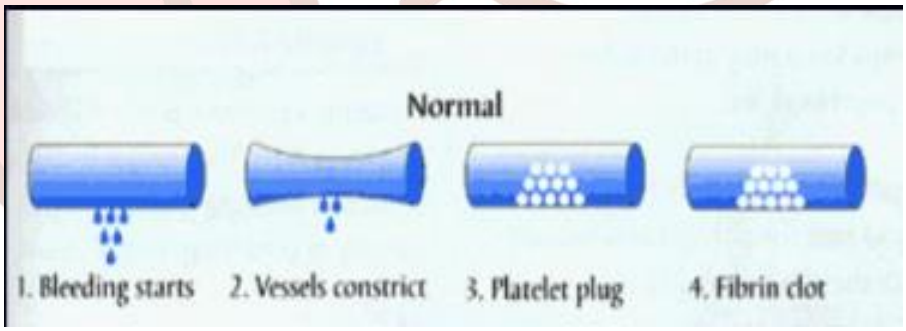


3.AGGLUTINATION→ Agglutination is the clumping together of platelets. Aggregated platelets are agglutinated by the actions of some platelet agglutinins and platelet-activating factor.

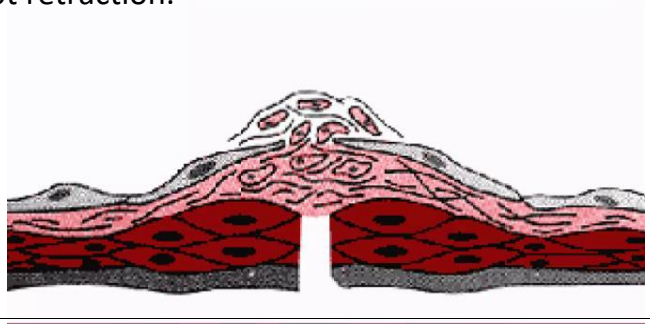
-FUNCTIONS OF PLATELETS:

Normally, platelets are inactive and execute their actions only when activated. Activated platelets immediately release many substances. This process is known as platelet release reaction. Functions of platelets are carried out by these substances. Functions of platelets are:

1. ROLE IN BLOOD CLOTTING Platelets are responsible for the formation of intrinsic prothrombin activator. This substance is responsible for the onset of blood clotting.



2. ROLE IN CLOT RETRACTION In the blood clot, blood cells including platelets are entrapped in between the fibrin threads. Cytoplasm of platelets contains the contractile proteins, namely actin, myosin and thrombosthenin, which are responsible for clot retraction.



3.ROLE IN PREVENTION OF BLOOD LOSS (HEMOSTASIS) Platelets accelerate the hemostasis by three ways :

i. Platelets secrete 5-HT, which causes the constriction of blood vessels.

ii. Due to the adhesive property, the platelets seal the damage in blood vessels like capillaries.

iii. By formation of temporary plug, the platelets seal the damage in blood vessels.

4. ROLE IN REPAIR OF RUPTURED BLOOD VESSEL Platelet-derived growth factor (PDGF) formed in cytoplasm of platelets is useful for the repair of the endothelium and other structures of the ruptured blood vessels.

5. ROLE IN DEFENSE MECHANISM By the property of agglutination, platelets encircle the foreign bodies and destroy them.

- ACTIVATORS AND INHIBITORS OF PLATELETS:

Activators	Inhibitors
1. Collagen, which is exposed during damage of blood vessels	1. Nitric oxide
2. von Willebrand factor	2. Clotting factors: II, IX, X, XI and XII
3. Thromboxane A2 4. Platelet-activating factor 5. Thrombin	3. Prostacyclin
6. ADP	4. Nucleotidases which breakdown the ADP
7. Calcium ions	
8. P-selectin: Cell adhesion molecule secreted from endothelial cells	
9. Convulxin: Purified protein from snake venom	

NOTE → N.B Aspirin inhibits cyclooxygenase. It therefore decreases the synthesis of both thromboxane A2 and prostacyclin. The endothelial cells can start to produce new cyclooxygenase within a few hours, while platelets cannot. Therefore, the daily intake of small amounts of aspirin reduces clot formation and prevents myocardial infarctions.

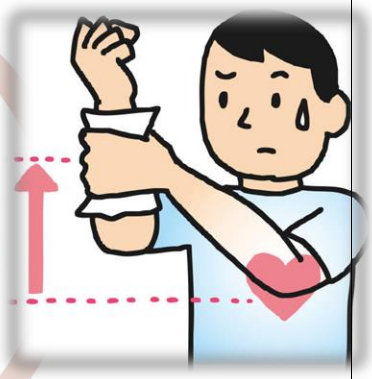
-DEVELOPMENT OF PLATELETS:

Platelets are formed from bone marrow. Pluripotent stem cell give rise to the colony forming unit-megakaryocyte (CFU-M). This develops into **megakaryocyte.**

- LIFESPAN AND FATE OF PLATELETS:

Average lifespan of platelets is 10 days. It varies between 8 and 11 days. Platelets are destroyed by tissue macrophage system in spleen. So, splenomegaly (enlargement of spleen) decreases platelet count and splenectomy (removal of spleen) increases platelet count .

-Hemostasis

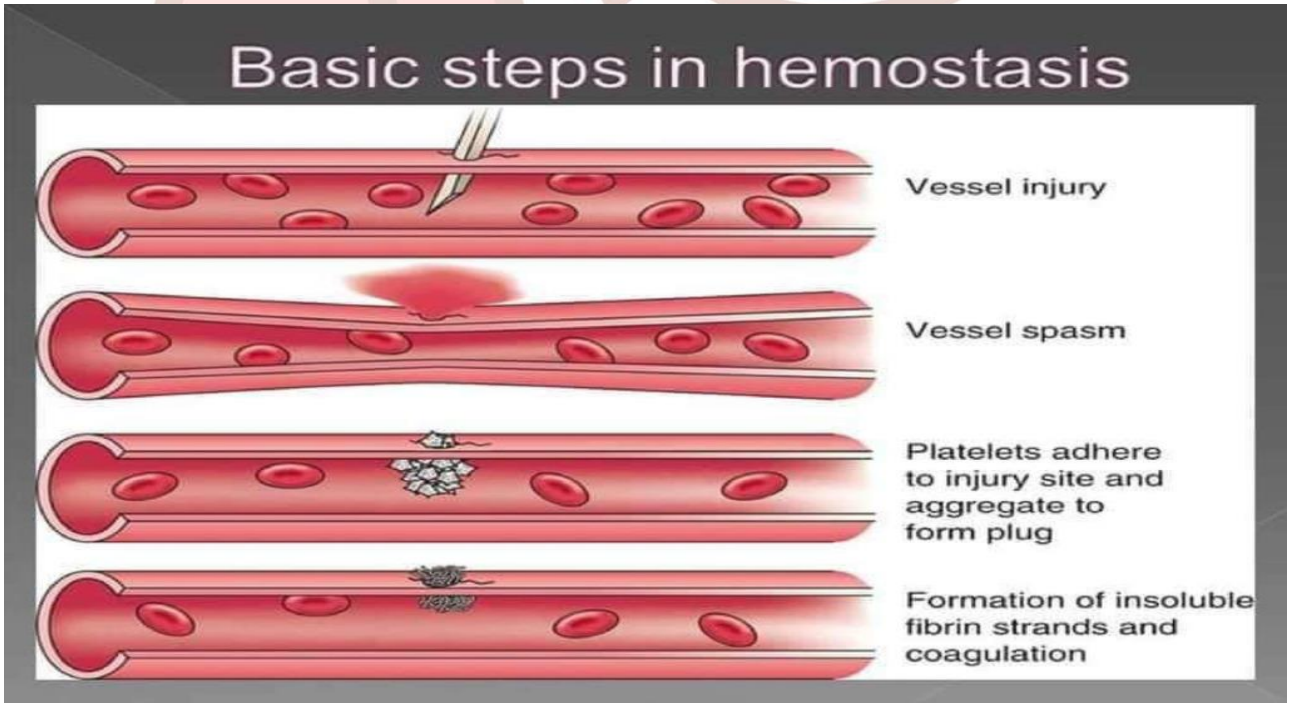


- DEFINITION: Hemostasis is defined as arrest or stoppage of bleeding

-STAGES OF HEMOSTASIS: When a blood vessel is injured, the injury initiates a series of reactions, resulting in hemostasis.

It occurs in three stages:

1. Vasoconstriction 2. Platelet plug formation 3. Coagulation of blood.



- VASOCONSTRICTION→ Immediately after injury, the blood vessel constricts and decreases the loss of blood from damaged portion. Usually, arterioles and small arteries constrict. Vasoconstriction is purely a local phenomenon. When the blood vessels are cut, the endothelium is damaged, and the collagen is exposed. Platelets adhere to this collagen and get activated. The activated platelets secrete serotonin and other vasoconstrictor substances which cause constriction of the blood vessels. Adherence of platelets to the collagen is accelerated by von Willebrand factor. This factor acts as a bridge between a specific glycoprotein present on the surface of platelet and collagen fibrils.

