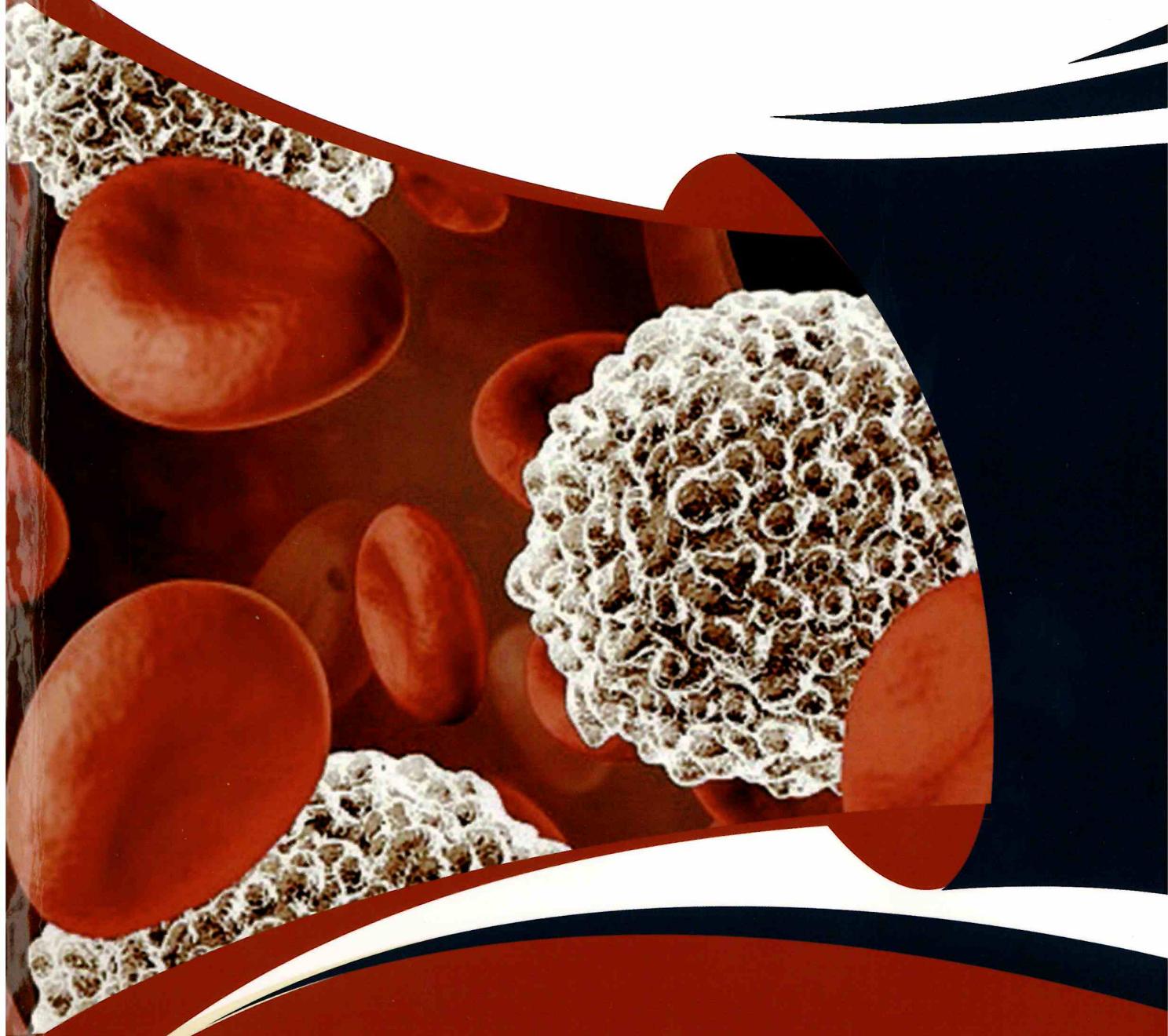




FACULTY OF
MEDICINE | كلية الطب



BLOOD, LYMPH AND IMMUNE SYSTEM

Alexandria Faculty Of Medicine

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CHAPTER I- INTRODUCTION AND PLASMA FUNCTIONS

After studying this chapter the student should be able to:

1. Identify the physical properties of blood including specific gravity, pH and viscosity
2. List blood cell types and identify components of plasma proteins
3. Discuss the functions of plasma proteins
4. Describe the role of plasma proteins in regulation of blood volume.

About 60% of the adult human body is fluid, mainly a water solution of ions and other substances. Although most of this fluid is inside the cells (intracellular fluid), about one third is in the spaces outside the cells (extracellular fluid). This extracellular fluid is in constant motion throughout the body. It is transported rapidly in the circulating blood and then mixed between the blood and the tissue fluids by diffusion through the capillary walls.

This chapter discusses the properties of blood, with focus on plasma.

I-A. Introduction

Blood is a connective tissue fluid that circulates through the body to maintain a constant environment around the body cells.

Blood is the main transportation vehicle of the body; it carries O₂ and nutrients to the tissues and returns CO₂ to the lung and other products of metabolism to the kidneys. It carries and distributes hormones and other agents that regulate cell functions. It functions also in regulation of body temperature, acid base equilibrium and water balance.

Blood plays a vital protective function against infection. Furthermore, injury to blood vessels is followed by blood clotting which stops further loss of this vital fluid. Thus blood helps to maintain homeostasis and to coordinate activities of various organs.

Blood is a highly complex fluid in which cellular elements are suspended. The fluid portion is the plasma, which forms 55% of blood volume (which averages around 5 litres). The cellular elements include red blood cells, white blood cells and platelets, and this form the remaining 45%. Blood constitutes about 8% of body weight.

Physical properties of blood

1. Colour

Arterial blood is bright red due to presence of oxyhemoglobin. Venous blood is dark red (bluish) due to presence of reduced hemoglobin.

2. Viscosity

Blood is 5 times more viscous than water. This high viscosity is due to presence of cells (mainly RBCs) and plasma proteins. Blood viscosity decreases in cases of anemia and hypoproteinemia and increases in cases of polycythemia and dehydration.

Blood viscosity is essential for maintaining normal arterial blood pressure (ABP) by preventing rapid flow of blood from arteries to veins.

3. pH

The pH of arterial blood is 7.4±0.02. That of venous blood is 7.38±0.02.

4. Specific gravity

Specific gravity of whole blood is 1060. Specific gravity of cells is 1090, while that of plasma is 1030.

I-B. Blood constituents

A. Blood plasma: it is the fluid component in which blood cells are suspended.

B. Formed blood elements (Figure 1)

- Red blood corpuscles (RBCs) or erythrocytes.
- White blood cells (WBCs) or leukocytes including the *granular leukocytes* (neutrophils, eosinophils and basophils) and the *agranular leukocytes* (lymphocytes and monocytes).
- Blood platelets.

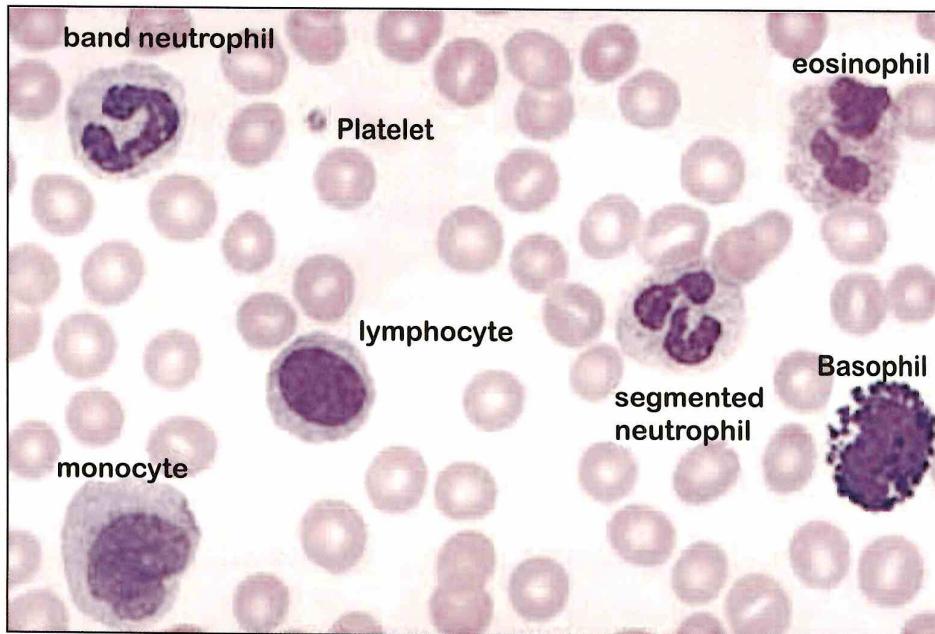


Figure 1. The formed blood elements

I-C. Plasma

Plasma is a remarkable solution containing numerous ions, and inorganic and organic molecules that aid the transport of other substances. Normal plasma volume is about 3 litres.

