



## Department of Anesthesia Techniques



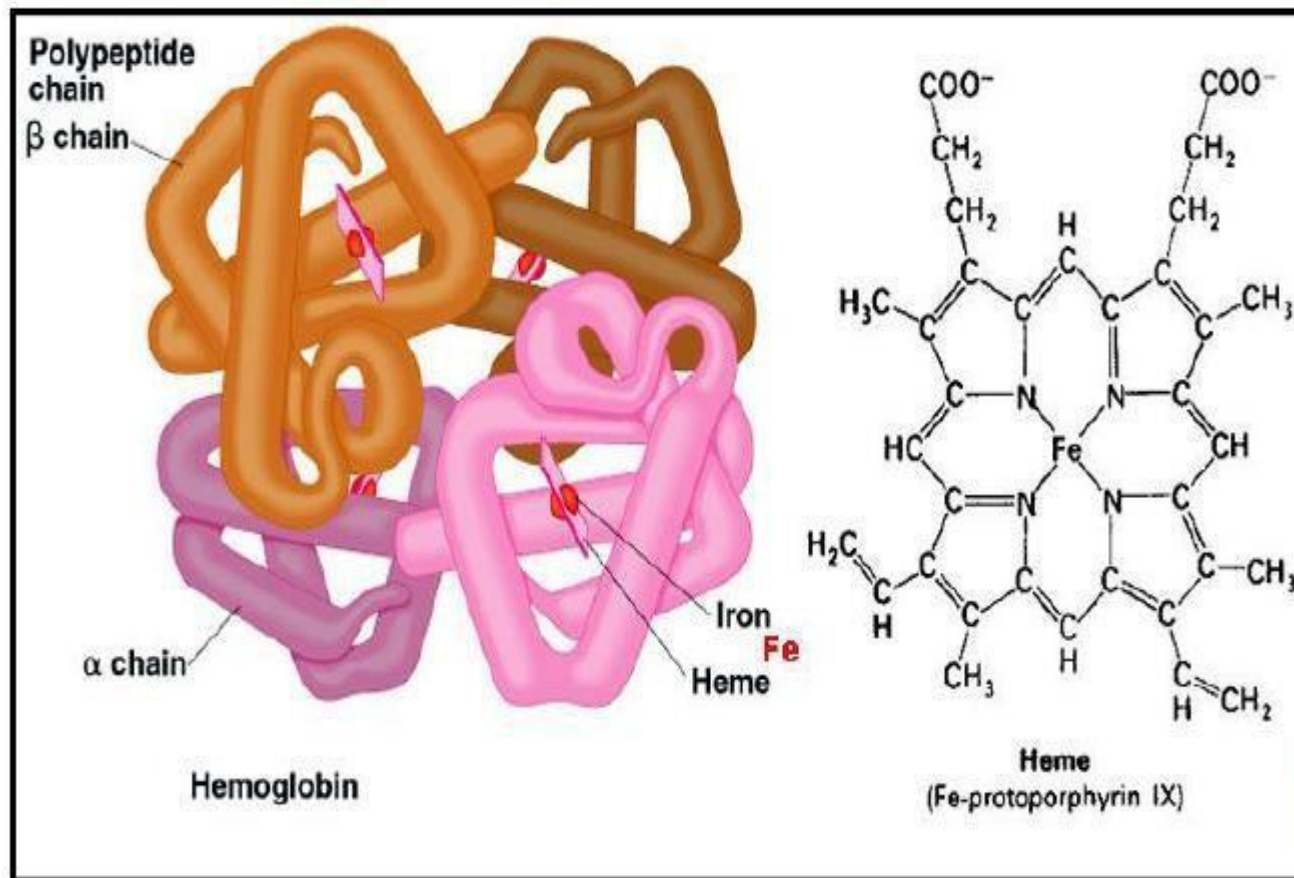
# Hemoglobin

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Lec. 5

# Hemoglobin Determination Sahli's Method or Acid Hematin method

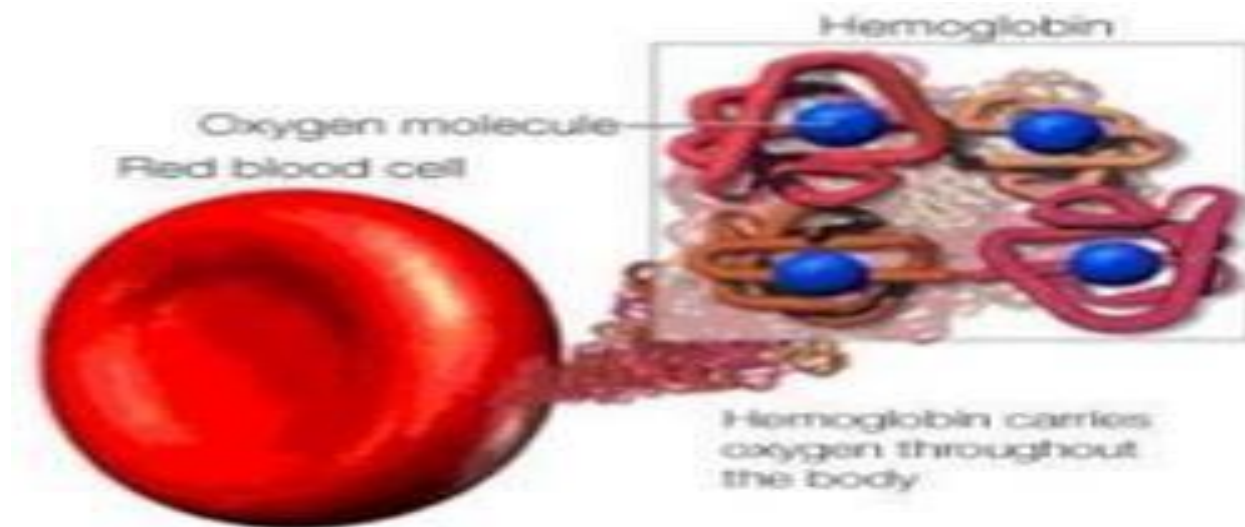


# Hemoglobin

- The name *hemoglobin* is the combination of *heme* and *globin*, reflecting the fact that each subunit of hemoglobin is a globular protein with an embedded *heme* (or haem) group. Each heme group contains one iron atom, that can bind one oxygen molecule. The most common type of hemoglobin in mammals contains four such subunits.

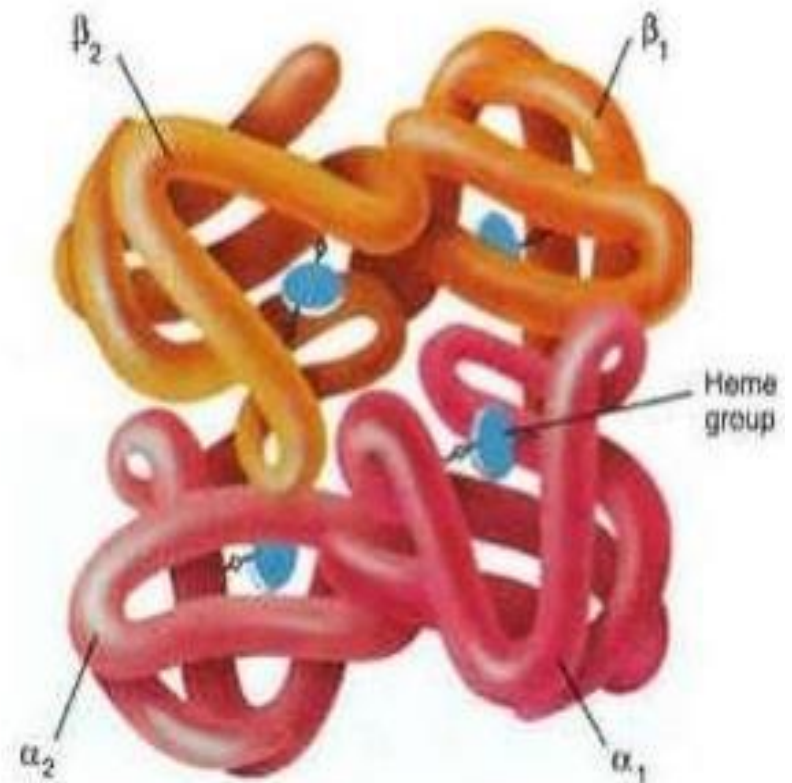
**Hemoglobin (Hb or Hgb) is a red color pigment present in red blood cells (RBCs) comprises Fe and Globin protein .**

- **Each gram of hemoglobin carries 1.34 ml oxygen**
- **Each gram of Hb contains 3.33 mg of iron**
- **Total Hb content in a healthy body is approximately 600 gms**



# Structure of Hemoglobin

- Hemoglobin molecule is a tetramer consisting of two pairs of similar polypeptide chains called **globin chains**.
- To each of the four chains is attached heme which is a complex of iron in ferrous form and protoporphyrin.
- The major (96%) type of hemoglobin present in **adults** is called **HbA** and it has
  - ✓ 2 alpha globin chains and
  - ✓ 2 beta globin chains ( $\alpha_2\beta_2$ ).





**The Estimation of hemoglobin in the blood is commonly prescribed in various physiological and pathological conditions and as both diagnostic and prognostic test especially in case of suspected Anemia which can be caused by various factors .**

**Nowadays in many laboratories, the Hemoglobin estimation is done by using Automatic Hematology Analyzers but still in many other labs the following method is Commonly used to determine the Hemoglobin concentration in patient's blood .**

**1-Sahli's Method or Acid Hematin Method**

**2-Cyanmethemoglobin Method ( CMG) Or Drabkin's Method**

# Sahli's Acid Hematin Method

Principle -

- Blood is mixed with an acid solution so that Hb is converted to **brown colored acid hematin**
- Diluted with water till brown colour matches that of brown glass standard
- Hb value is read directly from the scale

# Sahli's Method



Sahli's apparatus (1930 )



