Blood and Immunity

Blood is a viscous fluid which is pumped by the heart through a closed system of blood vessels. The normal total circulating blood volume is about 8% of the bodyweight (5600 ml in a 70 Kg man). 55% of this volume is plasma. Blood is composed of :

1. Plasma.

2. Cellular elements (red blood cells, white blood cells and platelets).

Functions of the Blood

1. Transport of O2, nutrients and hormones to the tissues.

2. Caries CO2 to the lungs and other products of metabolism to the kidneys for excretion.

3. Blood play a role in the regulation of body temperature.

4. Blood helps to maintain the pH and electrolytes concentrations of interstitial fluid within the range required for normal cell functions.

5. Blood has protective function such as combating invading microorganisms, mediating inflammation, initiating immune response to foreign materials and maintaining hemostasis.

Plasma

Plasma is a part of the extracellular fluid (ECF). The normal plasma volume is 4% of TBW (3500 ml in a 70 Kg man), plasma consist of an aqueous solution of proteins, electrolytes and small organic molecules.

The major type of plasma proteins are albumin (4.5 g/ dl), globulin (2.5 g/ dl) and fibrinogen (0.3 g/ dl).

Functions of plasma proteins

1. In colloid osmotic pressure, proteins exert an osmotic pressure of about 25 mmHg across the capillary wall, which is called the colliod osmotic pressure (oncotic pressure). It tends to pull water into the blood.

2. Buffer: plasma proteins are responsiple for 15% of buffering capacity of the blood because of weak ionization of (cooH) and (NH2) groups,

helping to keep the blood pH constant (at the normal plasma pH is 7. 4). CooH group act as hydrogen ion donor while NH2 group act as hydrogen ion acceptor. In alkaline solution, the protein CooH ionize act as acid and free protein anions, negatively charged while in acidic solution the protein NH2 group act as base, taking up hydrogen ions and the protein cary a positive charge.

3. Transport of other substances: In addition to lipids, hormones, plasma proteins also transport several metals and other substances such as transport copper by (Ceruloplasmin), bound hemoglobin (haptoglobin), T3and T4 hormones (thyroxine - binding globulin), non-heme iron is transported by transferrin present in β-globulin fraction. Calcium, Magnesium,some drugs, dyes and several cations and anions are transported by plasma albumin.

4. Defence function: circulating antibodies of the globulin fraction have play a special role in the body immune.

5. Bleeding arrest: **fibrinogen** and other plasma proteins are concerned with the **blood clotting.**

6. Protein reservoir

When **depleted tissue proteins**, the plasma protein can act as a **source** for rapid replacement of the tissue proteins. In addition to use **blood amino acid** to synthesis new tissue protein, also **plasma protein** become into the cell by **pinocytosis,** this proteins **split** into **amino acids** that back into the blood to synthesis cellular proteins. So the plasma proteins act as labile proteins storage medium.

Protein	Fraction	Concentration (mg/dl)	Molecular Weight (D)	Properties
Transthyretin	Prealbumin	15-35	62,000	Retinol transport, binds T_4
Albumin	Albumin	4000-5000	66,000	Colloid osmotic pressure, binding protein
Retinol-binding protein	α_1	3-6	21,000	Retinol transport
α_1-Antiprotease	α_1	200-400	54,000	Protease inhibitor
Thyroxine-binding globulin	α_1	<1.0	58,000	Major binding protein for T_3 and T_4
Transcortin	α_1	3-3.5	52,000	Binds glucocorticoids
α-Fetoprotein	α_1	0.002 (adults) 200-400 (fetus)		Elevated in adults with hepatoma
Ceruloplasmin	α_2	20-40	132,000	Contains copper
α_2-Macroglobulin	α_2	150-350	725,000	Protease inhibitor
Haptoglobin	α_2	50-300	100,000*	Binds hemoglobin
Transferrin	β	200-400	80,000	Binds iron
Hemopexin	β	50-120	60,000	Binds heme
Fibrinogen	β	150-400	340,000	Clot formation
C-reactive protein	γ	<0.2	125,000	Acute-phase reactant
Immunoglobulins	γ	700-1500	150,000-850,000	Very heterogeneous

Characteristics of Some Plasma Proteins, **Transthyretin**, **also called prealbumin.**

All the albumin and fibrinogen of plasma as well as **50 – 80%** of the **globulin** are formed in the **liver** and the remainder of the globulin is formed in the **lymphoid tissues,** mainly **globulin** that constitute the antibodies.