Constituents of plasma

1. Water (90%)
2. Inorganic constituents (1%)
e.g. sodium, chloride, calcium, potassium

3. Organic constituents (9%).

These include:

- a- Plasma proteins (7%)
- b- Nutrients & waste products (2%)

Plasma proteins

Their concentration ranges from 7-9 g/dL. Plasma proteins include:

- Albumin (~4.8 g/dL)
- Globulins (α , β , γ) (~2.7 g/dL)
- Fibrinogen (~0.2 g/dL)
- Prothrombin (~0.02 g/dL)

Sources of plasma proteins

1. Albumin, fibrinogen, prothrombin and most of alpha and beta globulins are formed in the liver.
2. Gamma globulins are manufactured in the lymphoid tissues.

Plasma proteins are continuously used by the tissues i.e. there is continuous turnover between proteins in the liver, plasma and tissues. They are dynamic and not static.

Functions of plasma proteins

1. Protein metabolism

Plasma proteins are constantly used by the tissues for their protein metabolism (thus they are dynamic).

2. Blood coagulation

Some of the plasma proteins (the clotting factors) are needed for blood coagulation. The most important of these are prothrombin and fibrinogen.

3. Immunity

Antibodies (gamma globulins) protect the body against pathogenic organisms and their toxins.

4. Transport of important substances

Plasma proteins function in the transport of hormones such as thyroid, adrenal and gonadal hormones from endocrine glands to target organs, thus preventing their rapid excretion through the kidney. They also serve as carriers for metals including iron, as well as fatty acids, amino acids, enzymes, drugs and carbon dioxide.

5. Regulation of blood volume

Plasma proteins (specially albumin) exert a colloid osmotic pressure of about 25 mmHg. As the capillary walls are relatively impermeable to plasma proteins, this osmotic force tends to pull water into the blood, thus maintaining blood volume.

6. Buffering action

The plasma proteins are responsible for 15% of the buffering power of the blood, which helps keeping the pH of tissues almost constant. Arterial blood has a pH of 7.4 ± 0.02 , while that of venous blood is 7.38 ± 0.02 .

Proteins are present in the blood as weak acids and their salts (proteinic acid and sodium proteinate).

7. Regulation of arterial blood pressure

Whole blood is five times and plasma is two times as viscous as water. Blood viscosity is due to red blood cells and plasma proteins. Blood viscosity is responsible partially for the peripheral resistance, which is the resistance that the blood meets during its passage through the peripheral narrow blood vessel. It prevents the rapid outflow of blood through the vessels, thus maintaining normal arterial blood pressure.

CHAPTER II. RED BLOOD CORPUSCLES

After studying this chapter the student should be able to:

1. Recognize the methods for examining and counting the blood elements.
2. Recognize the general and structural features of red blood corpuscles.
3. Correlate the structure to functional adaptation of red blood corpuscles.
4. Mention the types of bone marrow and describe the histological components of the myeloid tissue.
5. Define hemopoiesis and name the different colony forming units involved in formation of the blood elements.
6. List and describe different stages of erythropoiesis.
7. Describe the regulatory role of erythropoietin in erythropoiesis, and feedback mechanism.
8. Discuss the nutritional factors required for erythropoiesis, and their sources
9. List the key hormones needed for normal erythropoiesis
10. Describe globin structure and state the types of normal and abnormal hemoglobin.
11. Describe heme structure and importance of the heme molecule
12. Outline hemoglobin abnormalities that may occur in sickle cell disease, thalassemia and methemoglobinemia in light of their causes.
13. Describe biosynthesis of heme and discuss its regulation and abnormalities inc. porphyria.
14. Integrate structural abnormalities of hemoglobin to deviation of its function from normal.
15. Discuss importance of glycolysis and hexose monophosphate shunt for RBC metabolism.
16. Describe the fate of RBCs and catabolism of hemoglobin
17. Discuss the dynamics of iron absorption
18. List the proteins and carriers involved in iron transport and storage
19. Illustrate how iron balance is maintained in the body
20. Discuss iron metabolism

Red blood cells are the most abundant cells of the blood and are necessary for the delivery of oxygen to the tissues.

This chapter discusses red cell structure, function and formation (hemopoiesis), and the role and metabolism of hemoglobin.

II-A. Formed blood elements

Preparation of blood for laboratory study

1-Blood smear (blood film)

To examine or count the formed blood elements, a blood smear must be prepared. Stains used in blood smear e.g. Giemsa's stain are designed to differentiate blood cells by their nuclei and cytoplasmic granules.

2-Blood count

It is the *average number of a particular formed blood element per cubic millimeter blood*. Recently, blood count is done by an automated hemocytometer. Accordingly, we can perform:

- RBCs count.

- Total leukocytic count.

- Platelets count.

- *Differential leukocytic count*: it is the percentage of each type of leukocytes relative to the total number of WBCs. It is done by counting the different types of leukocytes in blood smear. This count can be of diagnostic significance as it is associated with certain disease states.

Histologically, blood is considered a specialized type of connective tissue. The plasma represents the abundant matrix while the formed blood elements represent the cells. However, blood lacks the fibrous component of connective tissue and its cells originate from the stem cells of the myeloid tissue present in red bone marrow.

II-B. Structure of red blood corpuscles

Red blood cells or erythrocytes (erythrum = red, cytes = cells) are so called because they are responsible for the red color of blood due to their content of hemoglobin. The circulating RBCs are *non-nucleated cells* as they lack the nucleus. Hence, they are rather called red blood corpuscles than true cells.

Average RBC count

In normal males is 5-5.5 millions/mm³ blood,

In normal females is 4.5-5 millions/mm³ blood.

Life span of RBCs

It is about 100-120 days. Old & deformed RBCs are recognized and phagocytosed by the macrophages in the spleen and liver.

LM picture of RBCs in blood film

- Shape: eosinophilic biconcave discs.
- Diameter: ranges between $7.2\text{-}7.8\mu\text{m}$. Almost the size of the smallest blood capillaries.
- Nucleus: absent.
- Cytoplasm: hemoglobin occupies about 33% of the corpuscular volume and is more concentrated at the periphery.

EM picture of RBCs (Figure 2)

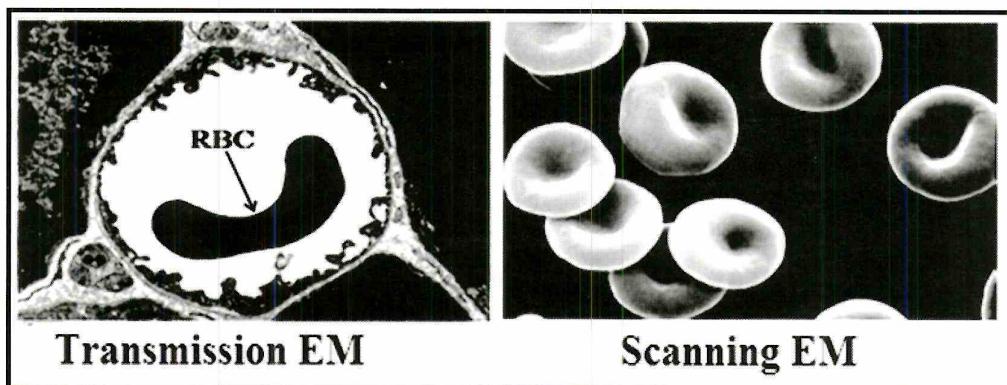


Figure 2. EM picture of RBCs

- Shape: membrane- bounded electron- dense biconcave discs.
- Cell organelles: no nucleus and no typical organelles.

RBC membrane

- It is covered from outside by prominent glycocalyx coat, which is responsible for the blood grouping.
- It is supported from inside by a well-developed cytoskeleton, which is responsible for the flexibility of RBCs. It is formed of a network of microfilaments (actin & spectrin), that are attached to the plasma membrane peripheral protein (ankyrin).

