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## BCH 471

### Experiment (7)

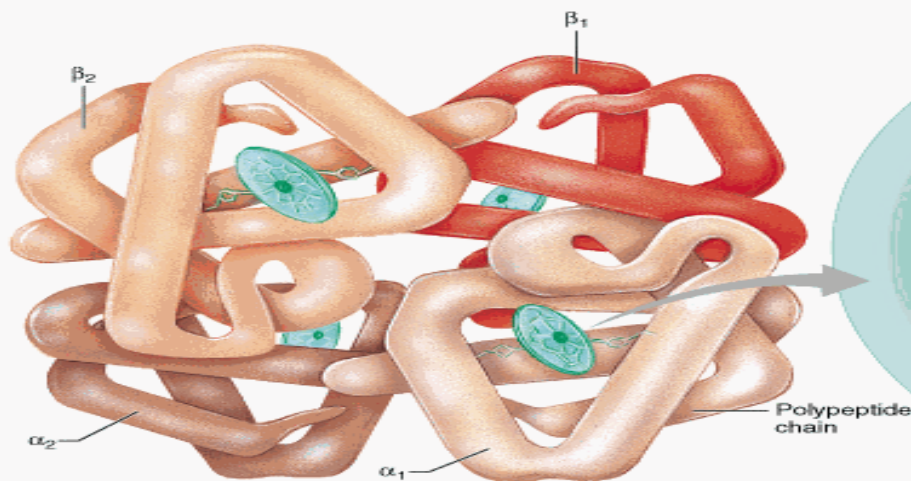
**HEMOGLOBIN AND ANEMIA,  
ERYTHROCYTE SEDIMENTATION RATE (ESR)  
AND HEMATOCRIT (HCT)**

# OBJECTIVES

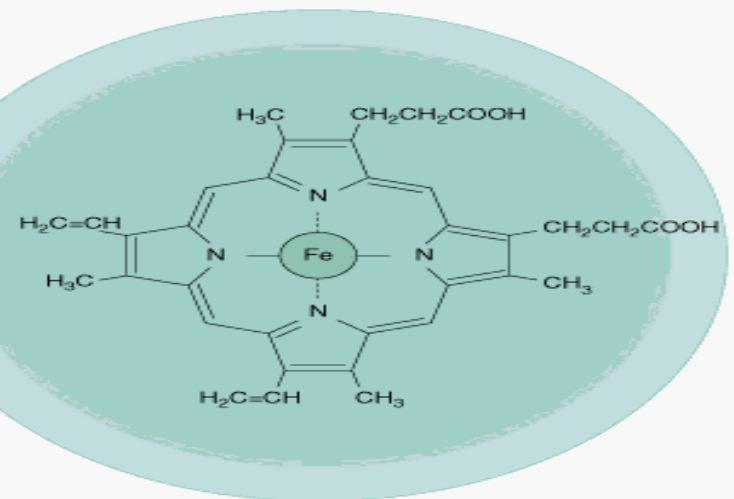
- 1) Quantitative determination of hemoglobin in a blood sample.
- 2) Determination of erythrocyte sedimentation rate (ESR).
- 3) Determination of hematocrit (HCT).
- 4) To assess the condition of a patient by such tests.

# HEMOGLOBIN STRUCTURE

- Hemoglobin (Hb) is a porphyrin–iron (II) protein in RBCs that transport oxygen from the lungs to the rest of the body and carbon dioxide back to the lungs.
- Hb is made up of 4 subunits of globin protein, with a heme (iron containing group).



