AL-Mustaqbal University College

Medical laboratory Techniques Department

Determination of Blood Hemoglobin (Hb)

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Blood

Blood is a specialized body fluid. It has four main components: plasma, red blood cells, white blood cells, and platelets.

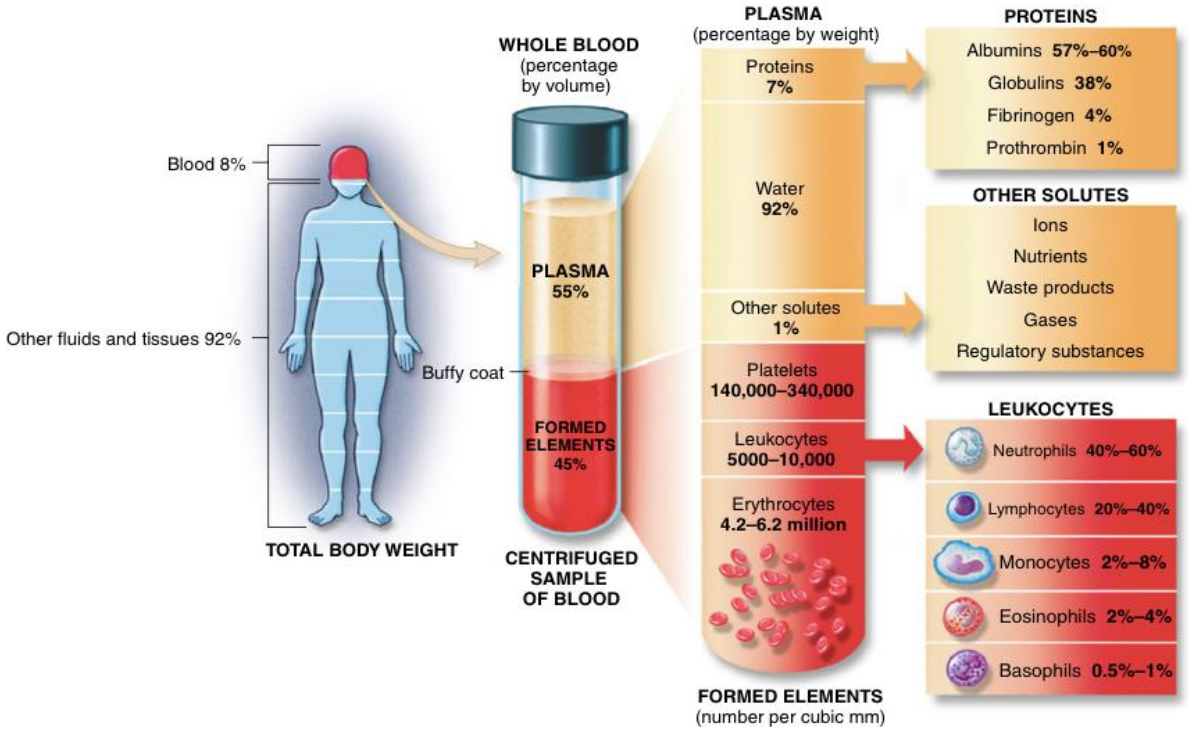


Figure (1) Component of Blood

Hemoglobin:

Hemoglobin is a large, complex protein molecule (globular protein) with a molecular weight of approximately 64000. It is comprising two major parts: **heme** and **globin proteins**.

The ***heme*** portion comprises a **prophyrin ring** with **iron** chelated in the center. The iron atom is the site of reversible oxygen attachment.

The globin portion consists of **four globin chain (α, β, Ɠ, δ).**

Each globin chain contains 141 or more amino acids.