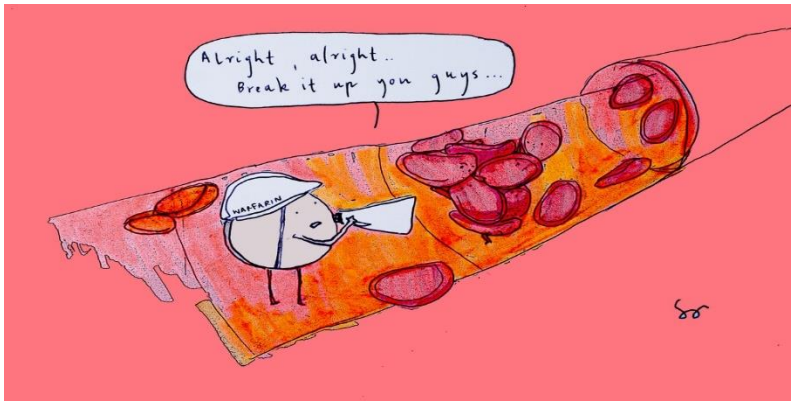
- PLATELET PLUG FORMATION→ Platelets get adhered to the collagen of ruptured blood vessel and secrete adenosine diphosphate (ADP) and thromboxane A2. These two substances attract more and more platelets and activate them. All these platelets aggregate together and form a loose temporary platelet plug or temporary hemostatic plug, which closes the ruptured vessel and prevents further blood loss. Platelet aggregation is accelerated by platelet activating factor.

- COAGULATION OF BLOOD→ During this process, the fibrinogen is converted into fibrin. Fibrin threads get attached to the loose platelet plug, which blocks the ruptured part of blood vessels and prevents further blood loss completely.

- Coagulation of Blood

- DEFINITION:

- Coagulation or clotting is defined as the process in which blood loses its fluidity and becomes a jelly-like mass few minutes after it is shed out or collected in a container.



- FACTORS INVOLVED IN BLOOD CLOTTING:

Coagulation of blood occurs through a series of reactions due to the activation of a group of substances. Substances necessary for clotting are called clotting factors. Thirteen clotting factors are identified :

Clotting Factor	Synonyms
Fibrinogen	Factor I
Prothrombin	Factor II
Tissue factor	Factor III; tissue thromboplastin
Calcium	Factor IV
Factor V	Proaccelerin; labile factor; Ac-globulin (Ac-G)
Factor VII	Serum prothrombin conversion accelerator (SPCA); proconvertin; stable factor
Factor VIII	Antihemophilic factor (AHF); antihemophilic globulin (AHG); antihemophilic factor A
Factor IX	Plasma thromboplastin component (PTC); Christmas factor; antihemophilic factor B
Factor X	Stuart factor; Stuart-Prower factor
Factor XI	Plasma thromboplastin antecedent (PTA); antihemophilic factor C
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor
Prekallikrein	Fletcher factor
High-molecular-weight kininogen	Fitzgerald factor; HMWK (high-molecular-weight kininogen)
Platelets	

- SEQUENCE OF CLOTTING MECHANISM:

→ **ENZYME CASCADE THEORY**: Most of the clotting factors are proteins in the form of enzymes. Normally, all the factors are present in the form of inactive proenzyme. These proenzymes must be activated into enzymes to enforce clot formation. It is carried out by a series of proenzyme-enzyme conversion reactions. First one of the series is converted into an active enzyme that activates the second one, which activates the third one; this continues till the final active enzyme thrombin is formed. Enzyme cascade theory explains how various reactions, involved in the conversion of proenzymes to active enzymes take place in the form of a cascade. Cascade refers to a process that occurs through a series of steps, each step initiating the next, until the final step is reached .

→ **Stages of Blood Clotting** : In general, blood clotting occurs in three stages:

1. Formation of prothrombin activator .

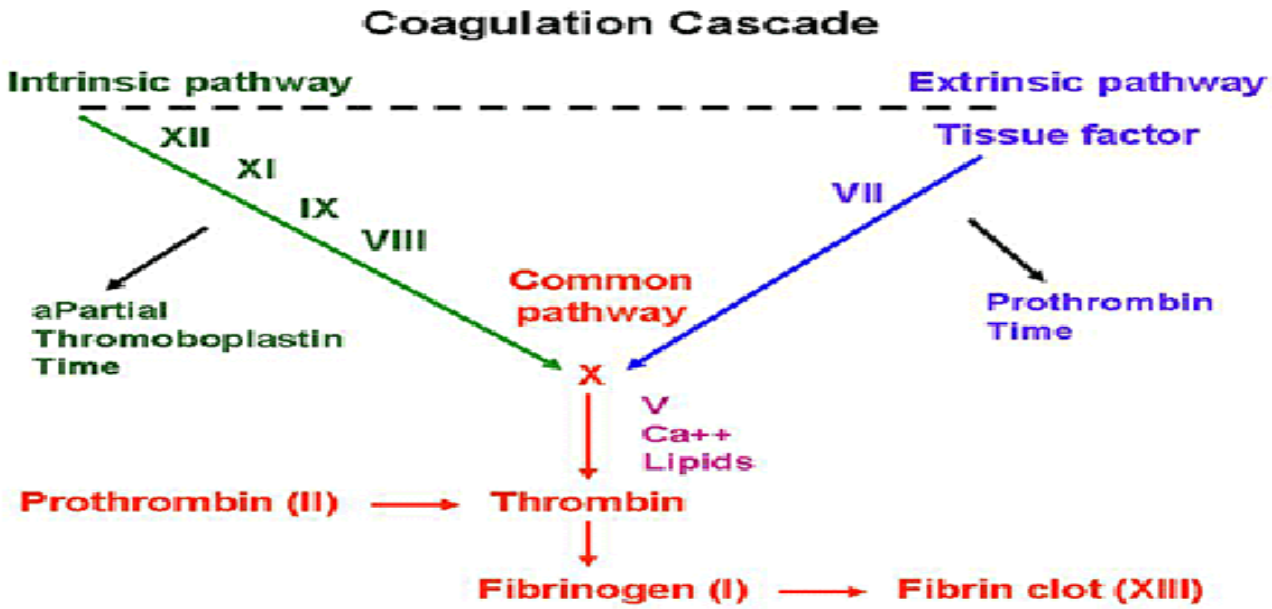
2. Conversion of prothrombin into thrombin .

3. Conversion of fibrinogen into fibrin.

-1. FORMATION OF PROTHROMBIN ACTIVATOR→

- Blood clotting commences with the formation of a substance called prothrombin activator, which converts prothrombin into thrombin. Its formation is initiated by substances produced either within the blood or outside the blood. Thus, formation of prothrombin activator occurs through **two pathways**:

i. Intrinsic pathway ii. Extrinsic pathway



- 1. Intrinsic pathway: This system is called intrinsic, as the phospholipids involved in the reactions arises from platelets (PF3), i.e. it is present in plasma. Initiation of the pathway may occur either:

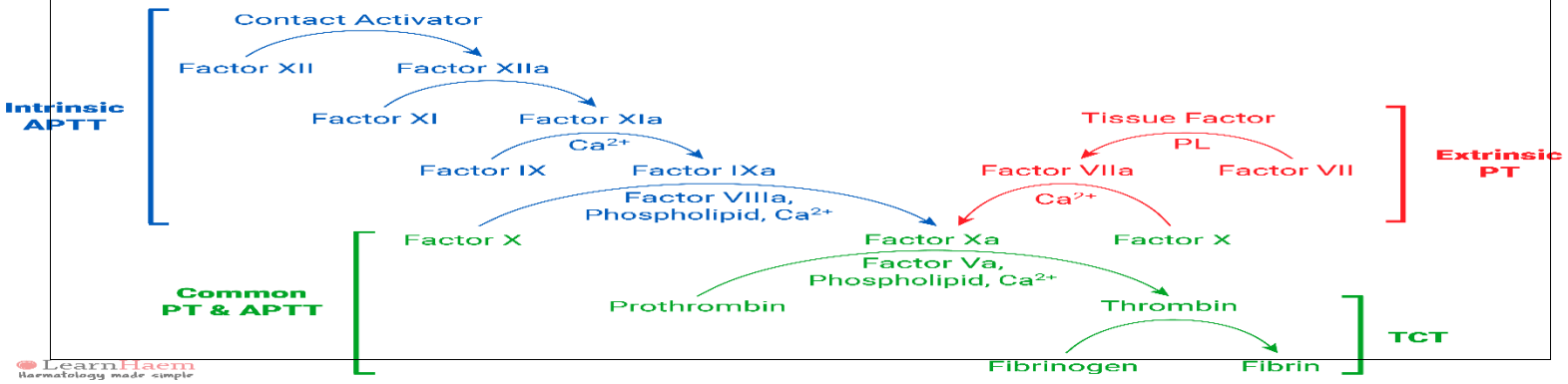
A)In vivo: by contact of blood with subendothelial collagen of damaged vessel.

B) In vitro: by contact of blood with:

•electronegative charged wet surfaces, e.g. glass of a test tube

• collagen fibers

Sequence of Events in Intrinsic pathway→



i. During the injury, the blood vessel is ruptured. Endothelium is damaged and collagen beneath the endothelium is exposed.

ii. When factor XII (Hageman factor) comes in contact with collagen, it is converted into activated factor XII in the presence of kallikrein and high molecular weight (HMW) kinogen.

iii. The activated factor XII converts factor XI into activated factor XI in the presence of HMW kinogen.

iv. The activated factor XI activates factor IX in the presence of factor IV (calcium).
v. Activated factor IX activates factor X in the presence of factor VIII and calcium.
vi. When platelet comes in contact with collagen of damaged blood vessel, it gets activated and releases phospholipids.

vii. Now the activated factor X reacts with platelet phos pholipid and factor V to form prothrombin activa tor. This needs the presence of calcium ions.

viii. Factor V is also activated by positive feedback effect of thrombin.