(a) Hemoglobin

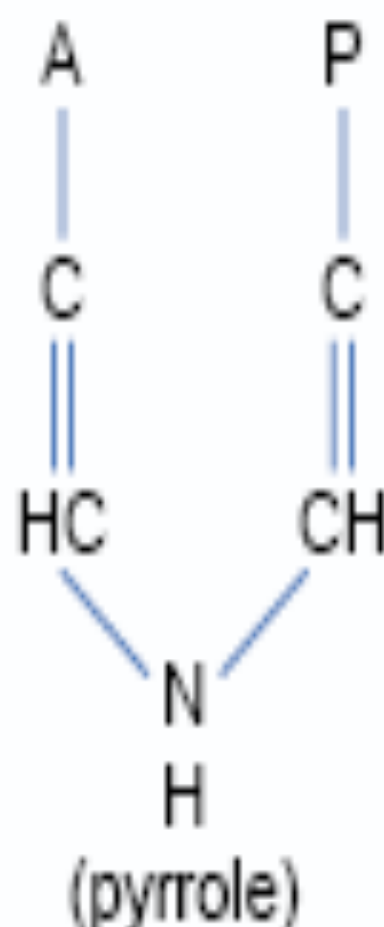


(b) Iron-containing heme group

# Hemoglobin Synthesis

- The circulation blood of normal adult contain about 750 g of Hb and of this about 7 – 8 g are degraded daily.
- **This amount has to be newly synthesized each day because:**
  - The globin part of Hb can be reutilized only after catabolism into its constituent amino acid.
  - The free heme is broken down into bile pigment which is excreted.
  - Iron alone is reutilized in the synthesis of Hb.
- **The rate of Hb synthesis (Rate of RBC formation) depends on**
  - The amount of oxygen reaching the blood
  - Capacity of the blood to carry oxygen ,which in turn depend on the amount of circulating hemoglobin

I. 2 succinyl-CoA + 2 glycine  $\longrightarrow$



II. 4 pyrrole  $\longrightarrow$  protoporphyrin IX

III. protoporphyrin IX +  $\text{Fe}^{++}$   $\longrightarrow$  heme

IV. heme + polypeptide  $\longrightarrow$  hemoglobin chain ( $\alpha$  or  $\beta$ )

V. 2  $\alpha$  chains + 2  $\beta$  chains  $\longrightarrow$  hemoglobin A

## Regulation of Hb Synthesis:

- Hb synthesis is stimulated by anoxia or hypoxia, whether due to oxygen deficiency or due to anaemia.
  - *Anoxia*: means a total depletion in the level of oxygen, an extreme form of hypoxia or "low oxygen"
- There is a strong evidence that the marrow response to the stimulus of hypoxia is dependent upon **erythropoietin**.
- **Erythropoietin is a** glycoprotein hormone formed in kidney in response to decrease oxygen carrying capacity (hypoxia or anemia), in order to stimulate the erythropoiesis