Hemopoiesis

Hemopoiesis means formation of the blood cells. This process occurs at the **different anatomical sites** from **emberyonic** to **adult** life.

1. In the **early few weeks** of emberyonic life, blood cells are

produced in the **yolk sac.**

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

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

4. By the age of **20 years** the **active red marrow** of long bones except humerus and femur have become **inactive**, yellow, fatty

marrow and no more blood cells produce.

5. **Beyond 20 years** blood cells normally formed in the **marrow** of flat or membranous bones as vertebrae, sternum, ribs and pelvis, also proximal ends of humerus and femur. Even in these bones the marrow becomes less productive with the age increases.

Bone marrow

The bone marrow is actually one of the largest organs in the body, approaching the size and weight of the liver. It is also one of the most active. Normally, 75% of the cells in the marrow belong to the white blood cell-producing myeloid series and only 25% are maturing red cells, even though there are over 500 times as many red cells in the circulation as there are white cells. This difference in the marrow reflects the fact that the average life span of white cells is short, whereas that of red cells is long.

Bone marrow is composed of

1. Active cellular marrow which is called red marrow.

2. Inactive marrow that infiltrated with fat which is called yellow marrow

Genesis of Blood Cells

The blood cells begin their lives in the bone marrow from single type of cell called the *pluripotential hematopoietic stem* cell, from which all the cells of the circulating blood are eventually derived. The hematopoietic stem cells (HSCs) differentiate into one or another type of **committed stem cells (progenitor cells)**. The different committed stem cells, when grown in culture, will produce colonies of specific types of blood cells. A committed stem cell that produces erythrocytes is called a colony-forming *unit-erythrocyte,* and the abbreviation CFU-E is used to designate this type of stem cell. Likewise, colony-forming units that form granulocytes and monocytes have the designation CFU-GM .

Growth and reproduction of the different stem cells are controlled by multiple proteins called **growth inducers**. One of these, *interleukin-3,* promotes growth and reproduction of virtually all the different types of committed stem cells, whereas the others induce growth of only specific types of cells.

The growth inducers promote growth but not differentiation of the cells. This is the function of another set of proteins called **differentiation**

inducers. Each of these causes one type of committed stem cell to differentiate one or more steps toward a final adult blood cell.

Formation of the growth inducers and differentiation inducers is itself controlled by factors outside the bone marrow. For instance, in the case of erythrocytes (red blood cells), exposure of the blood to low oxygen for a long time causes growth induction, differentiation, and production of greatly increased numbers of erythrocytes, infectious diseases cause growth, differentiation, and eventual formation of specific types of white blood cells that are needed to combat each infection.

Factor regulating hemopoiesis (hemopoietic growth factors)

1. **Erythropoietin**

stimulus for red blood cell production in low oxygen states is a circulating hormone called *erythropoietin,* a glycoprotein with a molecular weight of about 34,000. 90% of Erythropoietin is produced in the kidney and the remainder from other tissue like liver.

erythropoietin begins to be formed within minutes to hours, and it reaches maximum production within 24 hours. Yet almost no new red blood cells appear in the circulating blood until about 5 days later. From this fact, as well as from other studies, it has been determined that the important effect of erythropoietin is to stimulate the production of proerythroblasts from hematopoietic stem cells in the bone marrow, once the proerythroblasts are formed, the erythropoietin causes these cells to pass more rapidly through the different erythroblastic stages than they normally do. The rapid production of cells continues as long as the person remains in a low oxygen state or until enough red blood cells have been produced to carry adequate amounts of oxygen to the tissues despite the low oxygen; at this time, the rate of erythropoietin production decreases to a level that will maintain the required number of red cells but not an excess.