-2. Extrinsic Pathway: This system is called extrinsic as it requires the presence of phospholipids from outside blood vessel.

- It is initiated only in vivo by factor III: tissue thromboplastin (TPL) released from damaged tissues.

- TPL activates factor VII that directly activates factor X in the presence of Ca2+, TPL and PL and indirectly through activation of factor IX.

Sequence of Events in **Extrinsic Pathway**→

i. Tissues that are damaged during injury release **tissue thromboplastin (**factor III). Thromboplastin contains proteins, phospholipid and glycoprotein, which act as proteolytic enzymes.

ii. Glycoprotein and phospholipid components of thromboplastin convert factor X into activated factor X, in the presence of factor VII.

iii. Activated factor X reacts with factor V and phospholipid component of tissue thromboplastin to form prothrombin activator. This reaction requires the presence of calcium ions.

-2. CONVERSION OF PROTHROMBIN INTO THROMBIN→ Blood clotting is all about thrombin formation. Once thrombin is formed, it definitely leads to clot formation .

Prothrombin activator that is formed in intrinsic and extrinsic pathways converts prothrombin into thrombin in the presence of calcium (factor IV).

Once formed thrombin initiates the formation of more thrombin molecules. The initially formed thrombin activates Factor V. Factor V in turn accelerates formation of both extrinsic and intrinsic prothrombin activator, which converts prothrombin into thrombin. This effect of thrombin is called positive feedback effect

-3. CONVERSION OF FIBRINOGEN INTO FIBRIN→ The final stage of blood clotting involves the conversion of fibrinogen into fibrin by thrombin .

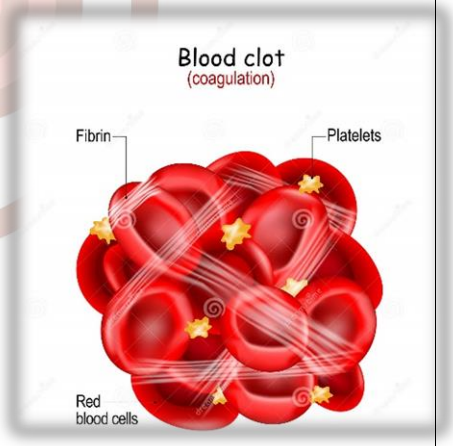
the thrombin acts as an enzyme to convert fibrinogen into fibrin fibers that enmesh platelets, blood cells, and plasma to form the clot.

Later these loose strands are modified into dense and tight

fibrin threads by fibrin-stabilizing factor (factor XIII) in the

presence of calcium ions. All the tight fibrin threads are

aggregated to form a meshwork **of stable clot** .



→Common Part in Both Pathways:

- Xa (activated by intrinsic and extrinsic pathways) catalyzes the conversion of prothrombin to thrombin in the presence of factor V, PL and Ca2+ .

- Finally, thrombin transforms soluble fibrinogen to insoluble fibrin.

- Thrombin in the presence of Ca2+ also activates factor XIII, which stabilizes the fibrin clot. Platelets, blood cells and plasma become entangled in the clot.

- Contraction of the platelets in the fibrin mesh causes clot retraction and squeezes serum out. - Serum is devoid of fibrinogen, prothrombin, factors V, VIII and XIII which become consumed during clotting.

- important Notes:

The extrinsic pathway is **very rapid** (15 sec.), while the intrinsic pathway is slow (1-6 min.).

- Injury of a blood vessel will trigger both the intrinsic system (by collagen) and the extrinsic system (by TPL).

- In the test tube, clotting occurs only by the intrinsic system (glass or addition of collagen).

- In intravenous thrombosis blood clotting occurs via the intrinsic system, which is initiated by the exposure of clotting factors to collagen

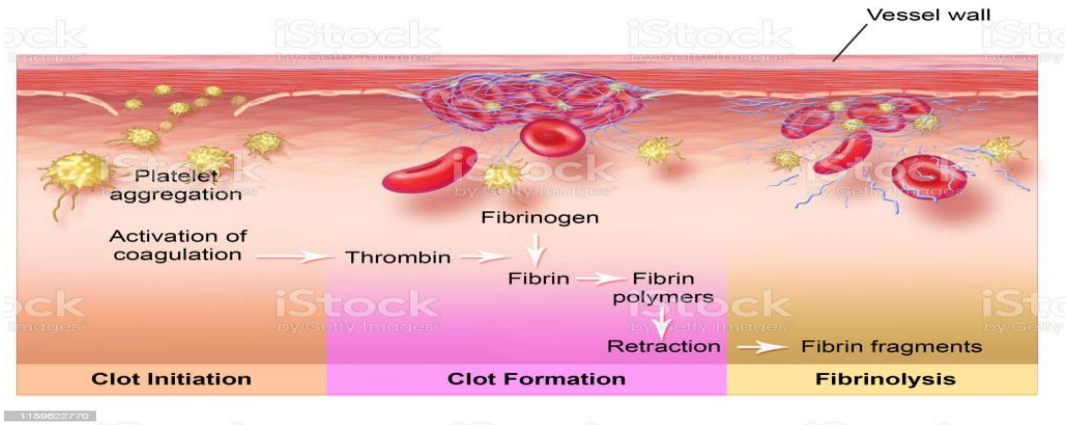
- BLOOD CLOT:

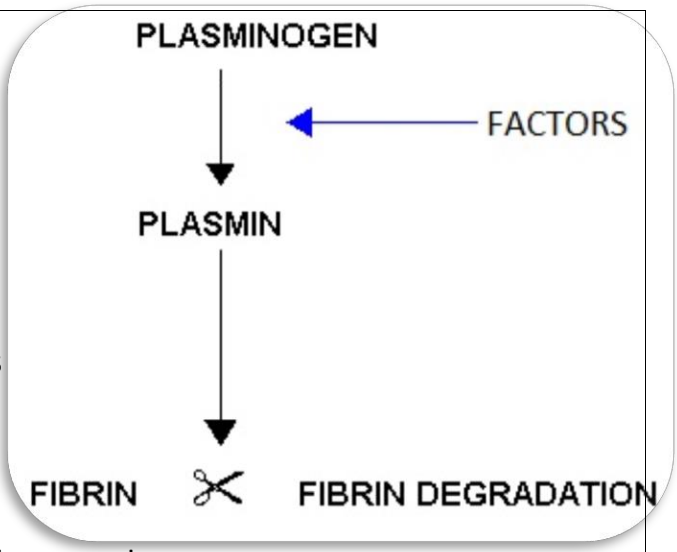
- the clot is composed of a **meshwork of fibrin** fibers running in all directions and entrapping blood cells, platelets, and plasma. The fibrin fibers also adhere to damaged surfaces of blood vessels; therefore, the blood clot becomes adherent to any vascular opening and thereby prevents further blood loss.

-Clot Retraction and Expression of Serum: Within a few minutes after a clot is formed, it begins to contract and usually expresses most of the fluid from the clot within 20 to 60 minutes. the fluid expressed is called serum because all its **fibrinogen and most of the other clotting factors** have been removed; in this way, serum differs from plasma. Serum cannot clot because it lacks these factors.

- FIBRINOLYSIS:

Lysis of blood clot inside the blood vessel is called fibrinolysis. It helps to remove the clot from lumen of the blood vessel. This process requires a substance called plasmin or fibrinolysin.



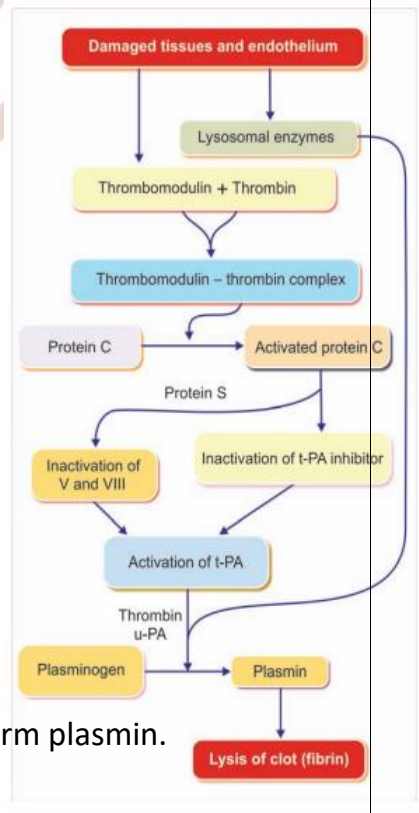


- Formation of Plasmin:

Plasmin is formed from inactivated glycoprotein called plasminogen. Plasminogen is synthesized in liver and it is incorporated with other proteins in the blood clot. Plasminogen is converted into plasmin by tissue plasminogen activator (t-PA), lysosomal enzymes and thrombin. The t-PA and lysosomal enzymes are released from damaged tissues and damaged endothelium. Thrombin is derived from blood. The t-PA is always inhibited by a substance called t-PA inhibitor. It is also inhibited by factors V and VIII. Besides t-PA, there is another plasminogen activator called urokinase plasminogen activator (u-PA). It is derived from blood.