Hemoglobin molecule contains **four heme groups** attached to each of **four globin chains** and may carry up to **four molecules of oxygen.**

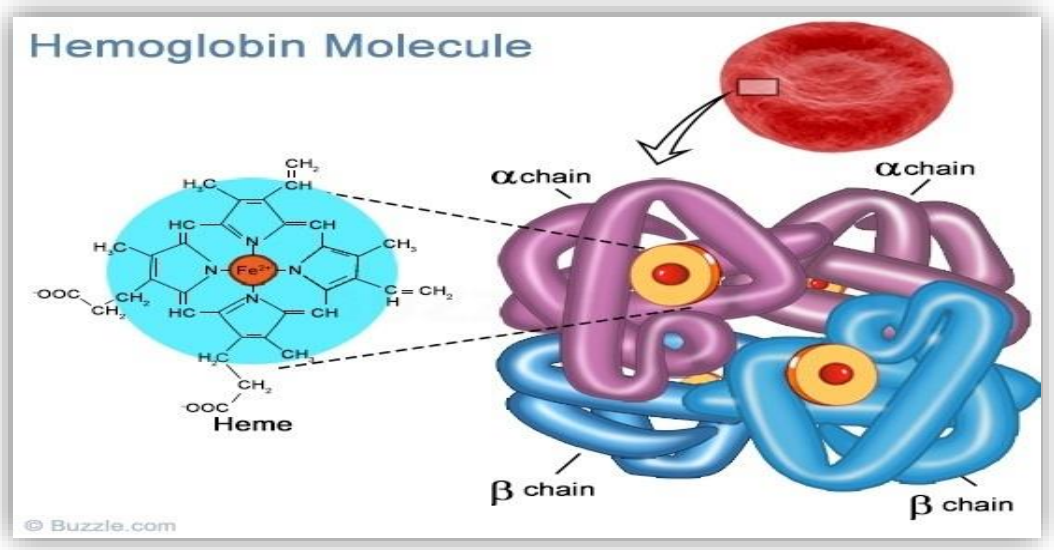


Figure (2) Structure of Hemoglobin Molecule

Type of Hemoglobin in Human Body:

1. **Hemoglobin A or A1 (HbA1):**

   which contains two (α-chain) and two (β-chain). It is found in normal human adults, it is symbiotically as (α2β2).

2. **Hemoglobin A2 (HbA2):**

   is found in normal adults, consists of two (α-chain) and two (Ɠ-chain). It is designated as (α2Ɠ2).

3. **Hemoglobin F (HbF):**

Predominated during fetal life but rapidly diminishes the first year of postnatal life. It consists of two (α-chain) and two (δ-chain). It is designated as (α2δ2).

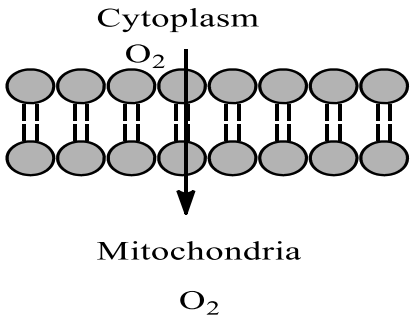
Role of Hemoglobin (Hb) in the Body:

Hemoglobin (Hb): is a hem protein whose primary function is to transport oxygen from the lungs to the body tissues and CO_2 transport back to the lungs.

Myoglobin:

is a heme protein found in skeletal and cardiac muscle. It can reversibly bind oxygen in a manner similar to the hemoglobin molecule. It is containing **one polypeptide chain** and **one heme group** per molecule, with a molecular weight of approximately 17000. The polypeptide chain contains 153 amino acids.

In the body, myoglobin acts as an oxygen carrier in the cytoplasm of muscle cell. Transport of oxygen from the muscle cell membrane to the mitochondria, it is main role.