In the absence of erythropoietin, few red blood cells are formed by the bone marrow. At the other extreme, when large quantities of erythropoietin are formed and if there is plenty of iron and other required nutrients available, the rate of red blood cell production can rise to perhaps 10 or more times normal. Therefore, the erythropoietin mechanism for controlling red blood cell production is a powerful one.

2. Colony – stimulating factors (CSFs): are secreted glycoproteins that bind to receptor proteins on the surfaces of hemopoietic stem cells, thereby activating intracellular signaling pathways that can cause the cells to proliferate and differentiate into a specific kind of blood cell such as

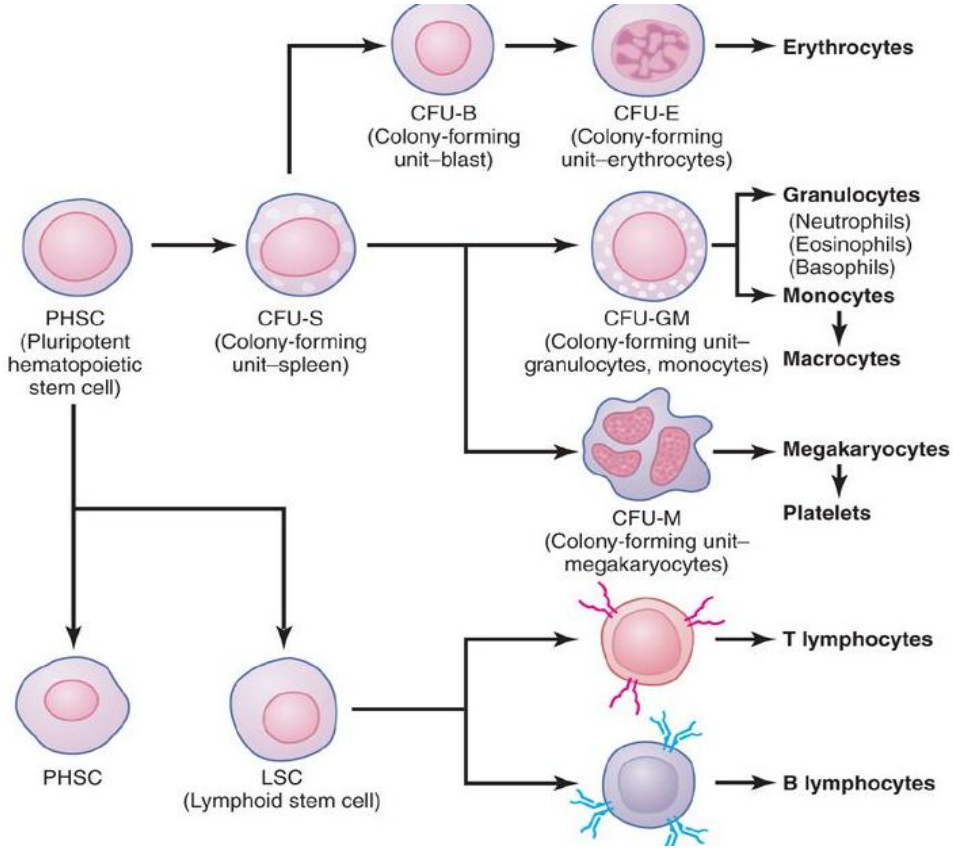
a. Macrophage colony-stimulating factor (MCSF) or CSF1

b. Granulocyte macrophage colony-stimulating factors (GM-CSF) or CSF2

c. Granulocyte colony-stimulating factors (G-CSF) or CSF3

3. Interleukins (ILs) such as IL 1,2,3,4,5,6,7,12,13, they act in sequence to convert uncommitted stem cells to committed stem cells

Also other factors are produced by macrophages, active T – lymphocytes, fibroblast and endothelial cells.