- Sequence of Events Involved in the Activation of Plasminogen→

1. During intravascular clotting, the endothelium of the blood vessel secretes a thrombin-binding protein, the thrombomodulin. It is secreted by the endothelium of all the blood vessels, except the minute vessels of brain.
2. Thrombomodulin combines with thrombin and forms a thrombomodulin-thrombin complex
3. Thrombomodulin-thrombin complex activates protein C
4. Activated protein C inactivates factor V and VIII in the presence of a cofactor called protein S .
5. Protein C also inactivates the t-PA inhibitor
6. Now, the t-PA becomes active
7. Activated t-PA and lysosomal enzymes activate plasminogen to form plasmin. Plasminogen is also activated by thrombin and u-PA.



- ANTICLOTTING MECHANISM IN THE BODY→

Under physiological conditions, intravascular clotting does not occur. It is because of the presence of some physicochemical factors in the body

1. Physical Factors:

i. Continuous circulation of blood.

ii. Smooth endothelial lining of the blood vessels.

2. Chemical Factors – Natural Anticoagulants:

i. Presence of natural anticoagulant called heparin that is produced by the liver.

ii. Production of thrombomodulin by endothelium of the blood vessels (except in brain capillaries). Thrombomodulin is a thrombin-binding pro tein. It binds with thrombin and forms a thrombomodulin-thrombin complex. This complex activates protein C. Activated protein C along with its cofactor protein S inactivates Factor V and Factor VIII. Inactivation of these two clotting factors prevents clot formation.

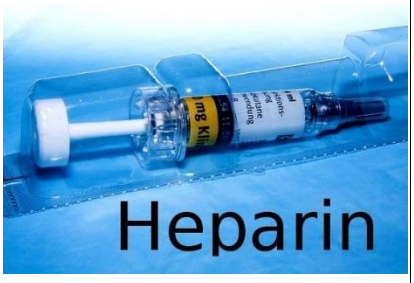
iii. All the clotting factors are in inactive state.

- ANTICOAGULANTS→

Substances which prevent or postpone coagulation of blood are called anticoagulants. Anticoagulants are of three types:

1. Anticoagulants used to prevent blood clotting inside the body, i.e. in vivo
2. Anticoagulants used to prevent clotting of blood that is collected from the body, i.e. in vitro.
3. Anticoagulants used to prevent blood clotting both in vivo and in vitro

-HEPARIN:



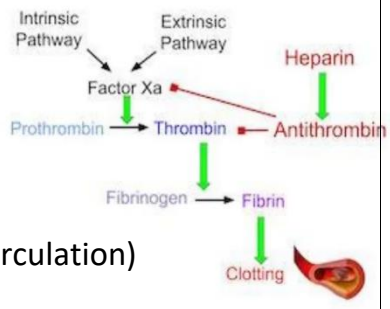
Heparin is a naturally produced anticoagulant in the body.

It is produced by mast cells which are the wandering cells

present immediately outside the capillaries in

many tissues or organs that contain more connective tissue. These cells are abundant in liver and lungs. Basophils also secrete heparin. Heparin is a conjugated polysaccharide. Commercial heparin is prepared from the liver and

other organs of animals. Commercial preparation is available in liquid form or dry form as sodium, calcium, ammonium or lithium salts.



-Mechanism of Action of Heparin→

i. Prevents blood clotting by its antithrombin activity.

It directly suppresses the activity of thrombin

ii. Combines with antithrombin III (a protease inhibitor present in circulation)

and removes thrombin from circulation.

iii. Activates antithrombin III

iv. Inactivates the active form of other clotting factors like IX, X, XI and XII

- Uses of Heparin→ Heparin is used as an anticoagulant both in vivo and in vitro

Clinical use: i. To prevent intravascular blood clotting during surgery.

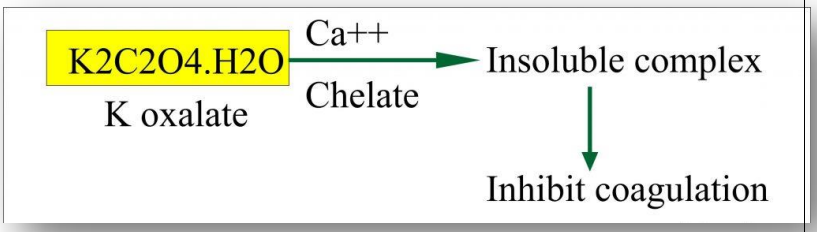
ii. While passing the blood through artificial kidney for dialysis.

iii. During cardiac surgery, which involves heartlung machine.

iv. To preserve the blood before transfusion.

\Use in the laboratory: Heparin is also used as anticoagulant in vitro while collecting blood for various investigations. About 0.1 to 0.2 mg is sufficient for 1 mL of blood. It is effective for 8 to 12 hours. After that, blood will clot because heparin only delays clotting and does not prevent it. Heparin is the most expensive anticoagulant .

-OXALATE COMPOUNDS:



-Mechanism of Action→

Oxalate combines with calcium and

forms insoluble calcium oxalate. Thus, oxalate removes calcium from blood and lack of calcium prevents coagulation.

- Uses→

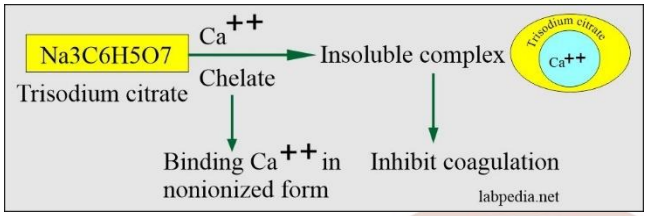
Oxalate compounds are used only as in vitro anticoagulants. 2 mg of mixture is necessary for 1 ml of blood. **Since oxalate is poisonous, it cannot be used in vivo**

- CITRATES:

Sodium, ammonium and potassium citrates are used as anticoagulants.

- Mechanism of Action→

Citrate combines with calcium in blood to form insoluble calcium citrate. Like oxalate, citrate also removes calcium from blood and lack of calcium prevents coagulation.



-Uses→ Citrate is used as in vitro anticoagulant

i. It is used to store blood in the blood bank as:

a. Acid citrate dextrose (ACD): 1 part of ACD with 4 parts of blood

b. Citrate phosphate dextrose (CPD): 1 part of CPD with 4 parts of blood

ii. Citrate is also used in laboratory in the form of formol-citrate solution (Dacie's solution) for RBC and platelet counts.

-In vivo anticoagulants: They prevent blood clotting inside the body.

	Heparin	Dicumarol
Origin	Mast cells and basophils	Plant
Mode of Action	Facilitates the action of Antithrombin III	Competitive inhibition of vitamin K on its receptors in liver → inhibits formation of II, VII, IX, X
Site of Action	In vivo and in vitro	Only in vivo
Onset	Rapid	Slow
Duration	Short	Long
Administration	I.V. and I.M.	Orally
Antidote	Protamine sulphate 1% Fresh blood transfusion	Vitamin K Fresh blood transfusion

- COLLECTING BLOOD IN A CONTAINER WITH SMOOTH SURFACE:

-Collecting the blood in a container with smooth surface

like a **silicon-coated container prevents clotting**.

The smooth surface inhibits the activation of factor XII

and platelets. So, the formation of prothrombin activator is prevented.

- PROCOAGULANTS:

Procoagulants or hemostatic agents are the substances which accelerate the process of blood coagulation. Procoagulants are:

-THROMBIN→ Thrombin is sprayed upon the bleeding surface to arrest bleeding by hastening blood clotting

- SNAKE VENOM→ Venom of some snakes (vipers, cobras and rattle snakes) contains proteolytic enzymes which enhance blood clotting by activating the clotting factors.

- EXTRACTS OF LUNGS AND THYMUS→ Extract obtained from the lungs and thymus has thromboplastin, which causes rapid blood coagulation.

- **SODIUM OR CALCIUM ALGINATE**→ Sosium or calcium alginate substances enhance blood clotting process by activating the Hageman factor.

- OXIDIZED CELLULOSE→ Oxidized cellulose causes clotting of blood by activating the Hageman factor.

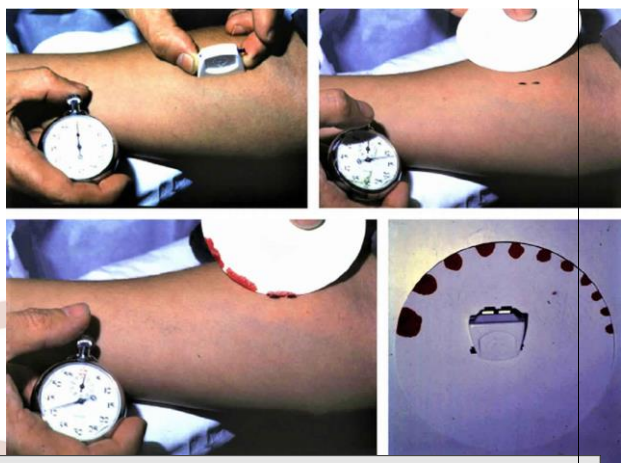
- TESTS FOR BLOOD CLOTTING (VIP FOR THE LAB)

Blood clotting tests are used to diagnose blood disorders. Some tests are also used to monitor the patients treated with anticoagulant drugs such as heparin and warfarin.

-TESTS:

1. Bleeding time 2. Clotting time 3. Prothrombin time 4. Partial prothrombin time 5. International normalized ratio 6. Thrombin time

Lets talk about them!!!!!!!!