Adaptation of RBCs structure to function

The histological characteristics of RBCs are designed to increase the gaseous carriage capacity of these corpuscles. These include:

- 1- The biconcave discoid shape increases the surface area of the RBCs by 20-30% more than if they had a spherical shape.
- 2- The lack of a nucleus and organelles allows more space in the cytoplasm for carrying the maximal amount of hemoglobin. This hemoglobin has been synthesized earlier during the process of erythrocytes formation in the bone marrow.
- 3- The concentration of hemoglobin at the periphery more than in the center of the corpuscle facilitates its binding to oxygen and carbon dioxide gases.
- 4- The structure of the cytoskeleton helps the RBCs to be squeezed as they pass through the narrowest blood capillaries without being damaged.
- 5- The corpuscular membrane is selectively permeable to oxygen and carbon dioxide gases to enhance their diffusion rates during the gaseous exchange processes.

II-C. The myeloid tissue

The myeloid tissue or the red bone marrow is the tissue responsible for formation of all formed blood elements in postnatal life.

Sites

- The central bone marrow cavity in young long bones.
- The marrow cavities between the bone trabeculae of cancellous bone.

Types of bone marrow

- 1- Red bone marrow: active in hemopoiesis.
- 2- Yellow bone marrow: inactive bone marrow in which the blood formation has stopped. Its yellow color is attributed to the presence of a large number of fat cells in its stroma. It can revert to the red type in stress conditions as hemorrhage and anemia.

Histological structure of myeloid tissue (Figure 3)

I-The stroma. It includes:

- **Reticular fibers:** they form a network supporting the myeloid cells and blood sinusoids.
- **Stromal cells:** they represent the fixed cell population of the marrow stroma. They include:
 - **Reticular stromal cells:** their function is to produce the reticular fibers and secrete growth factors that stimulate the marrow stem cells. In yellow bone marrow, these cells are changed to adipocytes by accumulating large fat droplets.
 - **Macrophages:** their function includes phagocytosis of aged RBCs as well as the malformed blood elements and storage of iron for further utilization in hemopoiesis.
 - Some **adipocytes:** they represent one of the largest cells in bone marrow.

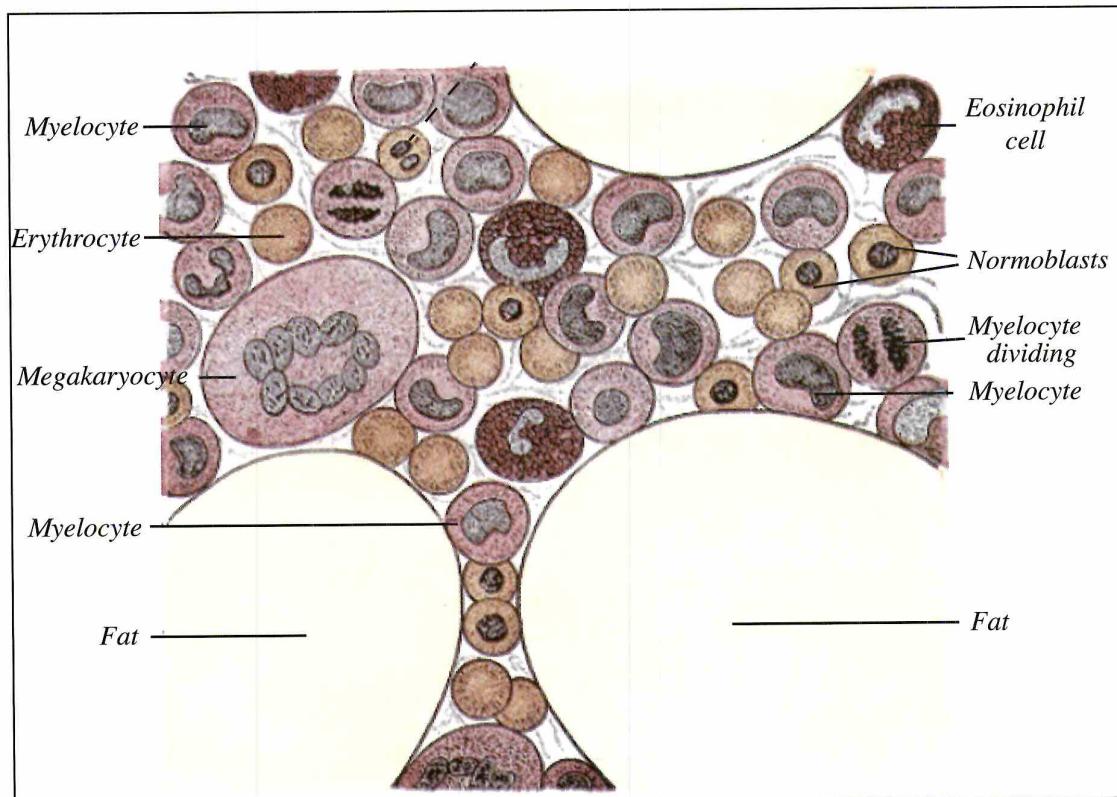


Figure 3. Diagram of myeloid tissue

II-The blood sinusoids

They are wide, irregular vascular channels lined by fenestrated endothelial cells.

III-The myeloid cells

These cells represent the free cell population of the bone marrow. They include:

1- Pluripotent hemopoietic stem cells (PHSCs):

- They have great ability to mitotic division.
- They are relatively small cells with faint basophilic cytoplasm and euchromatic nuclei.
- When they divide, they give rise to daughter cells; half of which remains as a reserve of the HPSCs population while the other half develops into the more differentiated multipotent hemopoietic stem cells (MHSCs).

2- Multipotent hemopoietic stem cells (MHSCs):

- They can undergo several cell divisions, giving rise to more differentiated cell colonies for each of the formed blood elements.
- Hence, there are 2 types of colony forming units (CFUs);
 - ***Colony forming unit- lymphocyte (CFU- Ly)***: complete their maturation giving rise to lymphocytes.
 - ***Colony forming unit- granulocyte, erythrocyte, monocyte, megakaryocyte (CFU- GEMM)***: further differentiate into the ***unipotent progenitors*** giving rise to:
 - i. CFU-Erythrocyte (CFU-E),
 - ii. CFU-Megakaryocyte (CFU-Meg),
 - iii. CFU-Neutrophil (CFU-G),
 - iv. CFU-Eosinophil (CFU-Eo),
 - v. CFU-Basophil (CFU-Ba),
 - vi. CFU-Monocyte (CFU-M).
- These cells are larger and show more basophilia in their cytoplasm as they contain abundant ribosomes that initiate the synthesis of the specific products of each blood element.

II-D. Erythropoiesis

Hemopoiesis (hematopoiesis) is the formation of balanced amounts of the different blood elements. The number of the daily formed blood elements must equalize the amount of the daily destroyed elements. Hemopoiesis includes: *erythropoiesis, thrombopoiesis, granulopoiesis, monopoiesis, and lymphopoiesis.*

Erythropoiesis

It is the formation of RBCs. The whole process for production of mature RBCs takes about 7 days. Erythropoiesis involves the following stages:

1. PHSCs.
2. MHSCs.
3. Colony forming unit- erythrocytes (CFU-E).
4. Proerythroblasts: they are the *first recognizable* erythrocyte precursor. They are large cells with basophilic cytoplasm.
5. Basophilic erythroblasts: at this stage hemoglobin synthesis is most active. The cytoplasm is *strongly basophilic* due to abundant polysomes.
6. Polychromatophilic erythroblasts: hemoglobin is accumulating in large quantities in the cytoplasm, ribosomes are still abundant. Accordingly, the cytoplasm shows eosinophilic areas alternating with basophilic spots. These cells represent the *last stage* in which the cells undergo repeated cell divisions. The coming phases involve *morphological maturation* of the erythroblasts.
7. Orthochromatophilic erythroblasts (normoblasts): the synthesis of hemoglobin is completed and ribosomes are much reduced in number. The nucleus becomes small and condensed. It is gradually pushed towards the periphery, extruded from the cell to be phagocytosed by the bone marrow macrophages.
8. Reticulocytes: they are non-nucleated immature RBCs. *They differ from mature RBCs in that:*
 - They are slightly larger.
 - Their cytoplasm contains remnants of ribosomes and other organelles which, on staining with cresyl blue stain, form a reticulate pattern.
 - They represent about 1% of all RBCs in a normal blood film. An increase in this percentage indicates an accelerated rate of erythropoiesis e.g. to compensate for anemia or hemorrhage.
9. Mature RBCs.