Red Blood Cells (Erythrocytes)

The normal red blood cells (RBCs) are non – nucleated , biconcave discs. The red cell membrane is flexible and exhibits a remarkable deformed into any shape, RBCs able to change its shape and can be pass through narrow

capillaries and return. The biconcave shape of the RBC provides a high surface area to volume ratio, mean diameter 8µm and thickness is 2.5µm. The normal range in adult male is 4.5 – 6.5 million / cu.mm and in adult female is 3.5 – 5.8 million / cu.mm.

A major function of red blood cells is to transport *hemoglobin*, which in turn carries oxygen from the lungs to the tissues. When it is free in the plasma of the human being, about 3 percent of it leaks through the capillary membrane into the tissue spaces or through the glomerular membrane of the kidney into the glomerular filtrate each time the blood passes through the capillaries. Therefore, hemoglobin must remain inside red blood cells to effectively perform its functions in humans.

The red blood cells have other functions, they contain a large quantity of *carbonic anhydrase*, an enzyme that catalyzes the reversible reaction between carbon dioxide (CO_2) and water to form carbonic acid (H_2CO_3), increasing the rate of this reaction several thousand fold. The rapidity of this reaction makes it possible for the water of the blood to transport enormous quantities of CO_2 in the form of bicarbonate ion from the tissues to the lungs, where it is reconverted to CO_2 and expelled into the atmosphere as a body waste product. The hemoglobin in the cells is an excellent *acid-base buffer* (as is true of most proteins), so the red blood cells are responsible for most of the acid-base buffering power of whole blood.

The life span of the normal RBCs in the circulation is 120 days, after that the old RBCs are destroyed by macrophages in the mononuclear phagocyte system as liver, spleen and bone marrow. The basic substances needed for normal RBCs production are amino acids (proteins), iron, vitamin B_{12}, folic acid and vitamin $B_{6.}$

Erythrogenesis (Erythropoiesis)

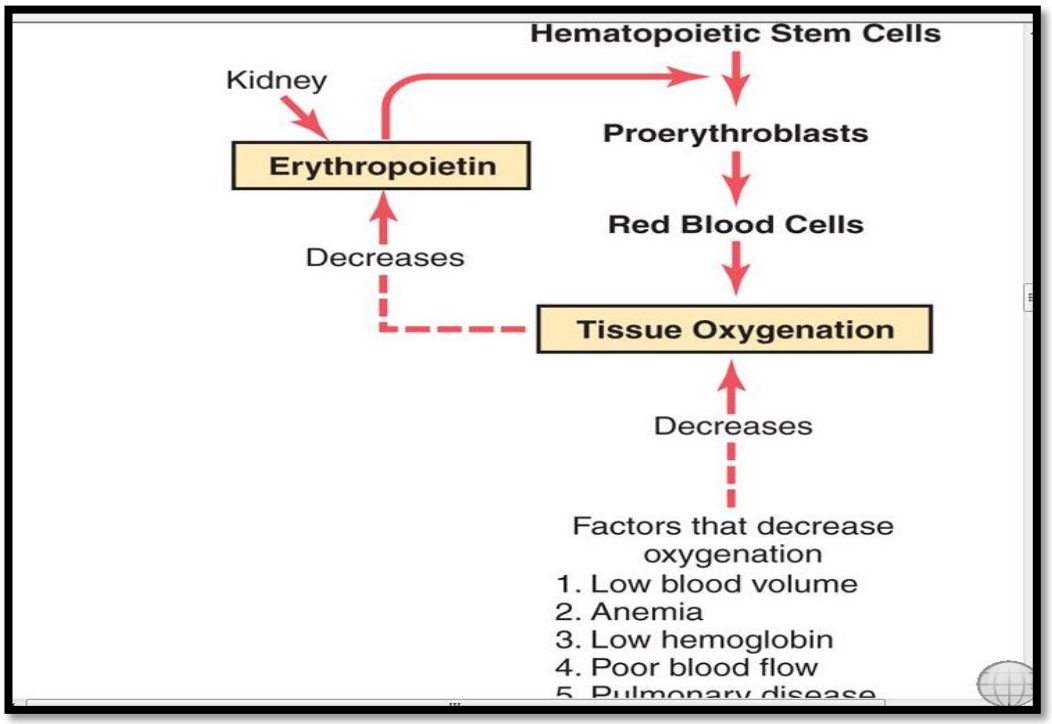
Erythrogenesis is the process of erythrocytes formation. Clinical measurment of erythrogenesis activity by reticulocytes count, because of reticulocytes passes to the blood and after 1 – 2 days in the blood it becames mature erythrocyte. The normal range of reticulocytes in adult is 0.5 – 2.5 %.