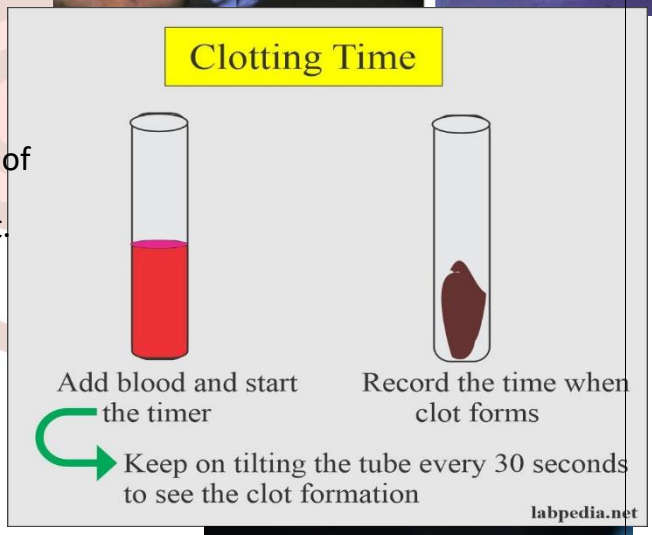


- BLEEDING TIME:

Bleeding time (BT) is the time interval from oozing of blood after a cut or injury till arrest of bleeding. Usually, it is determined by Duke method using blotting paper or filter paper method.

Its normal duration is 3 to 6 minutes.

It is prolonged in purpura.



- CLOTTING TIME:

Clotting time (CT) is the time interval from oozing of blood after a cut or injury till the formation of clot.

It is usually determined by capillary tube method.

Its normal duration is 3 to 8 minutes.

It is prolonged in hemophilia.

- PROTHROMBIN TIME:

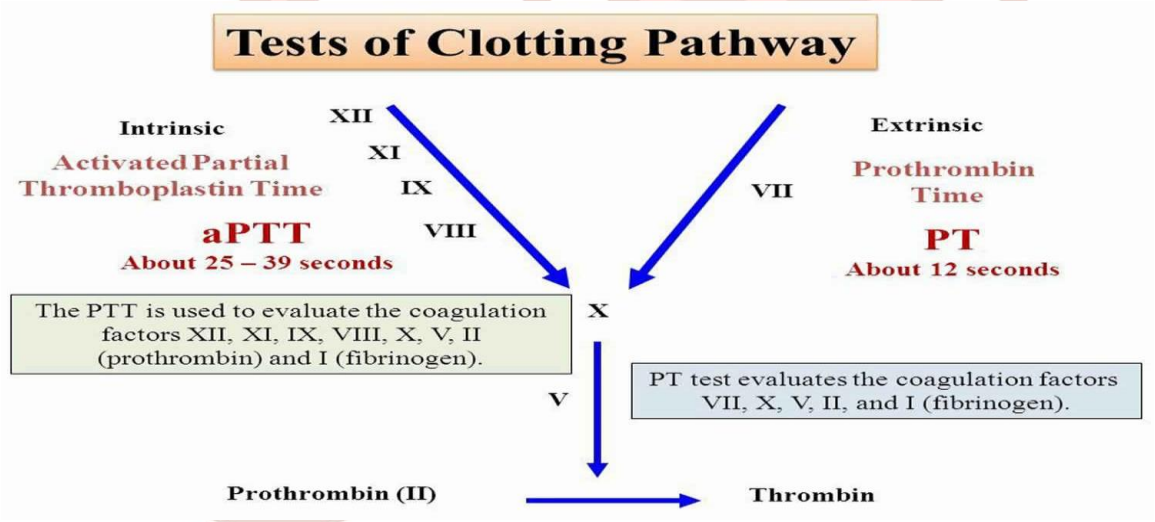
Prothrombin time (PT) is the time taken by blood to clot after adding tissue thromboplastin to it. Blood is collected and oxalated so that, the calcium is precipitated and prothrombin is not converted into thrombin. Thus, the blood clotting is prevented.

Then a large quantity of tissue thromboplastin with calcium is added to this blood. Calcium nullifies the effect of oxalate. **The tissue thromboplastin activates prothrombin and blood clotting occurs**. During this procedure, the time taken by blood to clot after adding tissue thromboplastin is determined. Prothrombin time

indicates the total quantity of prothrombin present in the blood. Normal duration of prothrombin time is 10 to 12 seconds. It is prolonged in deficiency of prothrombin and other factors like factors I, V, VII and X. However, it is normal in hemophilia.

- PARTIAL PROTHROMBIN TIME OR ACTIVATED PROTHROMBIN TIME:

Partial prothrombin time (PPT) is the time taken for the blood to clot after adding an activator such as phospholipid, along with calcium to it. It is also called activated partial prothrombin time (APTT). **This test is useful in monitoring the patients taking anticoagulant drugs**. It is carried out by observing clotting time after adding phospholipid, a surface activator and calcium to a patient's plasma. Phospholipid serves as platelet substitute. Commonly used surface activator is kaolin. Normal duration of partial prothrombin time is 30 to 45 seconds. It is prolonged in heparin or warfarin therapy (since heparin and warfarin inhibit clotting) and deficiency or inhibition of factors II, V, VIII, IX, X, XI and XII.



- THROMBIN TIME:

Thrombin time (TT) is the time taken for the blood to clot after adding thrombin to it. It is done to investigate the presence of heparin in plasma or to detect fibrinogen abnormalities. This test involves observation of clotting time after adding thrombin to patient's plasma. Normal duration of thrombin time is 12 to 20 seconds. It is prolonged in heparin therapy and during dysfibrinogenemia (abnormal function of fibrinogen with normal fibrinogen level).

-Abnormalities of Hemostasis:

- 1. Hemophilia→

Hemophilia is a group of **sex-linked inherited** blood disorders, characterized by prolonged clotting time.

However, the bleeding time is normal. Usually, it affects the males, with the females being the carriers.

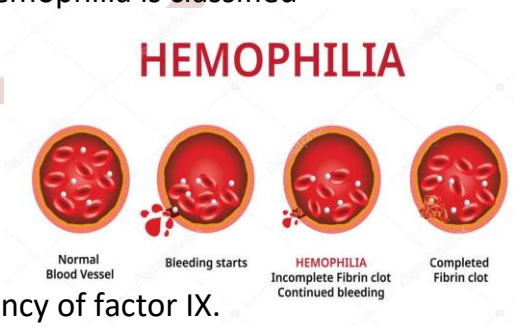
Because of prolonged clotting time, even a mild trauma causes excess bleeding which can lead to death. Damage of skin while falling or extraction of a tooth may cause excess bleeding for few weeks. Easy bruising and hemorrhage in muscles and joints are also common in this disease.

-Causes of hemophilia→

Hemophilia occurs due to lack of formation of prothrombin activator. That is why the coagulation time is prolonged. The formation of prothrombin activator is affected due to the deficiency of factor VIII, IX or XI.

- Types of hemophilia→

Depending upon the deficiency of the factor involved, hemophilia is classified into three types:



i. Hemophilia A or classic hemophilia:

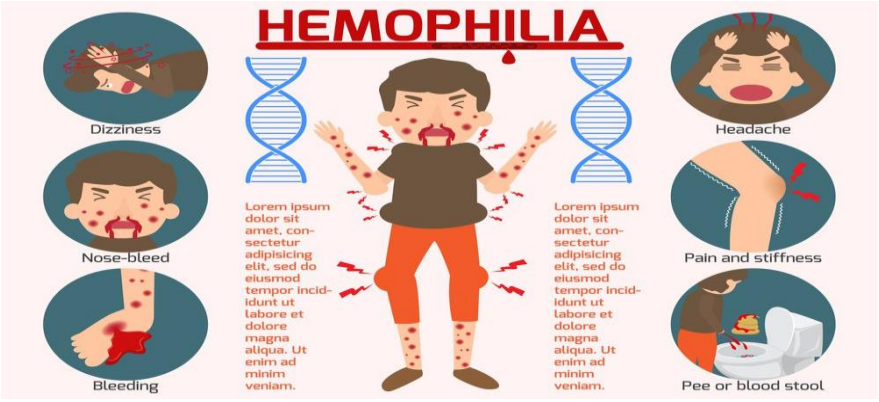
Due to the deficiency of factor VIII. 85% of people with hemophilia are affected by hemophilia A.

ii. Hemophilia B or **Christmas disease**: Due to the deficiency of factor IX.

15% of people with hemophilia are affected by hemophilia B

iii. Hemophilia C or factor XI deficiency: Due to the deficiency of factor XI. It is a very rare bleeding disorder

- Symptoms of hemophilia→

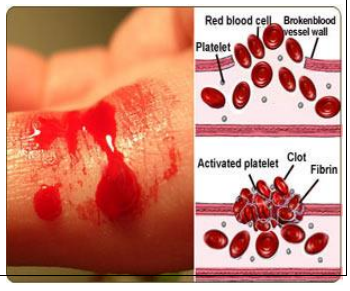


-Treatment for hemophilia→ Effective therapy for classical hemophilia involves replacement of missing clotting factor.

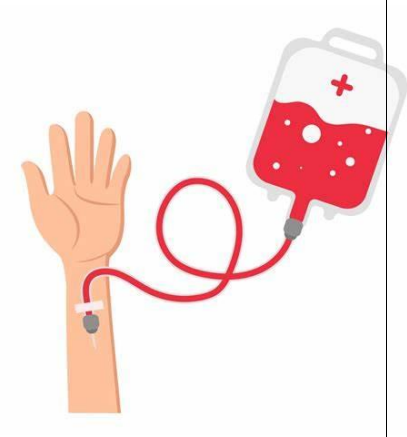
- 2. Purpura→ Purpura is a disorder characterized by prolonged bleeding time. However, the clotting time is normal. Characteristic feature of this disease is spontaneous bleeding under the skin from ruptured capillaries. It causes small tiny hemorrhagic spots in many areas of the body. The hemorrhagic spots under the skin are called purpuric spots (purple colored patch like appearance). That is why this disease is called purpura. Blood also sometimes collects in large areas beneath the skin which are called **ecchymoses**.