II-E. Role of red cells in oxygen transport

Red blood cells (erythrocytes) transport hemoglobin, which is the main carrier of O₂ in the circulation. In normal adults the red cells occupy about 45% of the volume of the blood; this percentage volume of red cells in relation to whole blood is the "hematocrit value".

Combination of hemoglobin with oxygen

The most important feature of the hemoglobin is its ability to combine loosely and reversibly with O₂. Hemoglobin binds O₂ to form oxyhemoglobin, O₂ is attached to the Fe²⁺ (ferrous iron) in the heme. The affinity of hemoglobin for O₂ is affected by changes in pH, temperature and concentration of certain substances in the red cells.

If blood is exposed to various drugs and other oxidizing agents, the Fe²⁺ in the hemoglobin molecule is converted to ferric iron (Fe³⁺) forming methemoglobin which is incapable of carrying O₂. Carbon monoxide reacts with hemoglobin to form carbon monoxyhemoglobin. Hemoglobin affinity for CO is much more than its affinity for O₂, thus reducing the O₂-Carrying capacity of blood.

Factors affecting formation of red blood cells (erythropoiesis)

The formation or regeneration of red blood cells is controlled by several factors that are essential for perfect red blood cells regeneration or erythropoiesis.

1. *Tissue oxygenation: role of erythropoietin*

Any condition that causes the quantity of O₂ transported to the tissues to decrease ordinarily increases the rate of red cell formation. The principal factor that stimulates red blood cell production is a circulating glycoprotein hormone called erythropoietin. Lack of O₂ (hypoxia) stimulates formation of erythropoietin. Most of the erythropoietin (90%) is formed in the kidney; the rest is formed mainly in the liver. Erythropoietin in turn enhances red blood cell production by the bone marrow until the hypoxia is relieved (Figure 4).

Erythropoietin stimulates production of proerythroblasts from haemopoietic stem cells in the bone marrow. In the absence of erythropoietin, few red blood cells are formed by the bone marrow. On the other hand, when O₂ transport to tissues rises above normal, the rate of erythropoietin formation decreases (negative feedback mechanism).

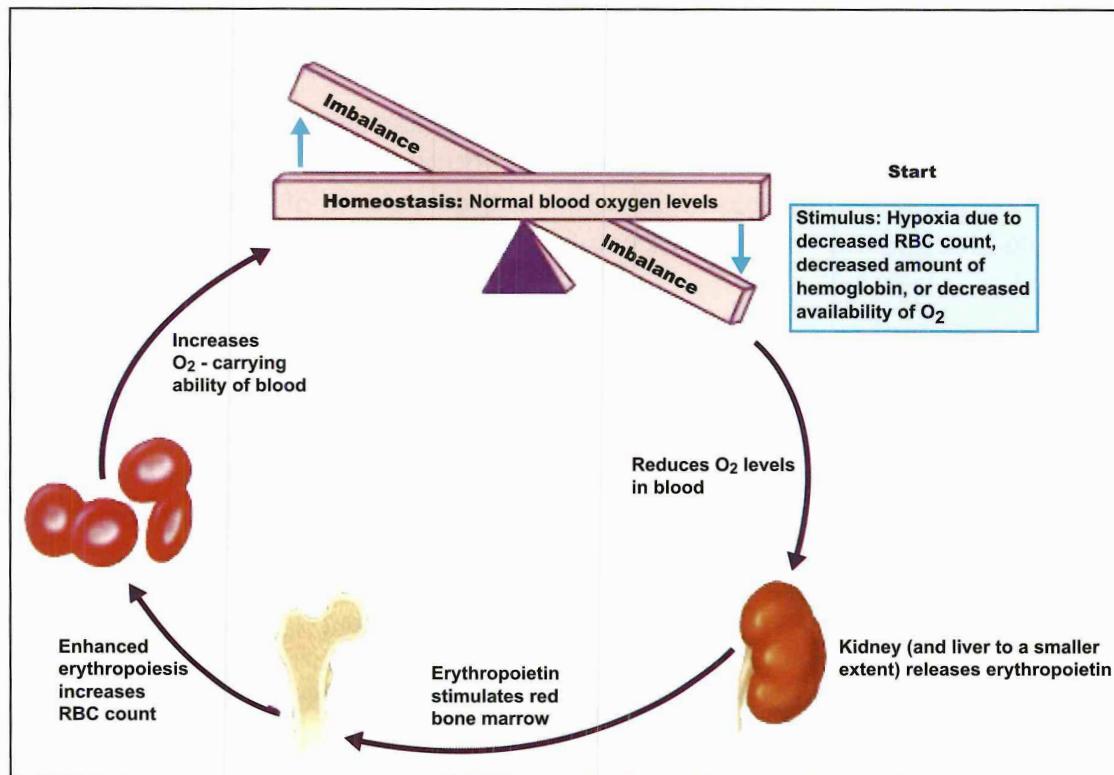


Figure 4. Role of erythropoietin in erythropoiesis

2. Nutritional factors

The cells of the bone marrow are among the most rapidly growing and reproducing cells of the body, thus they are greatly affected by the person's nutritional status. Especially important for formation and maturation of red blood cells are proteins, iron, vitamins, copper and cobalt.

a) Proteins

Animal proteins that are present in liver, kidney and muscles are superior in production of hemoglobin (globin fraction) compared to plant-derived proteins. Animal proteins contain the essential amino acids, which are neither stored nor formed in the body.

b) Iron

Sixty percent of the iron in the body is in hemoglobin (heme fraction), 3 % in myoglobin, and the rest is mostly in ferritin, which is present in enterocytes, hepatocytes and many other cells. Iron is also a constituent of cytochrome enzymes needed for intra-cellular oxidation.

c) Vitamins

The two vitamins, vitamin B12 and folic acid are required for formation of thymidine tri-phosphate, an essential building block of DNA. Thus they are

important for nuclear maturation and cell division of red cell precursors, and are called *maturity factors*. The other vitamins (including vitamin C) influence the general metabolism and growth of body tissues including the bone marrow i.e. they are not specific for red blood cells formation.

d) Copper and Cobalt

Copper and cobalt act as catalysts in hemoglobin synthesis.

3. Hormones

Hormones are required for erythropoiesis as they promote tissues metabolism in general. However, they are not specific except erythropoietin.

Thyroxine hormones are the most powerful stimulant of body metabolism and their deficiency for long time can cause anemia. Male hormones (androgens) and growth hormones stimulate body metabolism and erythropoiesis.

4. Liver

A healthy liver is essential for normal red blood cell formation. The liver manufactures globin, stores iron, copper, vitamin B12 and folic acid, and a small fraction of erythropoietin.

5. Bone marrow

It is the factory in which red cells and most other blood cells are formed. Destruction of bone marrow by irradiation, chemicals, drugs or bacterial toxins will lead to deficiency of all blood cells which is called "aplastic anemia".

II-F. Hemoglobin

Hemoglobin structure

1- Heme

It is an iron-porphyrin (Figure 5) compound composed of:

a. **Porphyrins:** these are cyclic compounds derived from the porphin nucleus made of 4 pyrrole rings linked by 4 methenyl bridges (-CH=) labelled α , β , γ and δ .

The porphyrins found in nature are compounds in which side chains are substituted for the hydrogen atoms in porphin nucleus. There are different types of porphyrins. Only types I and III occur in nature:

1- Type I isomer. The substituent groups attached to the 4 pyrrole rings are symmetrically arranged e.g.(AP.AP.AP.AP).

2- Type III isomer. The substituent groups attached to the 4th pyrrole ring are arranged in the reverse order, e.g.(AP, AP, AP, PA).

The biologically important porphyrin in heme and cytochrome are type III isomers.

b. Iron: It is present in ferrous state (Fe^{++}) and is linked to 4 nitrogen atoms of 4 pyrrole rings. Also, there are 2 additional bonds called 5th and 6th coordination bonds. These two bonds are located on each side of the heme plane (perpendicular to the heme plane).

The 5th position is linked to nitrogen atom of imidazol ring of proximal histidine, while the 6th position is bound to oxygen in HbO_2 and empty in deoxyhemoglobin (Hb) (unoccupied). The 4 pyrrole rings are attached to side chains called methyl, Vinyl, methyl, Vinyl, methyl, propionyl, propionyl, methyl. (M,V,M,V,M,P,P,M)..