Regulation of Erythrogenesis

The main factor stimulating RBCs production is hypoxia (O_2 deficiency at the tissue level), therefore any condition that causes decrease of O_2 transport to the tissues lead to increase of RBCs production such as

1. Low blood flow.
2. Anemia.
3. Low hemoglobin.
4. Poor blood flow.
5. pulmonary disease.

Hypoxia does not act directly on bone marrow but stimulates the secretion of important regulating hormone like erythropoietin. Other factors are stimulating erythropoietin as cobalt, epinephrine, norepinephrine, prostaglandine and androgen.



Function of the erythropoietin mechanism to increase production of red blood cells when tissue oxygenation decreases.

Maturation of Red Blood Cells-Requirement for Vitamin B_{12} and Folic Acid

A common cause of red blood cell maturation failure is failure to absorb **vitamin B_{12} (Cyanocobalamin)** from the gastrointestinal tract. This often occurs in the disease *pernicious anemia,* in which the basic abnormality is an *atrophic gastric mucosa* that fails to produce normal gastric secretions. The parietal cells of the gastric glands secrete a glycoprotein called *intrinsic factor,* which combines with vitamin B_{12} in food and makes the B_{12} available for absorption by the gut. It does this in the following way:

1. Intrinsic factor binds tightly with the vitamin B_{12}. In this bound state, the B_{12} is protected from digestion by the gastrointestinal secretions.
2. Still in the bound state, intrinsic factor binds to specific receptor sites on the brush border membranes of the mucosal cells in the ileum.

3. Vitamin B_{12} is transported into the blood during the next few hours by the process of pinocytosis, carrying intrinsic factor and the vitamin together through the membrane. Lack of intrinsic factor, therefore, decreases availability of vitamin B_{12} because of faulty absorption of the vitamin.

Once vitamin B_{12} has been absorbed from the gastrointestinal tract, it is first stored in large quantities in the liver and then released slowly as needed by the bone marrow. The minimum amount of vitamin B_{12} required each day to maintain normal red cell maturation is only 1 to 3 micrograms, and the normal storage in the liver and other body tissues is about 1000 times this amount. Therefore, 3 to 4 years of defective B_{12} absorption are usually required to cause maturation failure anemia.

Folic acid (Pteroylglutamic Acid) is a normal constituent of green vegetables, some fruits, and meats (especially liver). However, it is easily destroyed during cooking. Also, people with gastrointestinal absorption abnormalities, such as the frequently occurring small intestinal disease called *sprue,* often have serious difficulty absorbing both folic acid and vitamin B_{12}. Therefore, in many instances of maturation failure, the cause is deficiency of intestinal absorption of both folic acid and vitamin B_{12}.

Hematocrit (HCT) or Packed ell volume (PCV)

The PCV or HCT is the volume of RBCs per unit volume of the whole blood. The normal range in adult male is 40 – 54 % and in the adult female is 37 – 47 % . PCV is affected by

1. Number of RBCs.

2. Shape of the RBCs.

3. Plasma volume.

Clinical Uses of PCV

PCV is used to detect

a. Anemia.

b. polycythemia.

c. hemoconcentration and hemodilution.