Methods of Determination the Hemoglobin:

1. Determination of oxygen.
2. Determination of carbon monoxide capacity.
3. Determination of iron content.
4. Drabkin reagent

Drabkin Reagent

Hemoglobin is converted to Cyanomethemogloin according to the following reaction:

Fe^{2+}(Hemoglobin) + Fe^{3+}(potassium ferricyanide) ⟶ methemoglobin

Methemoglobin + potassium cyanide ⟶ Cynomethemoglobin

Procedure:

- **Working reagent**: dilute Drabkin reagent

(49 ml distilled water +1 ml concentrated reagent

Wave length	540 nm/546nm
Temperature	25^0C/30^0C/37^0C
Cuvette	1cm light path

Reagent	Sample	Blank	Standard
Working reagent	5 ml	5ml	-----
Sample	20µl	-----	-----
Standard	-----	----	5ml

- Mix well and after 5 min incubation, measure the absorption. The color is stable up to 30 minutes.
- **Standard**: Methemoglobin 18 g/dl.
- **Ready to use do not reaction with drabkin reagent.**

Calculation:

Hemoglobin concentration using standard

$$\text{Conc. of Hb in sample} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}\right) \times \text{Conc.of standard}$$

Normal ranges of Hb

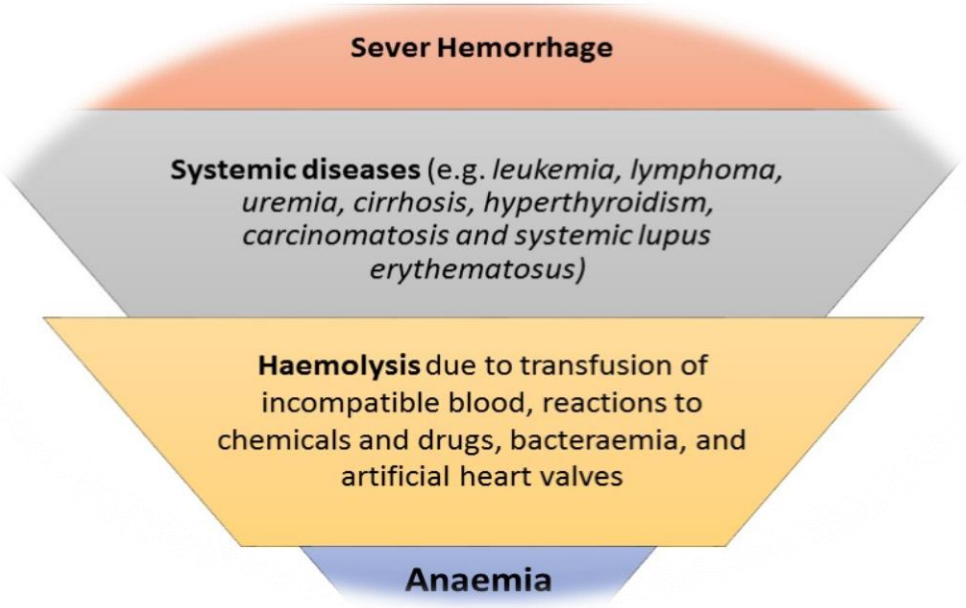
1. Female: 12–16 g/dL (7.4–9.9 mmol/L SI units)
2. Males: 13–18 g/dL (8.1–11.2 mmol/L SI units)
3. Pregnancy: Decreased (dilutional)
4. Elderly: Slightly decreased
5. Newborn: Increased

Please note that:

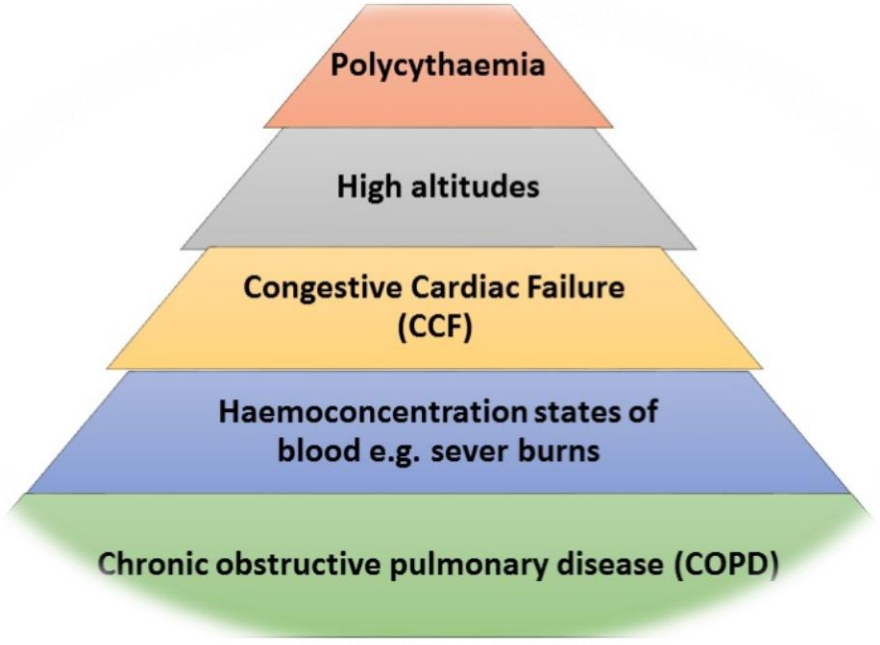
- Normal range for females is (120 – 160 g/L) and for males (130 – 180 g/L)