The transport of O_2 is based on a physical interaction between molecular O_2 and iron of heme to provide reversible association

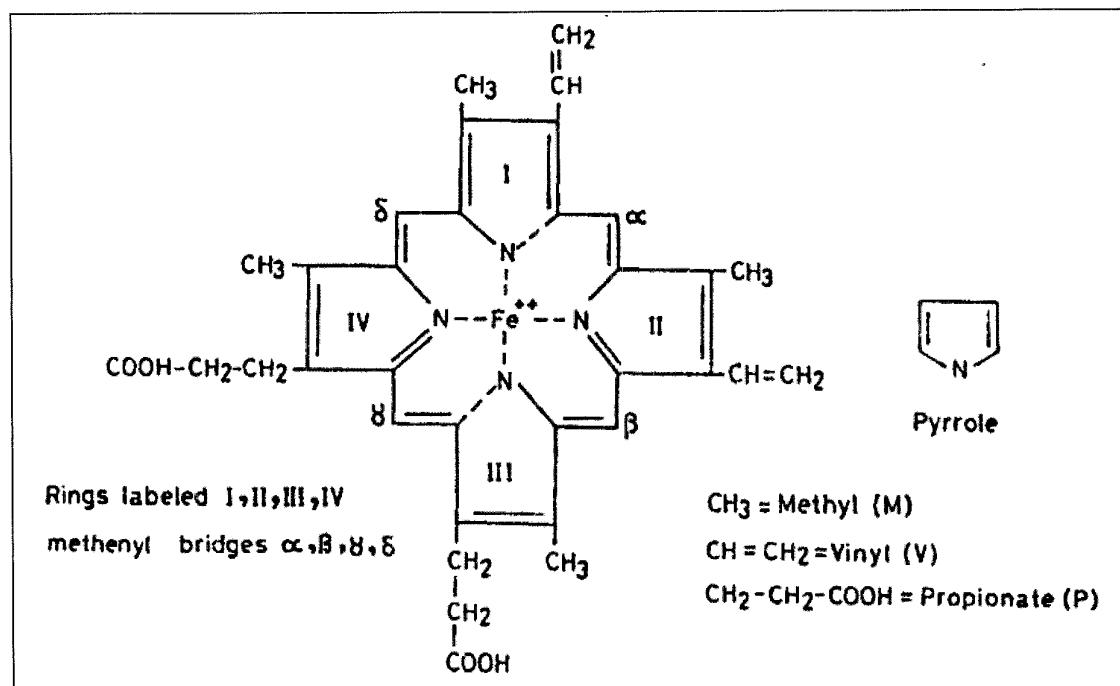


Figure 5. Structure of heme

The above-mentioned structure of heme can be drawn in a simplified manner as follows (Figure 6):

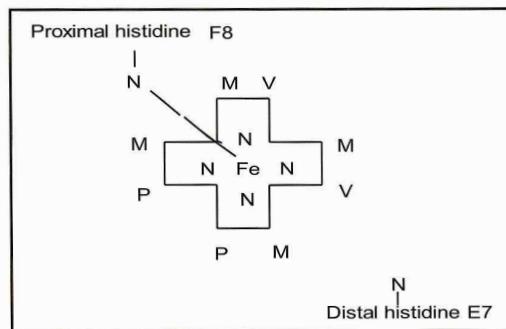


Figure 6. Structure of hemoglobin- simplified

2- Globin (protein part or apoprotein)

It is a simple protein (histone) which is characterized by its high content of histidine and lysine. It is composed of four polypeptide chains 2α and 2β chains. The α -chain contains 141 amino acids and β -chain contains 146 amino acids. Each β -polypeptide chain is folded into 8 right handed α -helices termed A-H starting from NH_2 -terminal, while α -subunit is folded into 7 α -helices. The ratio of heme to globin is 4:1. So each heme moiety is linked to one peptide chain.

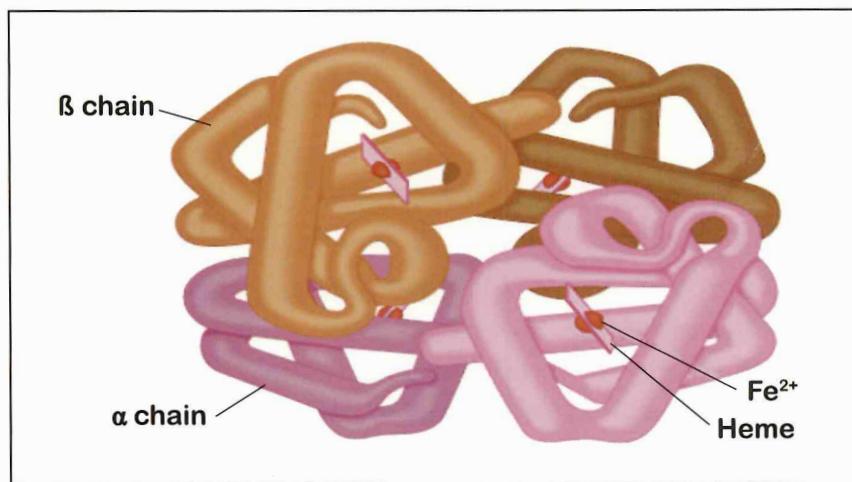


Figure 7. Hemoglobin

The myoglobin-hemoglobin family of proteins has produced a way in which Fe^{++} can be bounded to the proteins so as to produce an O_2 binding site. Hemoglobin protects the O_2 binding Fe^{++} from irreversible oxidation by providing environments in which the first step of an oxidation reaction (the binding of oxygen) is permitted, but the final step (oxidation) is blocked.

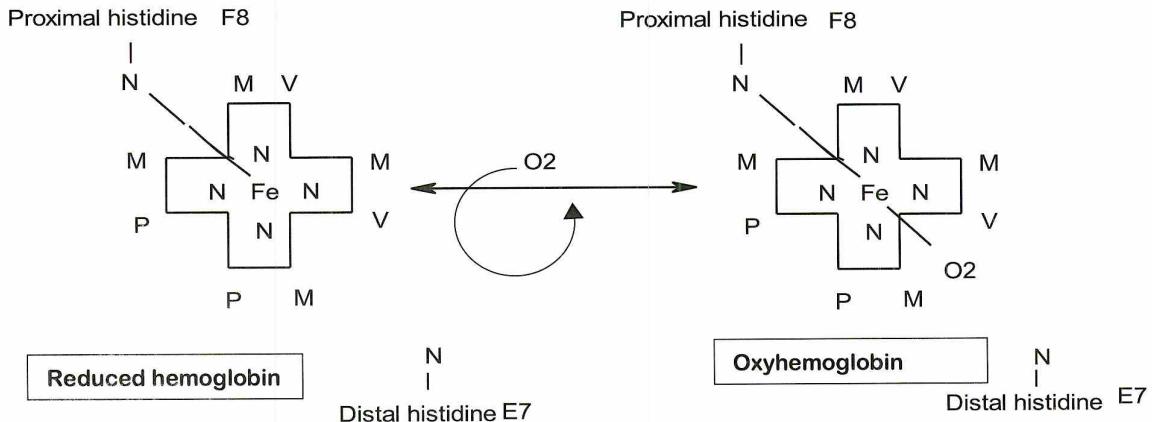


Figure 8. Oxyhemoglobin vs reduced hemoglobin

Types of normal hemoglobin

1. *Adult hemoglobin*. There are 2 types HbA_1 and HbA_2 .

a) Major adult hemoglobin: $Hb\ A_1$ ($\alpha_2\beta_2$)

Contains 2 alpha chains and 2 beta chains. This hemoglobin A_1 constitutes 95-97% of the total hemoglobin.

b) Minor adult hemoglobin: $Hb\ A_2$ ($\alpha_2\delta_2$)

Contains 2 α -chains and 2 δ -chains. HbA_2 forms about 2-4% of total hemoglobin. In the δ -chains there is more than one aminoacid different than those in β -chain e.g. arginine residue at the position 16 instead of glycine which is normally present in beta chain.

2. *Glycosylated hemoglobin (Hb A1c)*

It is a modified form of hemoglobin similar to hemoglobin A_1 but it contains glucose linked to ϵ amino group present on lysyl residues and at the NH_2 -terminal ends. The reaction is non-enzymatic and its rate depends on the concentration of glucose. It is present in normal value 5% of the total hemoglobin. This percentage is increased in prediabetic and diabetic patients up to 8-14%.