PCV is increased in polycythemia and dehydration but is decreased in anemia.

Polycythemia

Plycythemia means that the red blood cell count commonly rises to 6 to 7 million/mm^3, about 30 percent above normal value. There are two types of polycythemia

1. Physiological polycythemia

A common type of secondary polycythemia, called *physiologic 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/mm^3; this allows these people to perform reasonably high levels of continuous work even in a rarefied atmosphere.

2. Polycythemia Vera (Erythremia)

This type is occurred in pathological condition in which the red blood cell count may be 7 to 8 million/mm^3 and the hematocrit may be 60 to 70 percent instead of the normal 40 to 45 percent. Polycythemia vera is caused by a genetic aberration in the hemocytoblastic cells that produce the blood cells. The blast cells no longer stop producing red cells when too many cells are already present. This causes excess production of red blood cells in the same

manner that a breast tumor causes excess production of a specific type of breast cell. It usually causes excess production of white blood cells and platelets as well.

Erythrocyte Sedimentation Rate (ESR)

ESR is the rate at which the RBCs sediment by rouleax formatrion (RBCs aggregate and become arranged one on the top of the other). ESR is not specific test but it helps in the diagnosis of the diseasis. The normal range in adult male is 0 – 15mm / hr and in the adult female is 0 – 20mm / hr.

Factors affecting ESR

1. Composition of plasma protein
Increase concentration of fibrinogen or globulin lead to increase rouleaux formation result in increase in the ESR.

2. Concentration of RBCs
Icrease RBCs concentration or high PCV lead to icrease the viscosity of the blood lead to increase to the sedimentation, result in decrease in the ESR.

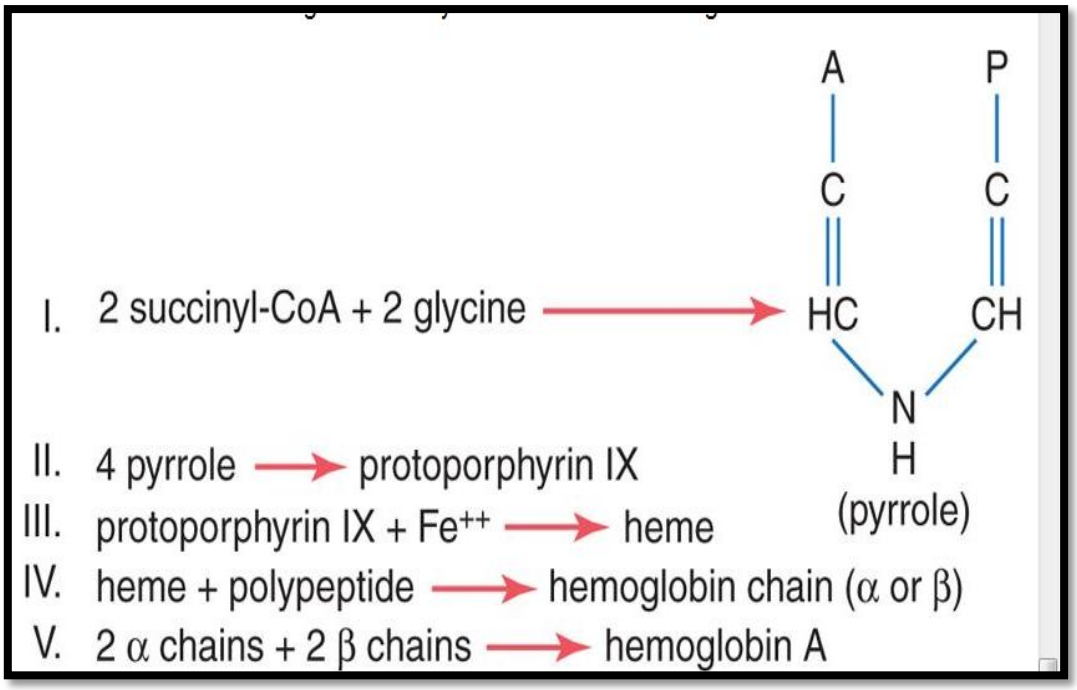
3. Shape of RBCs
RBCs with abnormal or irregular shapes as in sickl cell anemia and spherocytosis, this interfer with the rouleaux formation or unable to form rouleaux lead to decrease in the ESR.