Tissue hypoxia



Kidney secrete erythropoietin into blood



Increase erythropoiesis



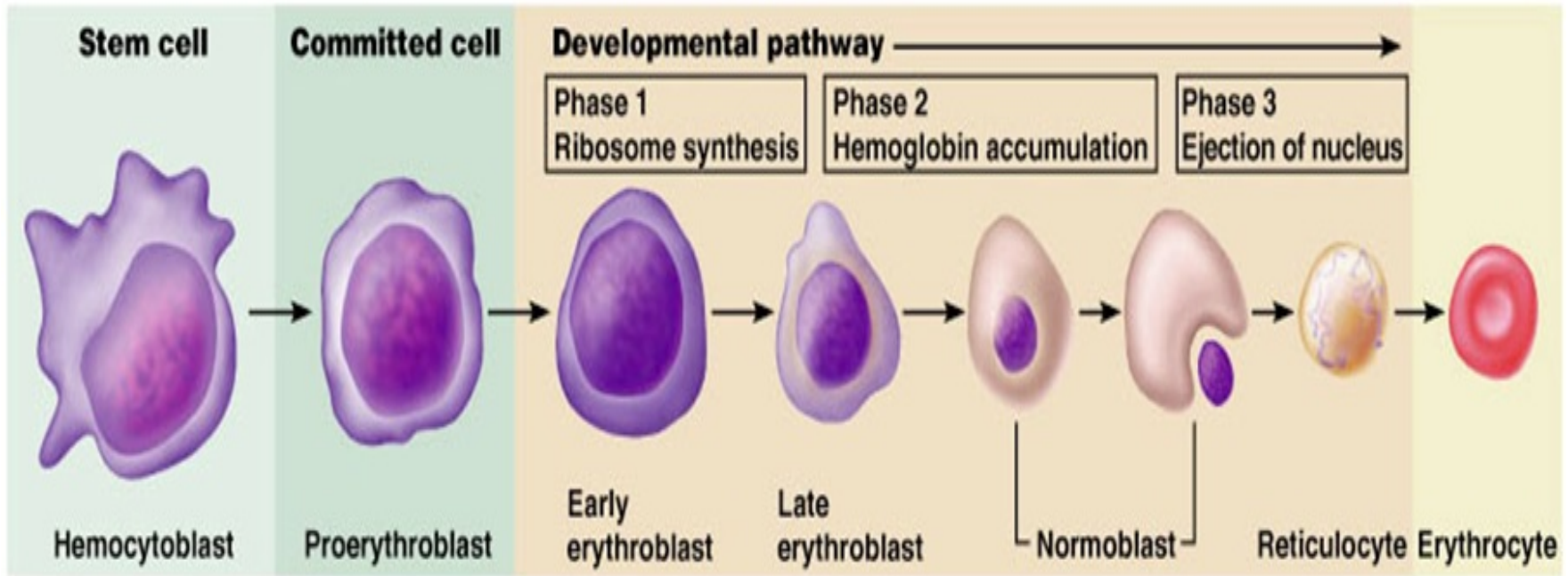
Increase number of RBC



Increase oxygen carrying capacity

Return to homeostasis when oxygen is delivered to kidney, this cause negative feedback inhibition to stop the secretion of erythropoietin



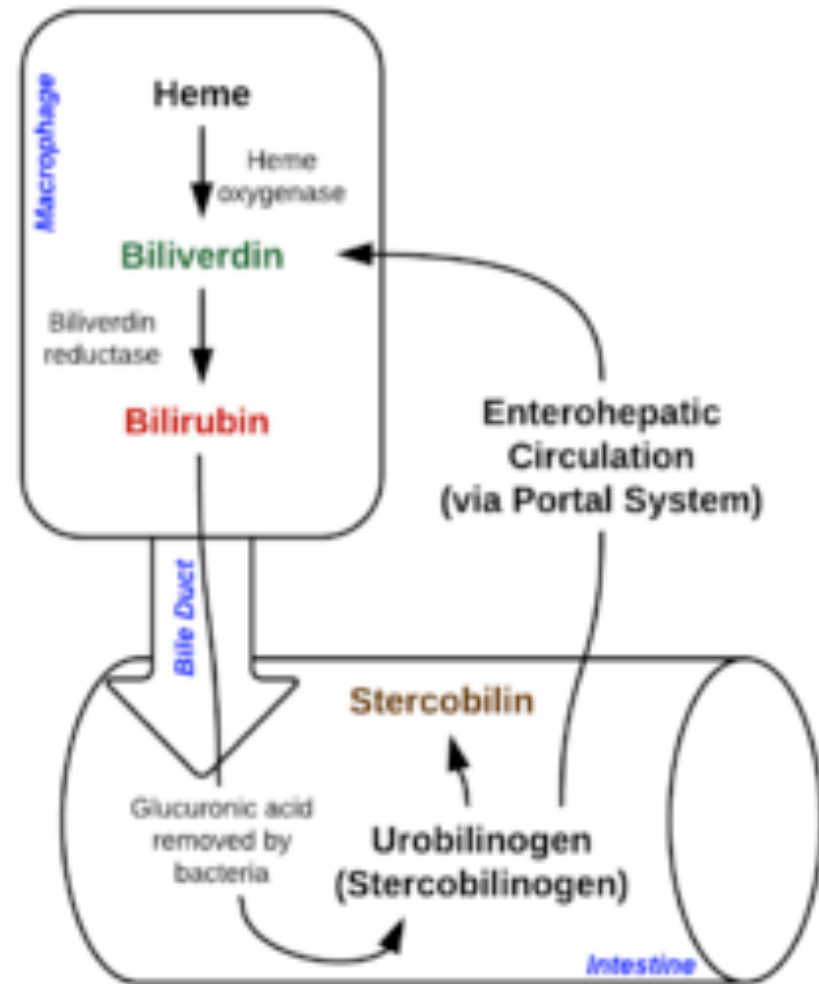


- The erythrocytes are derived from primitive nucleated cells in the bone marrow by successive processes of mitosis and maturation.
- A primitive stem cell divides to form two cells, one of which retains its behavior as a stem cell while the other successively divides to form the mature non-nucleated fully haemoglobinized erythrocyte