Thus, glycohemoglobin gives an idea about the blood glucose level during the last three months and is useful in the assessment of diabetic control.

3. *Fetal hemoglobin = HbF ($\alpha_2\gamma_2$)*

It is present normally in newborn and early fetal life. At age of 7 months 90 % of fetal hemoglobin is replaced by adult hemoglobin (HbA_1).

- It consists of 2 alpha chains and 2 gamma chains.

- In gamma chain there is more than one aminoacid different from those in β -chain e.g. *His₂₁ residue is ser₂₁*

- HbF has a great affinity for O₂ under physiological conditions, because γ -chains do not bind 2,3 BPG well. BPG is responsible for lowering the O₂ affinity of Hb and allowing Hb to release O₂ at the typical PO₂ of tissues.

Abnormal types of hemoglobin

1. Sickle cell disease (HbS)

The glutamic amino acid is replaced by valine amino acid at the position number 6 of beta chain, due to mutation in the structural gene ($\alpha_2\beta_2$).

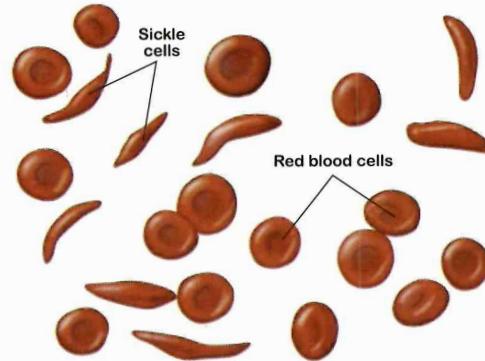


Figure 9. Sickle cells

Pathogenesis

The presence of valine (nonpolar amino acid) instead of glutamic (polar amino acid) at position 6 of β -chain will lead to formation of sticky patches on the surface of hemoglobins of both oxyhemoglobin S and deoxyhemoglobin S. However both the deoxyhemoglobin S and A contains a complementary patches (grooves). When the blood is deoxygenated, the sticky patches of deoxyhemoglobin S bind to the complementary patches of deoxyhemoglobin S, leading to polymerization of deoxyhemoglobin S leading to formation of long fibrous precipitates leading to sickling of RBCs.

Effects of sickling of RBCs

- a- Sickle shaped RBCs are fragile and easily hemolysed so anemia will occur (sickle cell anemia).
- b- Sickle shaped RBCs are easily trapped in small blood vessels leading to thrombus formation and damage of organs like brain, bone spleen, etc.

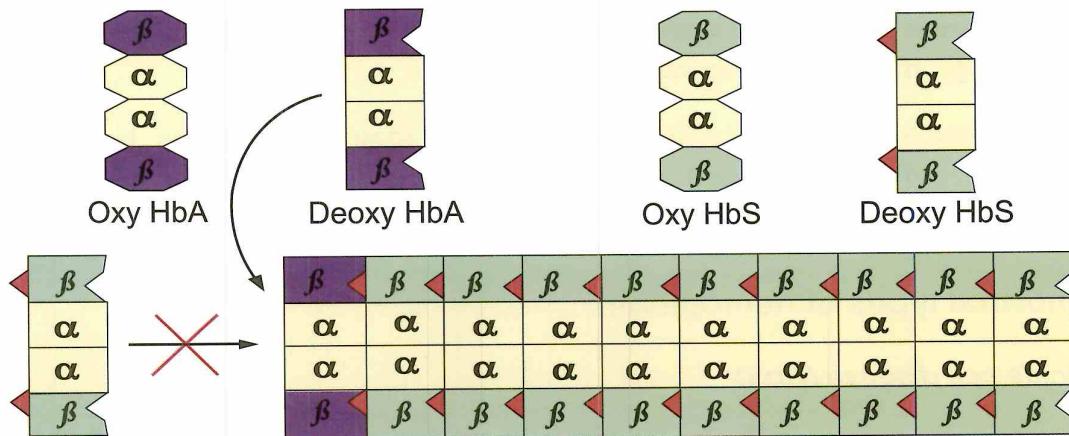


Figure 10. Polymerization of deoxyhemoglobin S.

The dissociation of oxygen from hemoglobin S (HbS) unmasks a sticky patch (red triangle) on the surface of its β -subunits (green) that can adhere to a complementary site on the β -subunits of other molecules of deoxyHbS. Polymerization to a fibrous polymer is interrupted deoxyHbA, whose β -subunits (lavender) lack the sticky patch required for binding additional HbS subunits.

Diagnosis

- a- Reticulocyte count between 10-20%.
- b- Sickle-shaped RBCs by microscopic examination.
- c- Electrophoresis. Shows HbS, no HbA. HbS moves slowly due to less negative charges on its molecules, so it appears in paper electrophoresis nearer to cathode than normal hemoglobin.

2. Thalassemias

These are hereditary hemolytic diseases in which the synthesis of either α - or β - globin chain is defective due to mutation affecting the regulatory gene.

Types

a- α -thalassemias: characterized by decreased or absent synthesis of α -chains of hemoglobin with compensatory increase in synthesis of other chains. Include 2 types:

- i. Pure β -chain HbH: Where there are 4 β -chains (HbH disease).
- ii. Pure γ -chain-Hb Bart's Where there are 4 γ -chains

b- β - thalassemias: Synthesis of β -chains is decreased or absent, whereas synthesis of α -chains is normal and will combine with δ -chains giving excess of HbA2 ($\alpha 2\delta 2$) or it may combine with γ -chains producing excess of HbF ($\alpha 2\gamma 2$).

The abnormal hemoglobin does not function as normal hemoglobin and has an abnormal O₂ dissociation curve.

Diagnosis

- Anemia: called Cooley's anemia or Mediterranean sea anemia.
- Electrophoresis: shows HbF, HbH, HbA2.

