



Chapter-06

HEMOGLOBIN AND ITS MEASURING METHODS

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ABSTRACT

Hemoglobin, a crucial protein in vertebrates, its intricate mechanism involves cooperative binding of oxygen, a phenomenon known as allosteric regulation. The structure, function, and regulation of hemoglobin, emphasizing the key techniques employed in its study. Understanding hemoglobin's structure involves unraveling its quaternary structure, which comprises four globin subunits and a heme prosthetic group in each. Techniques such as X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy have been instrumental in elucidating the three-dimensional arrangement of hemoglobin, offering insights into its allosteric behavior. Classical techniques like the Hill equation have been pivotal in quantifying the cooperative nature of oxygen binding. Additionally, modern techniques like computational modeling and molecular dynamics simulations contribute to a deeper understanding of the dynamic interactions within the hemoglobin molecule. Furthermore, hemoglobin extends beyond its canonical role in oxygen transport. Aberrations in hemoglobin structure, and seen in genetic disorders like sickle cell anemia and thalassemia

Keywords: Hemoglobin, Erythrocyte, Blood, Cyanmethemoglobin, Sahli's, Drabkin's, Anemia, Polycythemia.

6.1 INTRODUCTION

Hemoglobin (Hb) is a conjugated protein found in red blood cells that plays a fundamental role in transporting oxygen from the lungs to tissues and organs throughout the body. This protein also aids in carrying carbon dioxide. Hemoglobin (Hb) is synthesized inside erythrocytes in the red bone marrow. Hemoglobin contains two components; one is Heme and another is Globin (*Bunn H. F., 1972*). Heme contains iron and protoporphyrin and Globin contains amino acids chain.

6.2 MECHANISM OF HEMOGLOBIN

Hemoglobin (Hb) is a crucial protein in red blood cells that transport of oxygen from the lungs to tissues throughout the body and helps carry carbon dioxide and waste product back to the lungs for exhalation (*Adamson J. W and Finch C. A., 197*).

The mechanism of hemoglobin involves several key steps:

- **Oxygen binding in the Lungs:** In the lungs, oxygen concentration is high, hemoglobin binds to oxygen molecules. The iron ion (Fe^{2+}) at the center of the heme group in each of the four Globin chains of hemoglobin is capable of binding to oxygen molecules. Each hemoglobin molecule can bind to four oxygen molecules.

- **Formation of Oxyhemoglobin:** When a hemoglobin molecule is bound to oxygen, it forms a molecule called Oxyhemoglobin (HbO₂).
- **Transport of Oxygen to Tissues** As blood circulates through the body, Oxyhemoglobin releases oxygen to tissues where oxygen concentration is lower due to cellular respiration.
- **Oxygen Unloading in Tissues** In tissues with lower oxygen levels, hemoglobin undergoes a conformational change that facilitates the release of oxygen to the surrounding tissues. The release of the first oxygen molecule enhances the ability of hemoglobin to release the remaining three.
- **Carbon Dioxide Binding in Tissues:** Hemoglobin also helps transport carbon dioxide. In the tissues carbon dioxide binds to amino groups on the Globin chains forming carbamino-hemoglobin.

Hemoglobin is a conjugated protein with a quaternary structure. In humans, hemoglobin is responsible for transporting oxygen from the lungs to the tissues and carbon dioxide from the tissues back to lungs.

6.3 STRUCTURE OF HEMOGLOBIN

Hemoglobin consists of 4 subunits and each containing an iron-containing heme group. The heme group is crucial for the binding and transport of oxygen. The four subunits are arranged in a globular quaternary structure (*Scott A. F. et al., 1981*).

1. **Primary structure:** The human hemoglobin molecule consists of two alpha (α) and two beta (β) Globin chains. The primary structure is encoded by the deoxyribonucleic acid in the genes.
2. **Secondary structure:** The primary structure forms secondary structures like alpha helices and beta sheets. These structures contribute to the overall three-dimensional shape.
3. **Tertiary structure:** This is the three-dimensional structure of each individual subunit. The heme group, which contains an iron ion, is embedded in a hydrophobic pocket within each subunit.
4. **Quaternary structure:** The quaternary structure is the overall structure formed by the interaction of the four subunits. Hemoglobin has a globular quaternary structure, and the interaction between the subunits is crucial for the protein's function. The binding of oxygen to one subunit induces a conformational change that makes it easier for the other subunits to bind oxygen.

5. **Heme group:** Each subunit of hemoglobin contains a heme group, which consists of a porphyrin ring with an iron ion (Fe^{2+}) at its center. This iron ion is what binds to oxygen, forming a reversible complex. The iron ion can exist in either the ferrous (Fe^{2+}) or ferric (Fe^{3+}) state, but it binds oxygen more readily in the ferrous state.

6.4 PRINCIPLE OF SAHLI'S METHOD

Firstly, blood is mixed with 0.1N HCL, and then hemoglobin is changes into brownish colored that is called acid hematin. Sahli's hemoglobinometer used for the color match (*Brittin G. M. et al, 1969*). *Figure 6.1 depicts the Sahli's hemoglobinometer.*



Figure-6.1: Sahli's Haemometer (Source- Pathology by Ranjith AR., 2021)

6.4.1 Method

Sahli (Acid Hematin) qualitative method.