Clinical Significance:

1- Decrease Hemoglobin

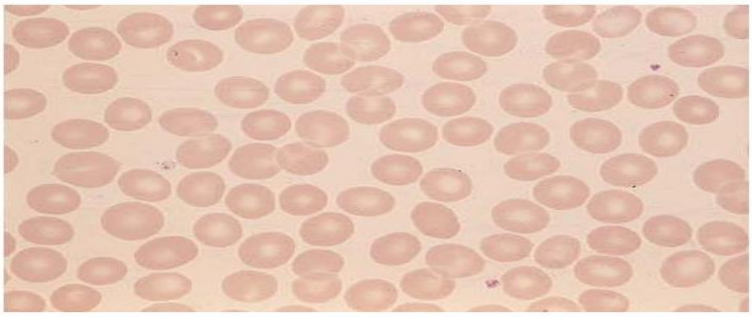


2- Increased Hemoglobin



Erythrocytes

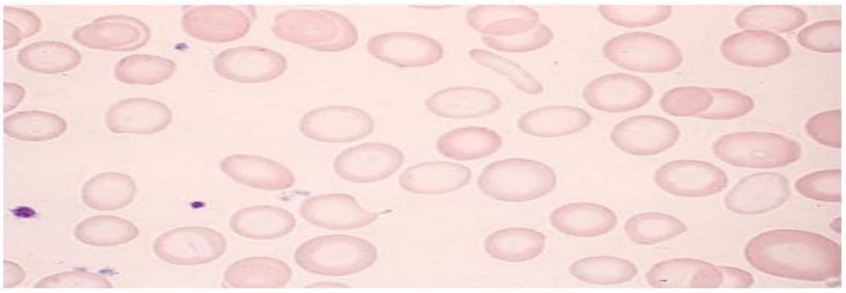
1. Normocytic erythrocytes (normal RBCs)



- *Cells are uniform size & shape*
- *Normal hemoglobin conc.*
- *Small, central pallor which is*
- *Less than one-third of the total cell volume.*

Erythrocytes

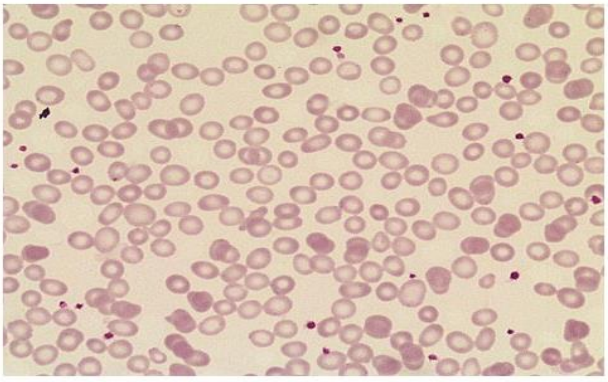
2. Hypochromic erythrocytes (low Hb in RBCs)



- *IDA*
- *Thalassemia*
- *Sideroblastic anemia*

Erythrocytes

3. Hyperchromic erythrocytes (high Hb in RBCs)



- *hereditaryspherocytosis*