- Thrombocytopenic purpura→ Thrombocytopenic purpura is due to the deficiency of platelets (thrombocytopenia). In bone marrow disease, platelet production is affected leading to the deficiency of platelets.

- 3. von Willebrand Disease→ von Willebrand disease is a bleeding disorder, characterized by excess bleeding even with a mild injury. It is due to deficiency of von Willebrand factor, which is a protein secreted by endothelium of damaged blood vessels and platelets. This protein is responsible for adherence of platelets to endothelium of blood vessels during hemostasis after an injury. It is also responsible for the survival and maintenance of factor VIII in plasma. Deficiency of von Willebrand factor suppresses platelet adhesion. It also causes deficiency of factor VIII. This results in excess bleeding, which resembles the bleeding that occurs during platelet dysfunction or hemophilia.



- Blood Transfusion



- Blood transfusion is the process of transferring blood or blood components from one person (the donor) into the bloodstream of another person (the recipient). Transfusion is done as a life-saving procedure to replace blood cells or blood products lost through bleeding.

- PRECAUTIONS: Certain precautions must be followed before and during the transfusion of blood to a patient.

- PRECAUTIONS TO BE TAKEN BEFORE THE TRANSFUSION OF BLOOD:

1. Donor must be healthy, without any diseases like:

a. Sexually transmitted diseases such as syphilis

b. Diseases caused by virus like hepatitis, AIDS, etc

2. Only compatible blood must be transfused

3. Both matching and cross-matching must be done

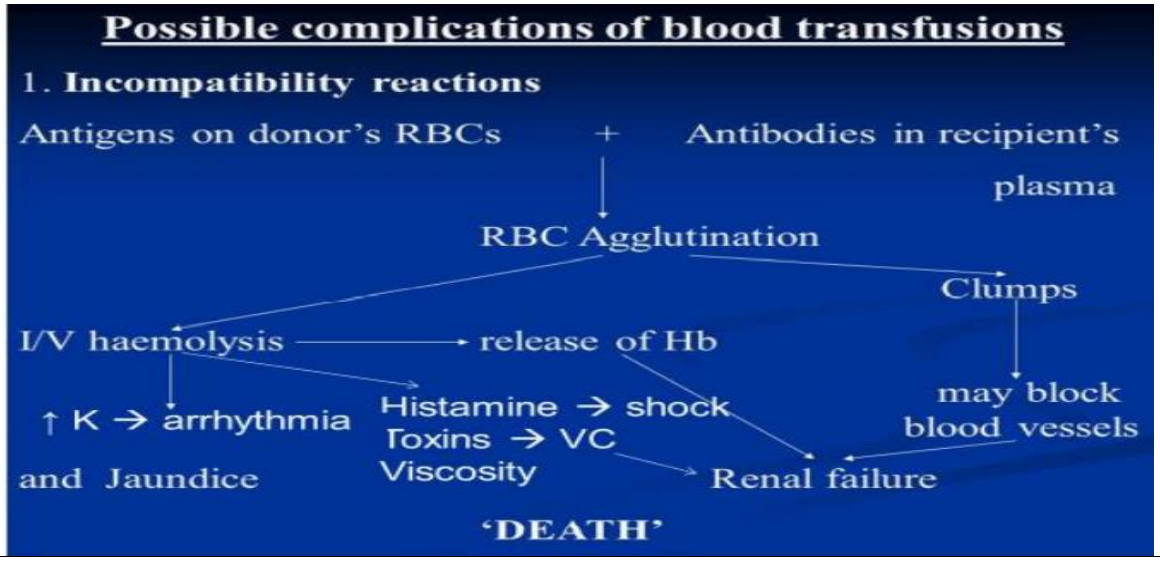
4. Rh compatibility must be confirmed.

- PRECAUTIONS TO BE TAKEN WHILE TRANSFUSING BLOOD:

1. Apparatus for transfusion must be sterile

2. Temperature of blood to be transfused must be same as the body temperature

3. Transfusion of blood must be slow. The sudden rapid infusion of blood into the body increases the load on the heart, resulting in many complications.



-Fresh frozen plasma (FFP) : is a blood product made from the liquid portion of whole blood.It is used to treat conditions in which there are low blood clotting factors (INR > 1.5) or low levels of other blood proteins. It may also be used as the replacement fluid in plasma exchange. Using ABO compatible plasma, while not required, may be recommended. Use as a volume expander is not recommended It is given by slow injection into a vein.

-Medical uses→

There are few specific indications for FFP. These generally are limited to the treatment of deficiencies of coagulation proteins for which specific factor concentrates are unavailable or undesirable. A usual dose of plasma is 10–20 mL/kg recipient weight.

-Indications for the use of FFP include the following:

Replacement of isolated factor deficiencies: FFP is used to treat

rare bleeding disorders when specific factor concentrates

are not available.

FFP is the usual treatment for factor V deficiency.

Reversal of warfarin effect: Warfarin

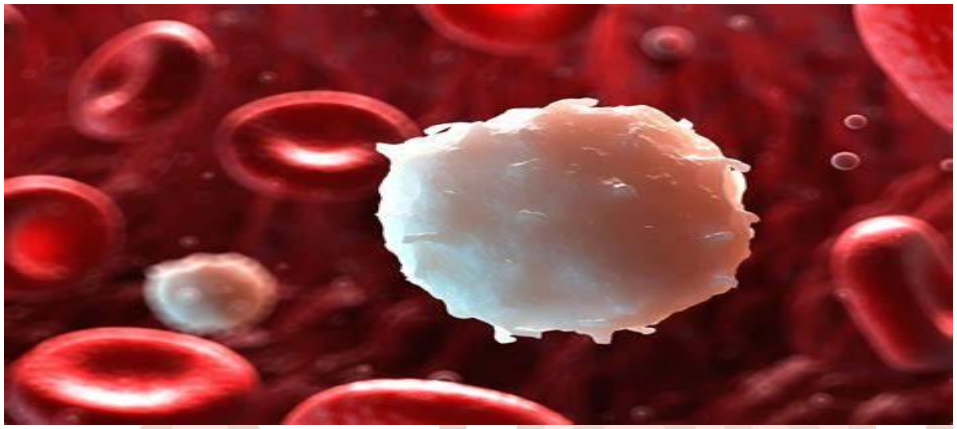
Patients who are anticoagulated with warfarin are deficient in the functional vitamin K dependent coagulation factors II, VII, IX, and X, as well as proteins C and S. These functional deficiencies can be reversed by the administration of vitamin K. For anticoagulated patients who are actively bleeding or who require emergency surgery, prothrombin complex concentrate (ideally, four factor PCCs) should be used if available.FFP/PF24/thawed plasma should only be used if more effective alternative treatments are not available. The ASA task force recommends starting with 5–8 mL/kg of FFP for warfarin reversal and rechecking laboratory values.

Use in antithrombin III deficiency: FFP can be used as a source of antithrombin III in patients who are deficient of this inhibitor and are undergoing surgery or who require heparin for treatment of thrombosis. There are purified, human derived, as well as recombinant forms of antithrombin III available in the US.

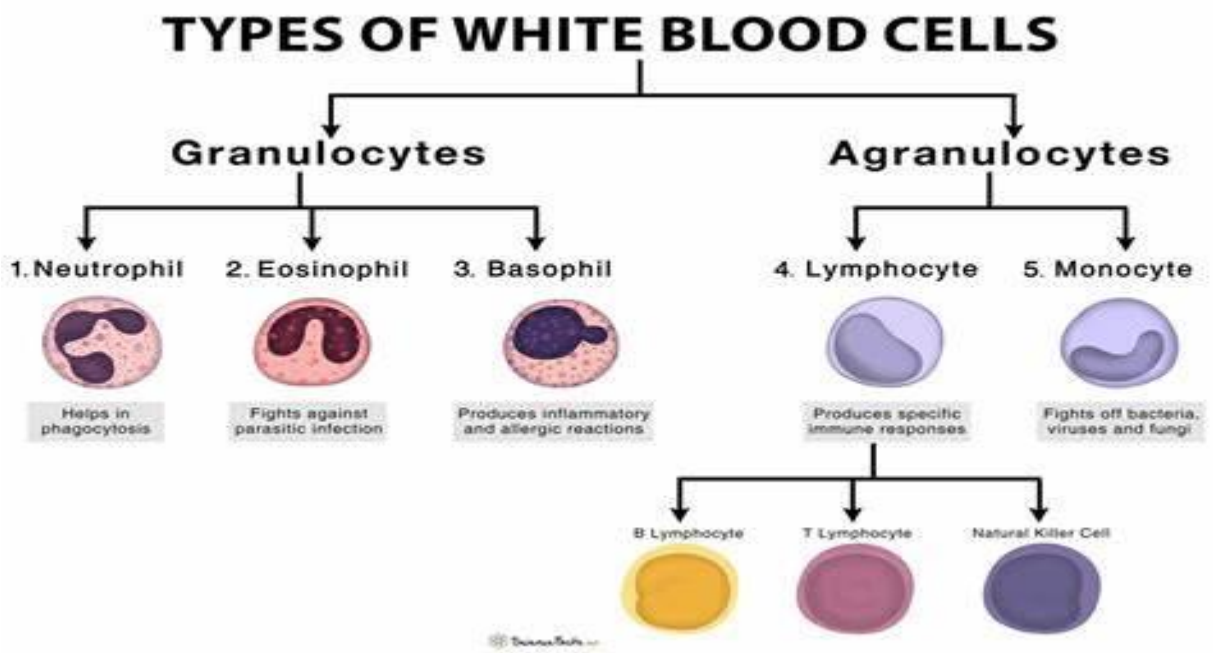
-WHITE BLOOD CELLS (WBCs)