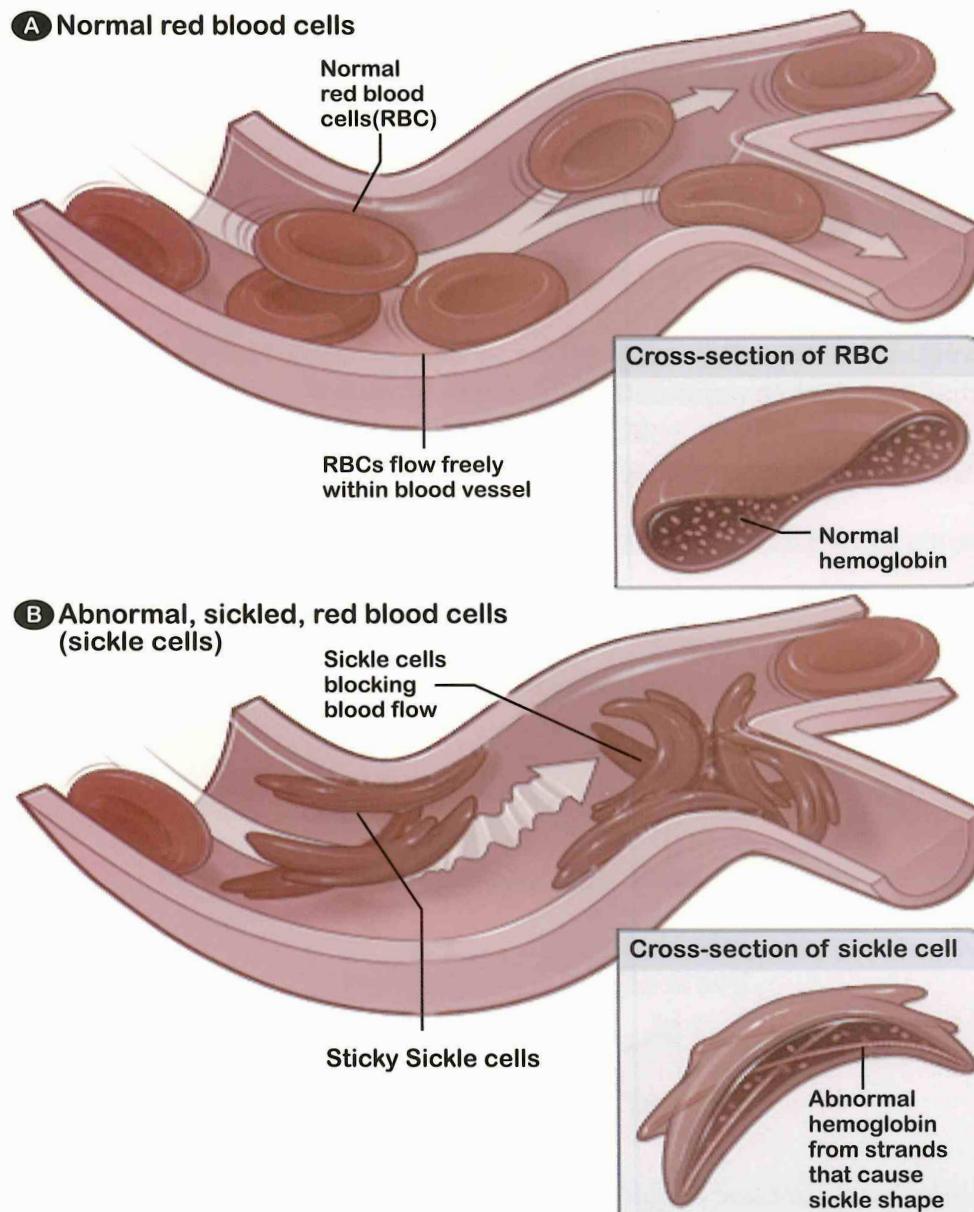


Figure 11. Sickled cells are trapped in small blood vessels

3- Methemoglobinemia

This is oxidized Hb, the Fe^{2+} normally present in heme being replaced by Fe^{3+} . The ability to react as an O_2 carrier is lost.

The normal erythrocyte contains small amounts of met-Hb, formed by spontaneous oxidation of Hb. Met-Hb is normally reconverted to Hb by reducing systems in the RBC, the most important of which is NADH-methemoglobin reductase.

Excess met-Hb may be present in blood because of increased production or diminished ability to convert it back to Hb.

Types and causes of methemoglobinemia

1-Congenital methemoglobinemia

a- Hemoglobin M (Hb-M):

A congenital condition due to mutation in globin biosynthesis in which distal or proximal histidine is replaced by tyrosine so heme iron is stabilized in the ferric state. Treatment with reducing agents as methylene blue is ineffective. The treatment is only conservative as **blood transfusion**.

b- Deficiency of NADH cytochrome b5 methemoglobin reductase system:

This is required for reducing heme Fe^{3+} back to the Fe^{2+} state. This system consists of NADH (generated by glycolysis), a flavoprotein named cytochrome b5 reductase (also known as met Hb reductase), and cytochrome b5.

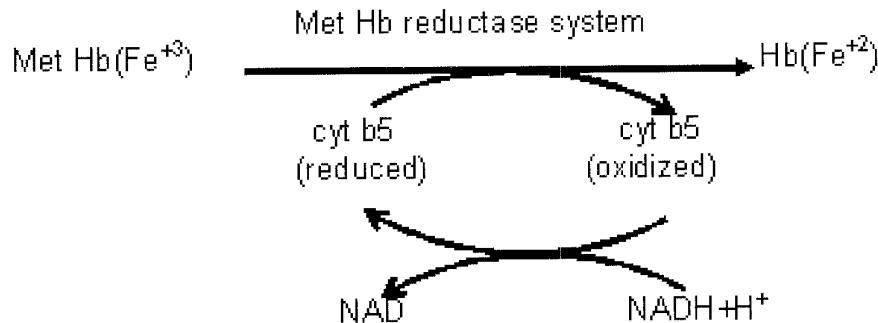


Figure 12. Reduction of met-hemoglobin by the NADH-cytochrome b5 methemoglobin reductase system

Treatment with reducing agents is effective in converting met Hb back to Hb in those patients.

2- Acquired (toxic) methemoglobinemia

Usually arises following the ingestion of large amounts of drugs e.g. phenacetin or the sulphonamides, excess of nitrites or certain oxidizing agents present in the diet may also cause it. Treatment with injection of reducing agents like vitamin C or glucose or methylene blue is effective in reversing acquired methemoglobinemia.

Diagnosis of methemoglobinemia

1. Cyanosis.
2. Examination of blood color(brownish).
3. Pulse oximetry to test saturation of blood oxygen.
4. DNA sequencing of the globin chain can be used to identify HbM.

Heme biosynthesis

Heme is the iron protoporphyrin, synthesized mostly in the bone marrow (85%) and liver because of the requirements for incorporation into hemoglobin and the cytochromes, respectively.

The initial and last three enzymatic steps are catalyzed by enzymes that are present in mitochondria whereas the intermediate steps are taking place in cytoplasm.

Steps of heme synthesis:

1. Synthesis of δ -aminolevulinic acid (ALA)

In the mitochondria by *ALA synthase* enzyme, *succinyl COA* condenses with *glycine* to form *α -amino- β -ketoadipic acid* in presence of vitamin *B₆* which activate glycine. By the same enzyme α -amino- β -ketoadipic acid is decarboxylated to form *δ -aminolevulinic acid (ALA)*

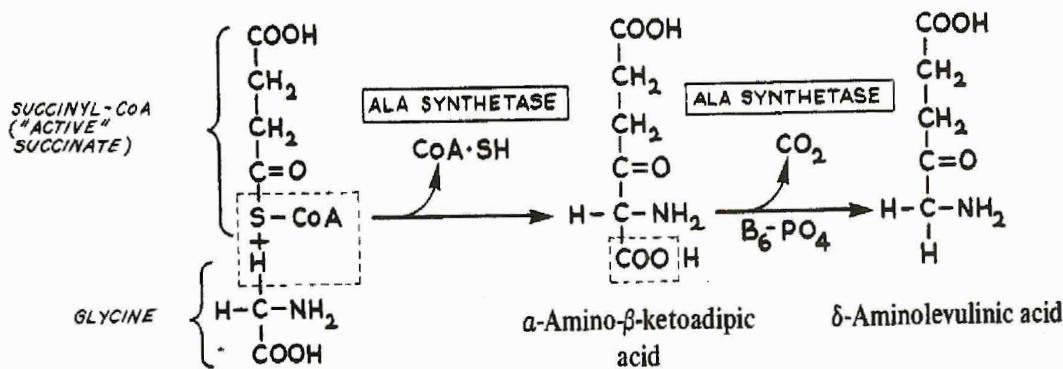


Figure 13. Synthesis of δ -aminolevulinic acid

2. Synthesis of porphobilinogen (PBG).

In the cytoplasm 2 molecules of ALA condense together with removal of 2 molecules of water by **ALA dehydratase** enzyme to form **porphobilinogen (PBG)** ALA dehydratase is zinc containing enzyme inhibited by lead.

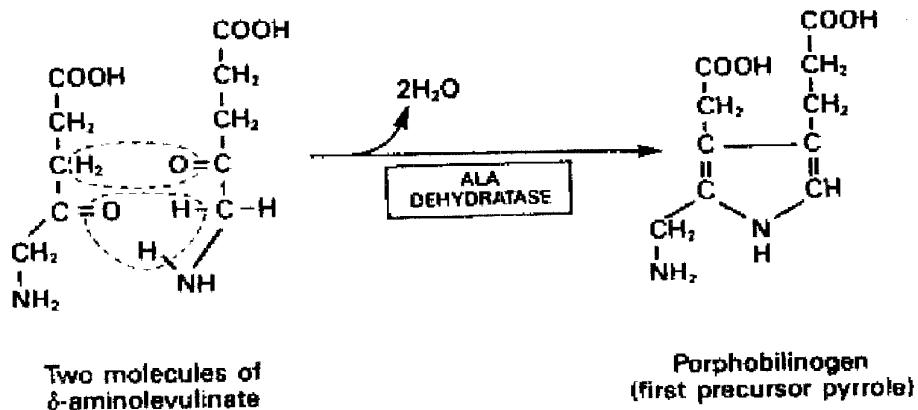
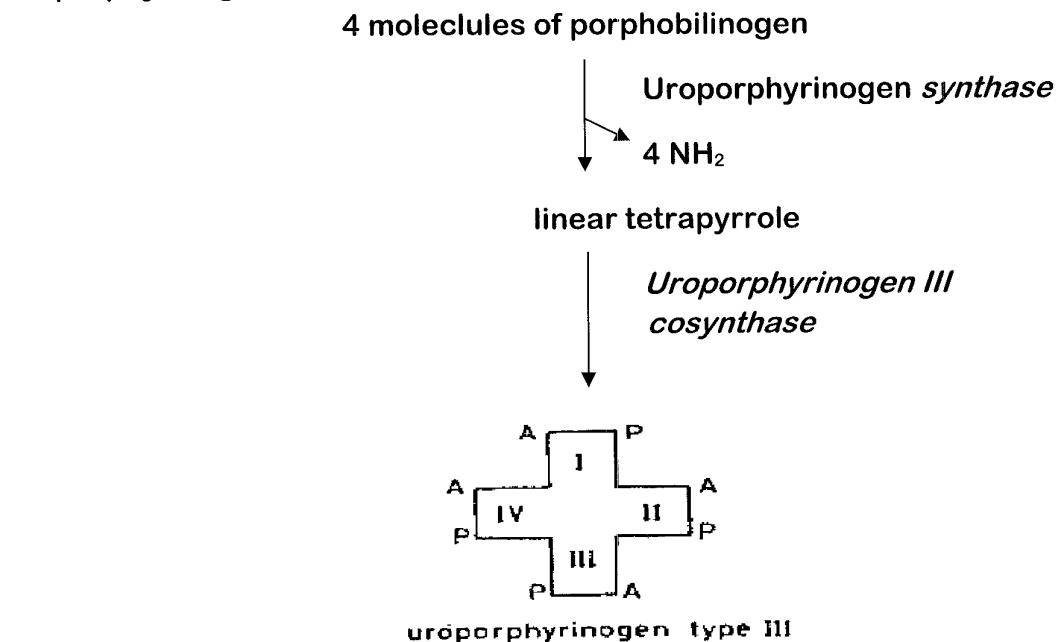