# Sahli's Acid Hematin Method

- Equipments-
- Sahli hemoglobinometer
- Sahli pipette (marked at 20 microlite or 0.02 ml)
- Stirrer
- Dropping pipette
- Reagents
- N/10 hydrochloric acid
- Distilled water

# APPARATUS & EQUIPMENTS REQUIRED FOR SAHLI'S METHOD / ACID HEMATIN METHOD

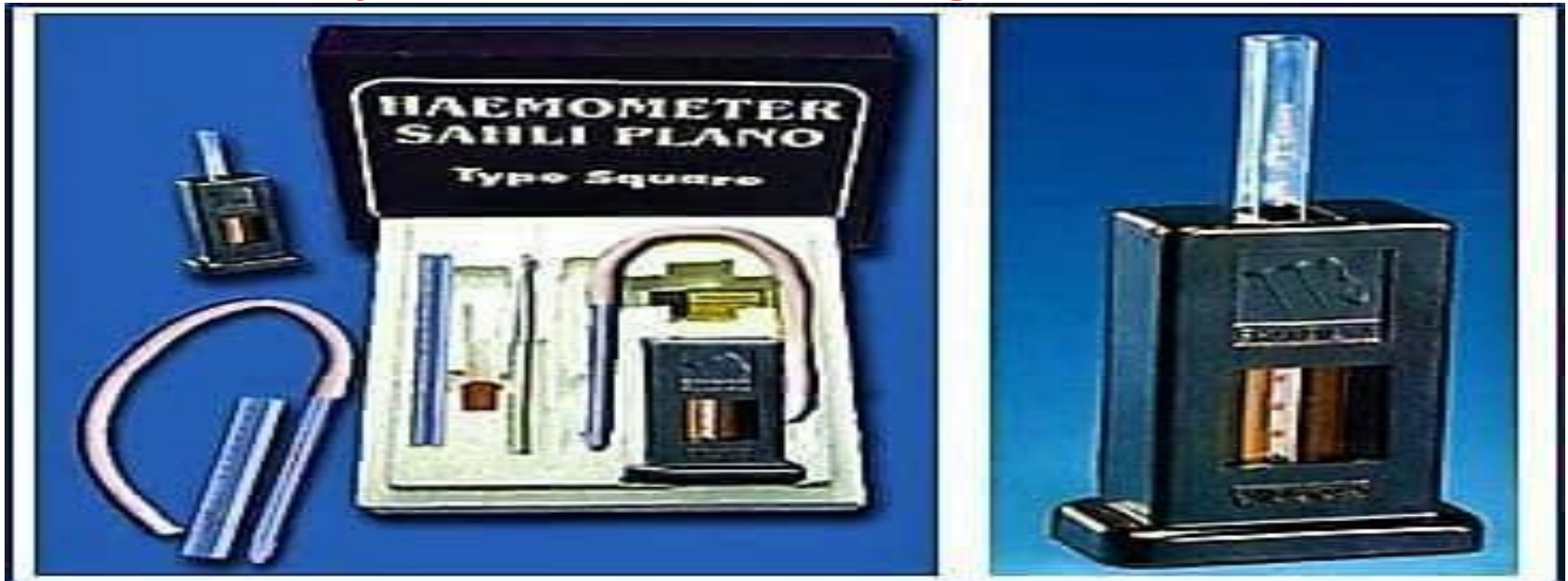
## Sahli's Apparatus

A- Hemoglobin pipette (0.02 ml or 20  $\mu$ l capacity )

B- Sahli's graduated Hemoglobin tube

C- Thin glass rod Stirrer for Hemoglobin Tube

D- Sahli's Comparator box with brown glass standard .



# Materials used

- 1. Sahli's haemoglobinometer.
- 2. Two Pasteur pipettes (one for HCl and one for distilled water).
- 3. Glass rod to stir ( stirrer )
- 4. 0.1 N - Hydrochloric acid
- 5. Distilled Water
- 6. Comparison tube.
- 7. Pipette ( Hemoglobin pipette with rubber tubing and mouthpiece )

# Sahli's Acid Hematin Method

- Place N/10 HCl into Hb tube upto 2 grams.
- Blood sample in Sahli's Hb pipette upto 20 micro litre.
- Add blood sample to acid solution.
- Mix with a stirrer.
- Allow to stand for 10 minutes.
- Add distilled water drop by drop till the colour of the solution matches to brown glass standard.
- Take the reading of the lower meniscus from the graduated tube in grams.





# Sahli's Acid Hematin Method

## Advantages

- Easy to perform
- Quick
- Inexpensive
- Can be used as a bedside procedure
- Does not require technical expertise

## DISADVANTAGES OF SAHLI'S METHOD / ACID HEMATIN METHOD

- 1-**Visual intensity may be different for different individuals by this method, we are not able to measure the inactive hemoglobin .
- 2-**This method estimates only oxy Hemoglobin. Carboxyhemoglobin and methemoglobin cannot be estimated .
- 3-**The end point disappears soon so it is difficult to know the actual endpoint and also the Proper stable standard is not available
- 4-**The resulting solution is not a clear solution but a suspension due to the action of hydrochloric acid on the proteins and lipids .