Hemoglobin (Hb)

the basic chemical steps in the formation of hemoglobin: First, succinyl-CoA, formed in the Krebs metabolic cycle ,binds with glycine to form a pyrrole molecule. In turn, four pyrroles combine to form protoporphyrin IX, which then combines with iron to form the *heme* molecule. Finally, each heme molecule combines with a long polypeptide chain, a *globin* synthesized by ribosomes, forming a subunit of hemoglobin called a *hemoglobin chain* (α and β). Each chain has a molecular weight of about 16,000; four of these in turn bind together loosely to form the whole hemoglobin molecule.

Hb begins synthesis in proerythroblast and contineous slightly even into the reticulocytes stage. Mature RBCs can not synthesize Hb. Red blood cells have the ability to concentrate hemoglobin in the cell fluid up to about 34 grams in each 100 milliliters of cells. The concentration does not rise above this value because this is the metabolic limit of the cell's hemoglobin-forming mechanism In normal people, the percentage of hemoglobin is almost always

near the maximum in each cell.



Synthesis of Hb

The affinity of Hb for O_2 is affected by PH, temperature and concentration of RBCs with the 2, 3 diphosphoglycerate (product of glucose metabolism). Thus the increase in this factors lead to decrease in the affinity of Hb to O_2.

Hb is broken into its constituents (globin and heme). Globin is catabolized in the liver into amino acid the enters the circulation. Heme is converted into biliverdin , in turn converted into bilirubin, enters the liver where its conjucated and becomes water – soluble and secretsby liver into the bile.

Jundice is the yellowish discoloration of skin and mucous membrane due to increase bilirubin concentration in the body fluids, is detectable when plasma bilirubin above 2 mg / dl.

Normal Hb Types

1. Hb A : is the normal Hb in adult, consist of 2α and 2β chains, Hb is predominant type in adult 96 – 98 % of total Hb.

2. Hb A_2 : this type is presence in the normal adult, about 1.5 – 3 % of the total Hb, consist of 2 α and 2 δ (delta).

3. Hb F : the main type of Hb in fetus and new born, consist of 2α and 2 γ (gamma). In the normal adult is 0,5 – 1 % of total Hb.

4. Embryonic Hb

a. Gower 1 Hb : consist of 2 zeta and 2 epsilon chains .

b. Gower II Hb : consist of 2 alpha and 2 epsilon chains.

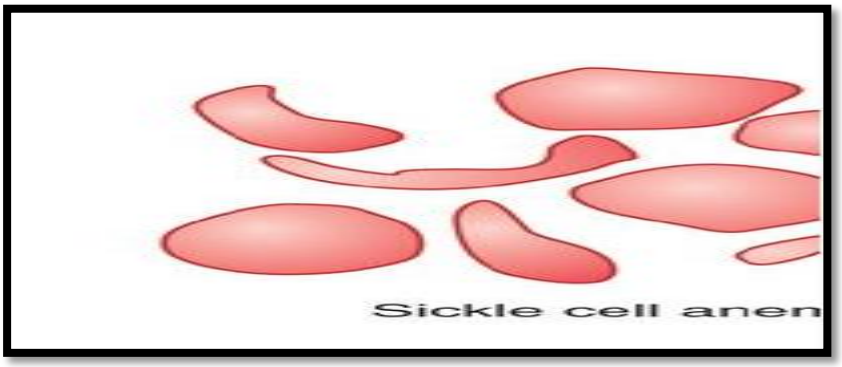
Genetic abnormalities of Hb (hemoglobinpathies)

1. Sickle – cell Hb or Hb S

Hb S has normal α chains but in each β chain glutamic acid at the sixth position is replaced by valine.Thus the RBCs become sickle and lose their deformability and hemolyze.