LEUKOCYTES:

- The total leukocytic count is 4,000–11,000/mm3 of blood

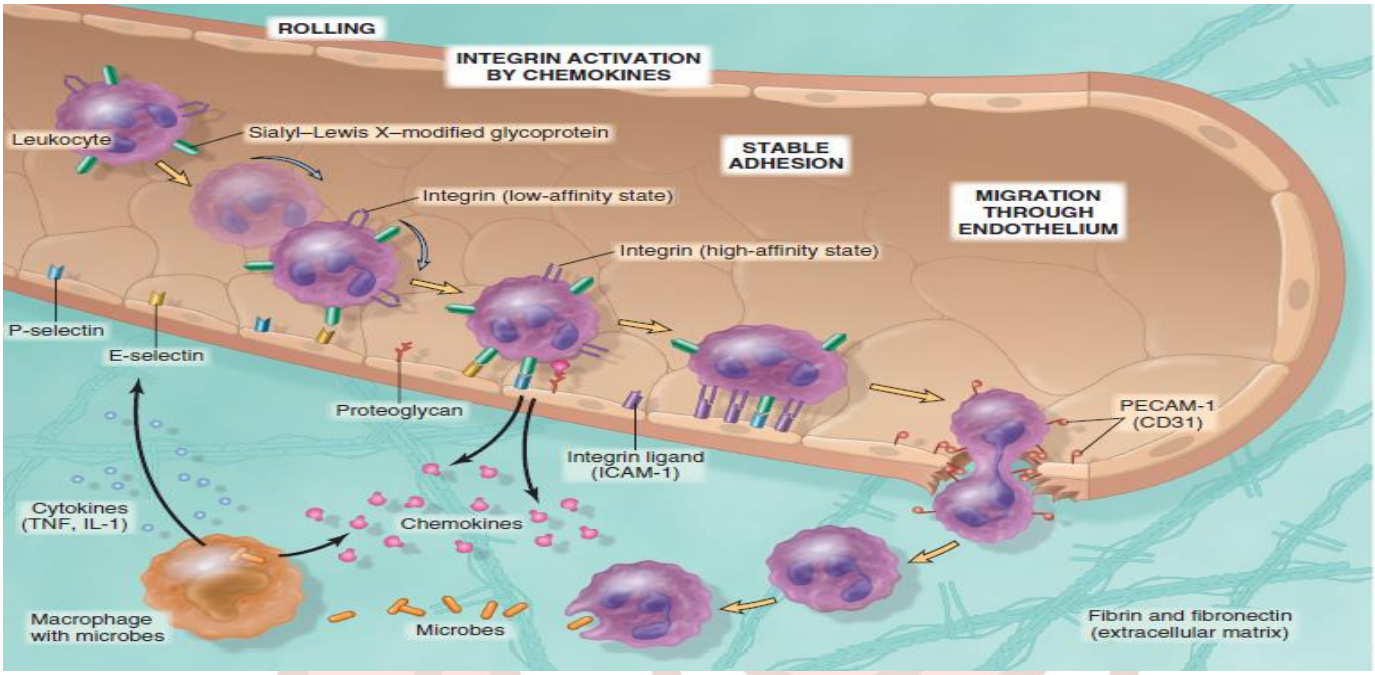


-Functions of Leukocytes→ Leukocytes are responsible for the defense of the body against pathogenic organisms and their toxins



A. Granular Leukocytes→ 1. Neutrophils: (60-70% of WBCs) - They represent the first line of defense against invading organisms.

-They perform their function by:



a. Margination: Neutrophils attach to the walls of capillaries.

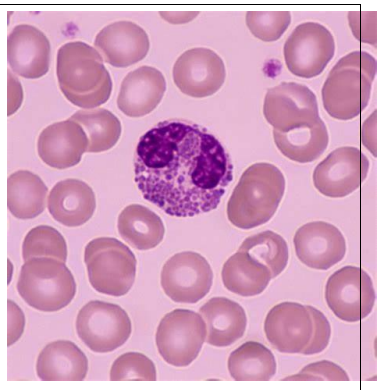
b. Diapedesis: Neutrophils squeeze themselves through the pores of capillaries and pass into tissue spaces.

c. Amoeboid movement: This movement allows neutrophils to reach invading organisms.

d. Chemotaxis: Breakdown products of inflamed tissues and bacterial toxins attract neutrophils to the infected area.

e. Phagocytosis: This is the most important property of neutrophils. It is their ability to ingest invading bacteria as well as necrotic tissue by engulfing them (endocytosis). It results in the formation of a phagocytic vacuole.

The neutrophil granules release their contents into the phagocytic vacuoles, killing the bacteria.- In more severe infection, pus is formed. It consists of necrotic tissue, dead neutrophils and dead macrophages.



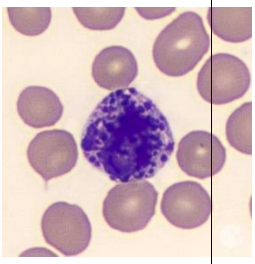
2.Eosinophils: (2-6% of WBCs)→

- Eosinophils increase in parasitic infestations.

They kill parasites that are too large to be engulfed through the release of toxic substances.

- They also increase in allergic conditions.

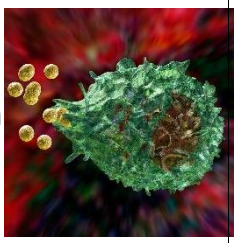
- Eosinophils are weakly phagocytic and show chemotaxis.



3. Basophils: (0-1% of WBCs) - Basophils are similar to mast cells. They contain histamine (increases capillary permeability) and heparin (naturally occurring anticoagulant).

B. Agranular Leukocytes:

- 1. Monocytes: (2-8% of WBCs)



- They are the largest type of leukocytes.

- They enter the blood from the bone marrow and circulate for 72 hours. Then they enter the tissues & become tissue macrophages. The tissue macrophage system was earlier known as the reticuloendothelial system.

- Monocytes migrate by amoeboid movement in response to chemotactic stimuli to the sites of inflammation soon after neutrophils.

- They phagocytose bacteria, dead neutrophils and remnants of destroyed tissues

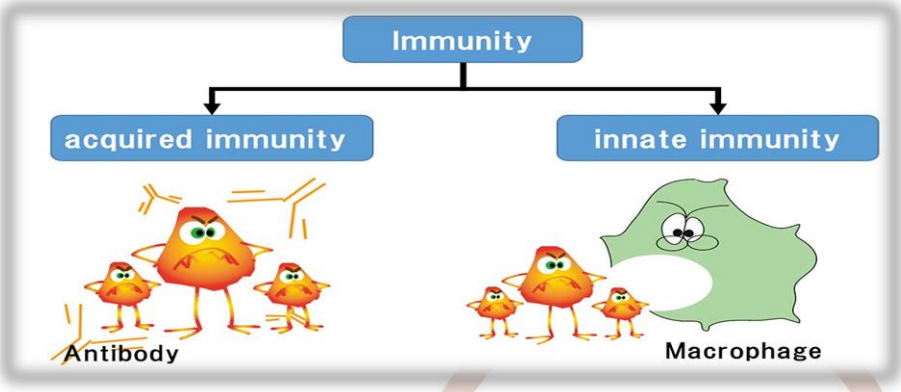
2. Lymphocytes: (20-30% of WBCs)

- They are formed in lymphoid tissues and enter the circulation via the lymphatic vessels.

- Lymphocytes play an important role in the body's defense through the immune system.

-IMMUNITY

- It is the ability of the body to resist almost all types of organisms and toxins that tend to damage tissues and organs. There are two types of immunity: innate (non-specific) and acquired (specific) immunity .



- I. INNATE IMMUNITY = NON-SPECIFIC IMMUNITY:

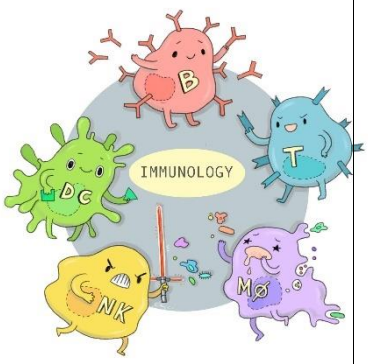
-Innate immunity is composed of non-specific mechanisms, which defend the body against invasion by most organisms. It is neither specific for particular infectious agents, nor is it improved by repeated encounters with the same agent. It includes the following mechanisms: i.e.,

1. Resistance of the skin to invasion by organisms.

2. Destruction of organisms swallowed by HCl and digestive enzymes in the stomach.

3. Phagocytosis of the bacteria by granulocytes and tissue macrophages.

- II. ACQUIRED IMMUNITY = SPECIFIC IMMUNITY→



It is the ability of the body to recognize the invading agent and to develop a powerful specific immune response against it.