# Haemoglobin Catabolism:

- In the reticuloendothelial system, erythrocytes are destroyed and haemoglobin is released.
- Globin is separated from haem and haematin is formed (the ferrous iron  $\text{Fe}^{+2}$  oxidized to ferric iron  $\text{Fe}^{+3}$ )
- The porphyrin ring is then opened and the iron is removed with formation of straight chain compound **biliverdin** which is converted to **bilirubine** by reduction
- The iron and amino acid of the globin are retained but pyrrole ring are excreted as bilirubin.



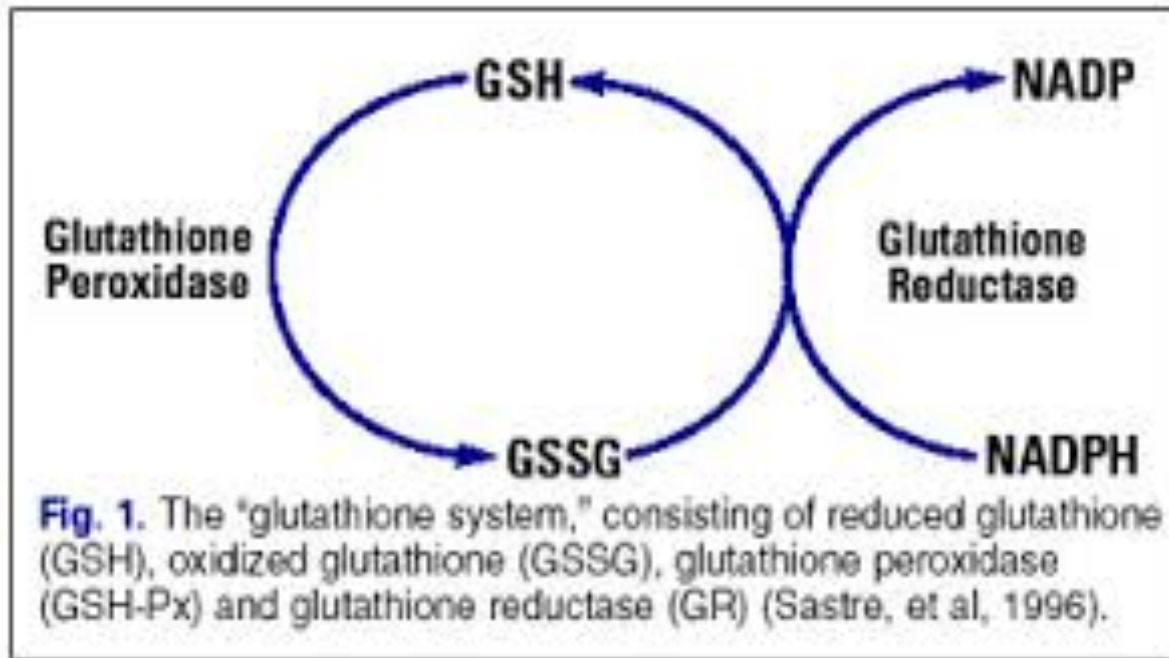
## The role of some factor affecting on the native of haemoglobin:

1) *Vitamins and cofactor*: Biotin (B7), pantothenic acid (B5), folic acid (B9), coenzyme A and pyridoxal phosphate are essential for haem synthesis .

2) *Trace metals* : Only copper and cobalt are known to play a role .