6.4.2 Requirements

Hemoglobin estimation is a common medical test. Hemoglobin estimation as follows:

Sahli hemoglobinometer: It consists of:

- (a) A hemoglobinometer
- (b) A graduated tube
- (c) Hb pipette (0.02 ml)
- (d) 0.1 N HCL
- (e) Distilled water

(f) Pasteur pipettes

6.4.3 Specimens

Hemoglobin (Hb) estimation is typically performed on blood specimens. The two main types of blood specimens commonly used for hemoglobin estimation are:

- Hemoglobin can be measured directly in whole blood. In this method fresh blood is collected, often from a vein in the arm. The sample is then analyzed to determine of the hemoglobin. The result is usually expressed in grams of hemoglobin per deciliter (g/dL)
- Capillary or venous blood mixed with EDTA. The specimen need not be a fasting sample.

6.4.4 Procedure

1. By using a dropper adds 0.1 N HCL in the test tube till the lowest mark (20% mark).
2. Draw blood up to 20ul without any mistake. Try to clean rest blood on the sides of the pipette.
3. Transfer blood in the graduated tube & mix well & keep for 10 minutes.
4. Dilute the mixture with distilled water and mix well carefully and check the color.
5. The matching should be done, and then read out at its lower point.

6.4.5 Normal Ranges

- (a) **Men:** 13-18 Hb, g/dl.
- (b) **Women:** 12-16.5 Hb, g/dl.
- (c) **Children (up to 1 year):** 11.0-13.0 Hb, g/dl.
- (d) **Children (up to 12 years):** 11.5-14.5 Hb, g/dl.
- (e) **Infants (full term cord blood):** 13.5-19.5 Hb, g/dl.

6.5 CYANMETHMOGLOBIN (CMG) PRINCIPLE

Firstly, blood is diluted with Drabkin's reagent (containing Potassium cyanide & potassium ferricyanide), hemoglobin binds with ferricyanide and converted into methemoglobin which is changes into Cyanmethemoglobin (HiCN). Check hemoglobin in

the spectrophotometer and colorimeter at about 540 nm (*Drabkin D. L. and Austin J. H., 1932*).

6.5.1 Method

Cyanmethmoglobin (CMG) quantitative method.

6.5.2 Requirements

Hemoglobin estimation is a crucial aspect of assessing a person's overall health the most common method for hemoglobin estimation is through a blood test. Here are the general requirements and steps for hemoglobin estimation.

1. **Drabkin's reagent:** In 1000 ml of distilled water –
 - (a) Potassium ferricyanide 400mg.
 - (b) Potassium dihydrogen phosphate: 280mg.
 - (c) Potassium cyanide: 100mg.
 - (d) Nonidet: 1ml.
 - The reagent is kept at 2-8°C.
2. **Cyanmethmoglobin (HiCN) standard:** it is common. Test measures at 540nm (Green Filter).
3. Hb pipette (20ul, calibrated) or Micro pipette (5ul, 10ul, 20ul, 50ul, 100ul and 1000ul.)
4. Test tubes (15x125mm).
5. Colorimeter & spectrophotometer.

6.5.3 Specimens

- Mostly blood specimens used for hemoglobin estimation include; Capillary or Venous blood mixed with EDTA. The specimen need not be a fasting sample.

6.5.4 Procedure

Table-6.1: By using pipette

Materials	Blank	Test
1. Drabkin's reagent, ml	5.0ml	5.0ml
2. Distilled water, ul	20ul	-
3. Blood sample, ul	-	20ul

Mix well & keep for 5 to 10 minutes.

1. Check absorbencies of 'test' with sitting blank to 100% T at 540nm (Green Filter).
2. Read absorbencies of standard 27 is fixed reading.

6.5.5 Calculations

Hemoglobin, g/dl = OD of TEST/OD of STD X 15

6.5.6 Normal Values

- **Men:** 13-18 Hb, g/dl.
- **Women:** 12-16.5 Hb, g/dl.
- **Children (up to 1 year):** 11.0-13.0 Hb, g/dl.
- **Children (up to 12 years):** 11.5-14.5 Hb, g/dl.
- **Infants (full term cord blood):** 13.5-19.5 Hb, g/dl.

6.6 CLINICAL SIGNIFICANCE

Increase: Increasing hemoglobin levels is of clinical significance for several reasons. Below are some clinical implications and reasons for the importance of increasing hemoglobin levels. Hemoconcentration due diarrhea & vomiting, High value seen in congenital heart disease in emphysema & in polycythemia.

Decrease: A decrease in hemoglobin to the normal value is called anemia. Hemoglobin basically decreases in pregnancy due to hemodilution. Iron deficiency is one of the leading causes of low hemoglobin levels. Inadequate intake of iron-rich foods or poor absorption of iron in the digestive tract, Deficiencies in vitamins such as vitamin B12 and folate can impair the production of red blood cells, leading to a decrease in hemoglobin levels. Chronic diseases like kidney disease, arthritis, and inflammatory disorders, can contribute to anemia by affecting the production or lifespan of erythrocytes. Disorders

affecting the bone marrow where erythrocytes are produced, can lead to decreased hemoglobin levels. Conditions like aplastic anemia, myelodysplastic syndromes, and leukemia are examples.

6.7 CONCLUSION

Hemoglobin is a protein that is essential for the transport of oxygen and carbon dioxide throughout the human body. Hemoglobin has a distinct structure that enables it to bind with oxygen in the lungs and release it in tissues, ensuring proper cell and organ function. Hemoglobin serves as an example of the intricate interplay between structure and function in biological molecules due to its regulation of hemoglobin synthesis and interaction with various factors such as pH and carbon dioxide.

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