It is carried out in 2 ways:

1. Humoral Immunity: <u>B-lymphocytes produce antibodies that</u> circulate in blood and react specifically with the foreign agent destroying it.

2. Cellular Immunity: <u>Formation of activated cytotoxic T-lymphocytes</u>, which can circulate in the blood, detect and attack the antigen specifically.

- Antigens:

-DEFINITION AND TYPES→ Antigens are the substances which induce specific immune reactions in the body. 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 antigen s or non-self antigens that enter the body from outside.

- These are substances that are able to induce an immune response and to react specifically with its products.

- Foreign organisms or toxins possess certain chemical structures that are specific to them and different from all other compounds. Such chemical structures are known as "antigenic determinants".

– Their molecular weight is more than 10,000. - Antigens usually have many antigenic determinants, which may be different from each other or be repeated molecular structures. - Antigenic determinants may be present on cell membranes or may be free, e.g., bacterial toxins.

A. Cellular Immunity (Cell Mediated Immunity)

-- This type of immunity is produced by the activated cytotoxic T lymphocytes. - It occurs when the body is exposed to viruses, fungi, few bacteria (e.g. the tubercle bacillus), cells from other individuals (tissue transplants) or tumor cells

- Cytotoxic T cells cannot recognize free antigens. They are activated when their T cell receptor "sees" the antigen to which they are specific in association with the MHC class I protein:

1. The antigen may be ingested by an antigen-presenting cell (macrophage), partially digested and presented on its cell membrane coupled with the MHC class I protein. In addition, the antigen-presenting cells also release interleukin-1 (IL-1), which activates T-lymphocytes.

2. T-helper cells are activated when they recognize the antigen in association with MHC class II proteins on the surface of antigen presenting cells. The T-helper cells secrete interleukins that activate T cytotoxic cells.

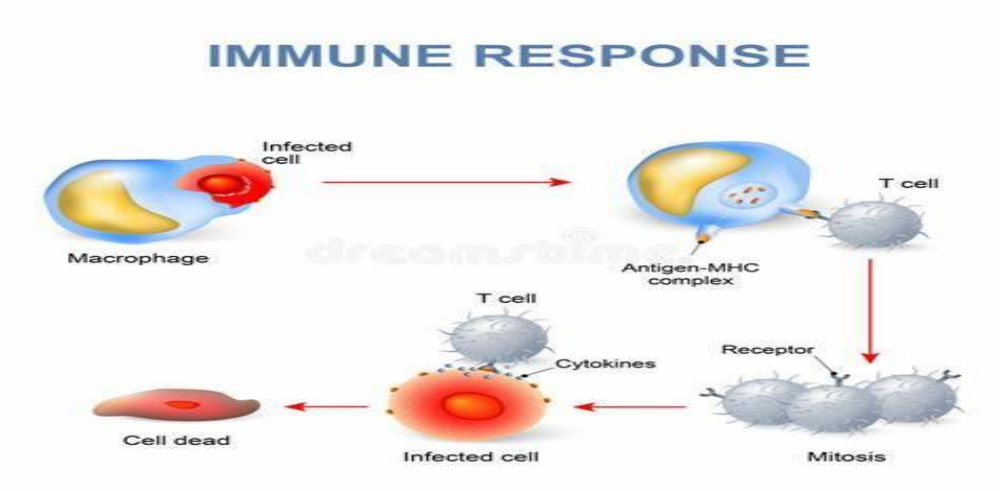
- When cytotoxic T cells are activated, they proliferate and differentiate into clones of:

1. Activated cytotoxic T cells: They destroy the antigen by:

• Directly attacking the cell expressing the antigen by inserting preforming molecules (perforins) into the membrane causing cell lysis.

•Secreting substances that attract macrophages to the site of the antigen, so that it is phagocytosed and killed.

2. Memory T cells: They remain dormant inside the body, and on second exposure to the same antigen these cells proliferate more rapidly and more powerfully than T lymphocytes.



B. Humoral Immunity→

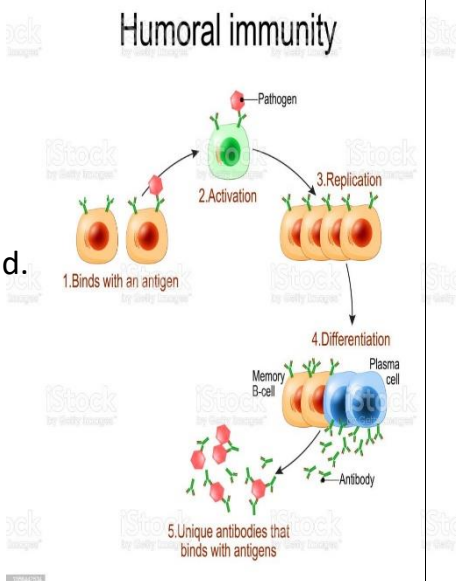
- B-lymphocytes are responsible for humoral immunity.

- Humoral immunity mainly acts against bacterial infections.

- B-lymphocytes are activated by the antigen when they come into direct contact with it. B-lymphocytes have membrane-bound. antibodies on their surface, which act as receptors for antigen recognition.

- Activated B-lymphocytes proliferate forming a clone of B-lymphocytes specific to this antigen.

These cells differentiate into:



1. **Plasma cells,** which contain a well-developed endoplasmic reticulum. The plasma cells secrete antibodies specific to the antigen. Secreted antibodies circulate in the blood. When they encounter the antigen, they combine with it and destroy it.
2. Memory B cells, which remain dormant until the antigen reenters the body. Then they launch a more rapid and more powerful secondary immune response.

-Mechanism of Action of Antibodies:

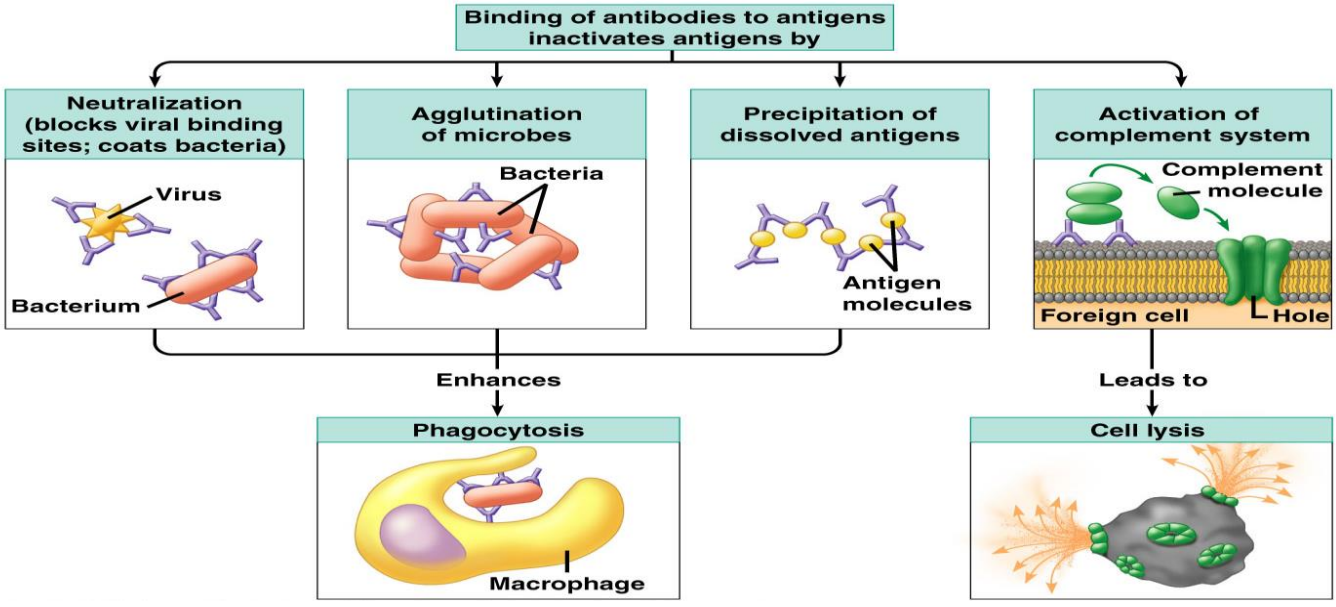
A. Direct attack of the antigen: Binding of antigen to its antibody causes:

1. Agglutination of bacteria, i.e., the antigens clumps together.

2. Precipitation of the antigens, i.e., when soluble antigens are bound to antibodies, they become insoluble and precipitate.

3. Lysis: Antibodies directly attack the cell membrane of bacteria, causing their rupture.

4. Opsonization: Some antibodies coat the microorganisms and make them "tasty" for Phagocytosis by neutrophils and macrophages.



B. Activation of the complement system.

-**INFLAMMATION** When tissue injury occurs, whether caused by bacteria, trauma, chemicals, heat, or any other phenomenon, multiple substances are released by the injured tissues and cause dramatic secondary changes in the surrounding uninjured tissues. This entire complex of tissue changes is called inflammation .

Inflammation is characterized by the following:

(1) vasodilation of the local blood vessels, with consequent increased local blood flow;

(2) increased permeability of the capillaries, allowing leakage of large quantities of fluid into the interstitial spaces;

(3) often, clotting of the fluid in the interstitial spaces because of increased amounts of fibrinogen and other proteins leaking from the capillaries;

(4) migration of large numbers of granulocytes and monocytes into the tissue; and

(5) swelling of the tissue cells.

AND THANK UUUUUUUUUUUUUUUUUUUUUUUU