Figure 14. Synthesis of porphobilinogen

3. Synthesis of uroporphyrinogen

In the cytosol four molecules of porphobilinogen condense together by uroporphyrinogen I synthase to form an open-chain tetrapyrrole which can spontaneously form uroporphyrinogen I, the linear tetrapyrrole in presence of uroporphyrinogen III cosynthase produces the asymmetrical isomer uroporphyrinogen III.



(A = Acetate group and P = Propionate group)

Figure 15 Synthesis of uroporphyrinogen

4 .Synthesis of coproporphyrinogen

In cytosol all acetate groups (A) are decarboxylated to methyl substituents (M)

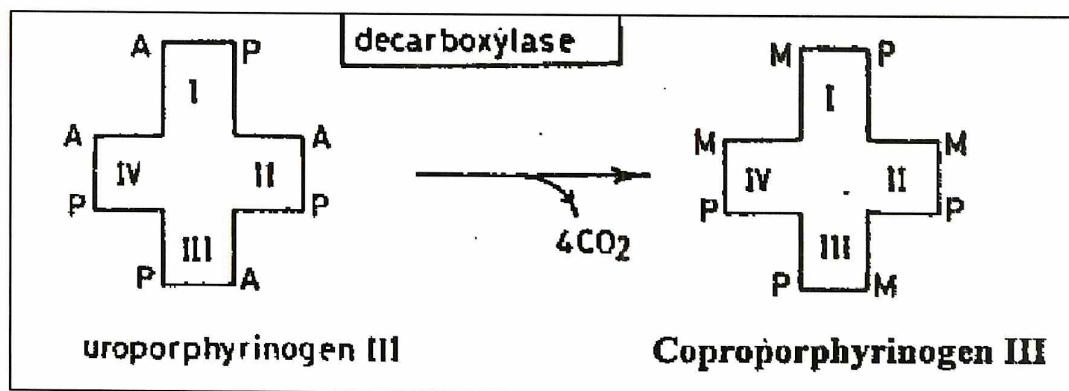


Figure 16. Synthesis of coproporphyrinogen

5. Synthesis of protoporphyrinogen III, protoporphyrin and heme

In mitochondrial ***coproporphyrinogen oxidase*** which acts only on type III oxidizes and decarboxylates two propionic side chains in rings I and II to vinyl groups(-CH=CH₂) and protoporphyrinogen III is formed.

Protoporphyrinogen (4 methylene bridges $-\text{CH}_2-$), by *oxidase* forms protoporphyrin (4 methenyl bridges $-\text{CH}=$), and then by *heme synthase (ferrochelatase)* heme is formed.

Regulation of heme synthesis

1. ALA synthase enzyme controls the rate-limiting step of Heme synthesis. **Heme** and also **hemin** act as a repressor of the synthesis of ALA synthase and act as feed-back inhibitor at this step, the inhibition occurs at an allosteric site.
 2. The block in heme biosynthesis in pantothenic acid or vitamin B6 deficiency occurs at very early step in heme synthesis (ALA synthase).
 3. ALA dehydratase is sulphhydryl enzyme and is very sensitive to inhibition by heavy metals as **mercury or lead**.

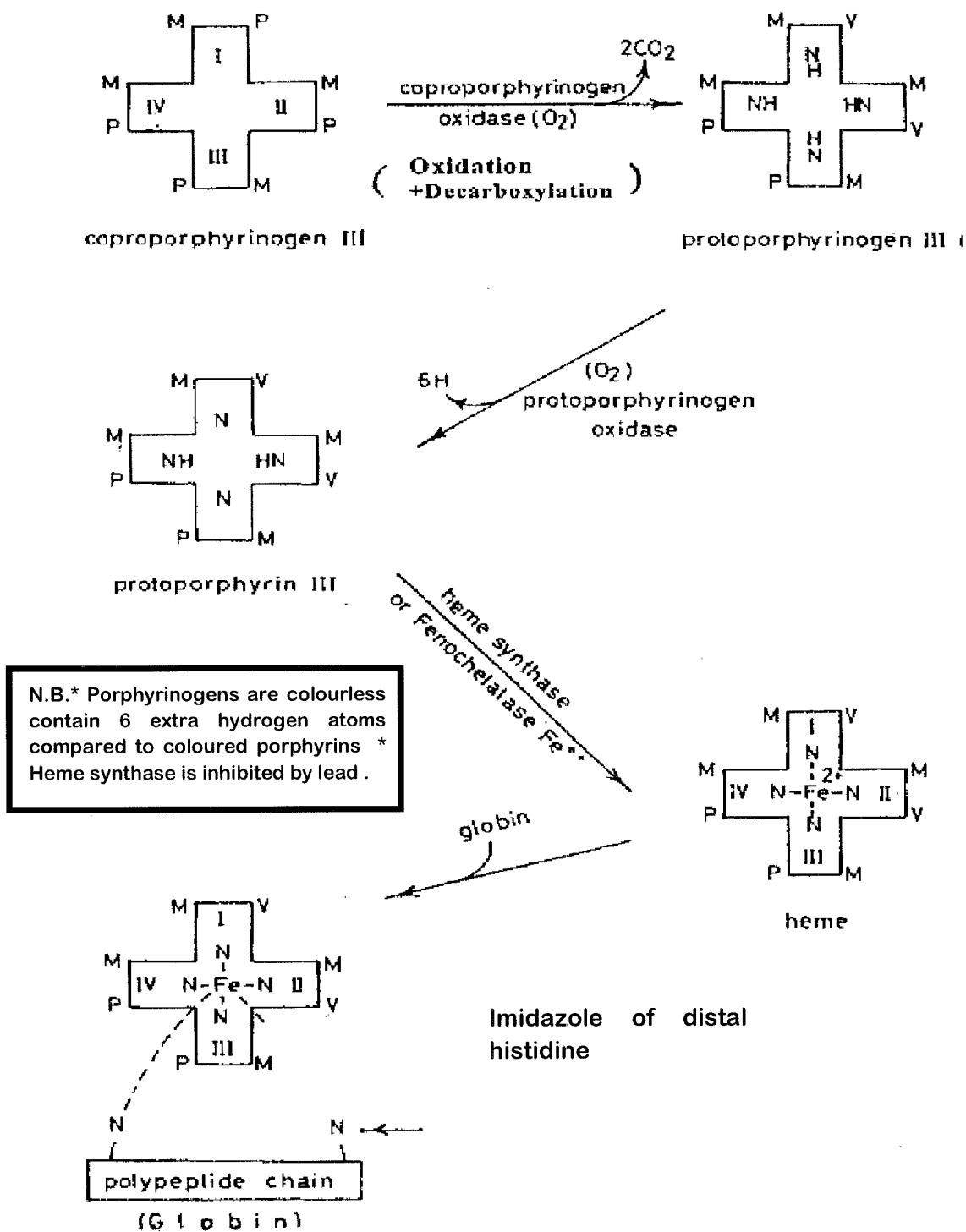


Figure 17 a . Synthesis of protoporphyrinogen III, protoporphyrin and heme