2. Thalassaemias

In this type the amino acid sequence is normal but polypeptide chains are impaired or absent like α and β thalassaemias are decreased or absent α and β polypeptides.



Sickle Cell Anemia

Anaemia

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

Types of anemia

1. Aplastic anemia

In this type bopne marrow may be destroyed and become unable to prpduce blood cells as in excessive X – ray exposure.

2. Hemolytic anemia

Hemolytic anemia is occurred due to excessive destruction of RBCs as a result of abnormalities of red cell membrane or Hb such as hereditary spherocytosis (RBCs are small , spherical in shape and fragile), sickle cell anemia.

3. Hemorrhagic anemia

a. Acute hemorrhagic anemia : In this type loss of large volume of blood over a short period. After rapid hemorrhage, replaces the plasma within 1-3 days.

b. Chronic hemorrhage: loss of small volume of blood over long period, thus this patient need to form a new RBCs.

4. Megaloblastic Anemia : deficiency of vitamin B12, folic acid, and intrinsic factor from the stomach mucosa, lead to slow reproduction of erythroblasts in the bone marrow. As a result, the red cells grow too large, with odd shapes that called megaloblasts.

Red cell indices

Red cell indices (absolute value of RBCs) are MCV, MCH, MCHC. They can be used to classify anemia according to the sizeof RBCs and their Hb concentration.

1. MCV (mean corpuscular or cell volume) : MCV is the average volume of red cell. The normal range in adult is 70 – 96 fl. Therefore:

a. If the MCV within the normal range, they called normocyte as in acute blood loss anemia.

b. If the MCV less than the normal value, so the RBCs called microcytes as in iron deficiency anemia.

c. If the MCV above the normal value , are called macrocyte as in B12 and folic acid deficiency anemia.

2. MCHC (mean corpuscular or cell Hb concentration) : It is the weight of Hb in 100 ml of packed red cell. The normal range is 31- 35g / L. Therefore:

a. If the MCHC within the normal value, so is called normochromic as in acute blood loss anemia.

b. If the MCHC less than the normal value, the RBC is hypochromic asin iron deficiency anemia .

3. MCH (mean corpuscular or cell Hb) : MCH is the average weight of Hb in RBC. The normal value is 27 – 32 pg (pictogram).

MCH express the Hb amount in absolute unit without taking cell size, thus its limited use and less useful in the classification of anemia

Platelets (Thrombocytes)

Platelets are the smallest cellular elements of the blood , disc - shaped. They are non nucleated granulated bodies, 2 – 4 µm in diameter, they are formed in bone marrow from megakaryocytes which are fragmented into the platelets and released into the circulation . The normal range in adult is 150,000 – 400,000 cells / cu.mm.

The life span is 7 – 10 days, then they are eliminated from the circulation mainly by the tissue macrophage system. The platelets serve multiple functions in the hemostasis and defence mechanism in the body.

Factors regulated platelets production:

The production is regulated:

1. Interlukins (IL 1, 3, 6)

2. Colony – stimulating factors that control the production of megakaryocytes.

Bilipid membrane arround the platelet contains several important glycoproteins they act as surface receptors for collagen, vessel wall von willebrand factor (VWF). The binding of VWF is necessary for platelet adhesion (the first step in platelet function).

The **cytoplasm** contain actin, myosin, glycogen, lysosome and two types of granules.

1. Dense granules : which contain non protein substances such as ADP, ATP, serotonin.

2. α – granules : which contain protein substances as clotting factors and platelet – derived growth factor (PDGF) which stimulates wound healing.