- (Copper is playing a role in the absorption of iron while Cobalt is essential constituent of vitamin B12 (Cobalamin) )

3) *Glucose -6-phosphatase dehydrogenase (G6PD)*: it is an enzyme responsible for the conversion the glucose in the pentose phosphate pathway (PPP) to form 6-phosphogluconate , this pathway provide **NADPH** which is used to produce reduced glutathion, which is necessary for cell integrity by neutralizing free radicals that cause oxidative damage.



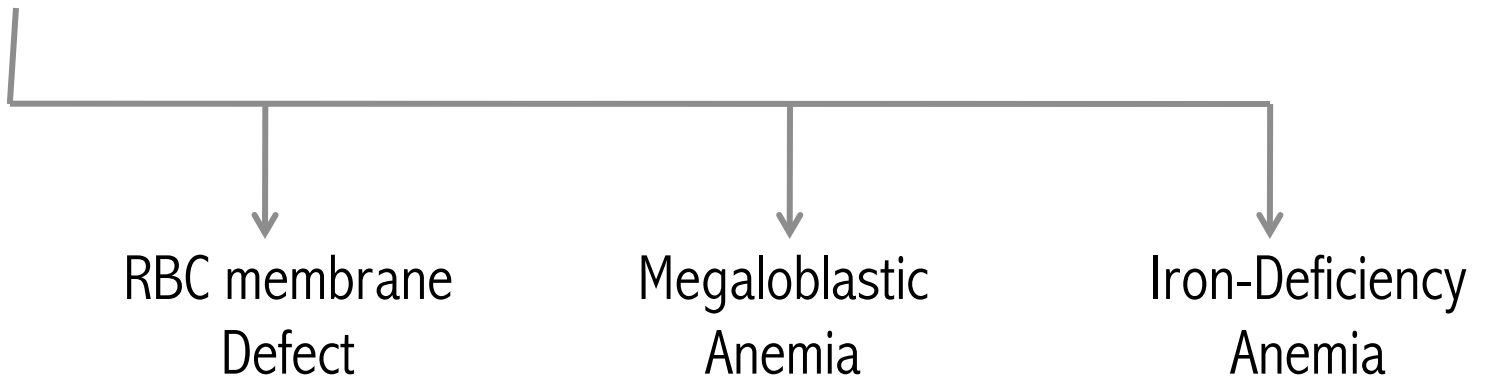
- Deficiency of G6PD lead to Decrease level of NADPH.
- Under oxidative stress, Hb is oxidized to met-Hb, which aggregates together causing hemolytic anemia.

## Anemia :

- It is in general decrease in the amount of RBC or the normal amount of Hb in blood. It can also be defined as a lowered ability of the blood to carry oxygen.

## Causes:

- I. Genetics
- II. Acquired



## Iron-deficiency anemia:

Deficiency of iron is essentially due to blood loss with failure to replace the iron stores because of :

- Dietary deficiency or
- Increase requirement or
- Defective absorption.

## Megaloblastic Anemia:

This may be due to deficiency of folic acid or cobaltamin (Vit. B12)

## RBC membrane defects:

- In this condition there is a defect of the erythrocyte membrane and an abnormality in the sodium pumps.
- The best-known disorders are **hereditary spherocytosis** and **hereditary elliptocytosis**.

# Estimation of blood haemoglobin:

## Principle:

- The ferrous (Iron II) in each haem in RBC is oxidized by ferricyanide to Fe(III)-methaemoglobin .
- A cyanide group ( $\text{CN}^-$ ) is then attached to the iron atom (because it is positively charge) by reaction with KCN to give the brown cyanomethamoglobin (stable) which can be estimated quantitatively

Normal Hb conc.: for men: 14 - 18 g/dl, for women : 12 - 16 g\dl

↑ Level of Hb is associated with polycythemia and dehydration

↓ Level of Hb is associated with aneamia

# METHOD

Pipette into clean dry test tubes

	Test	Blank
Hemoglobin reagent	2 ml	2 ml
Blood sample	0.01 ml ( 10 $\mu$ l)	_____

Mix, allow to stand at room temperature for 3 min and read the absorbance at 540 nm against hemoglobin reagent

- Hb conc (g/dl) = 29.4 x Abs of test

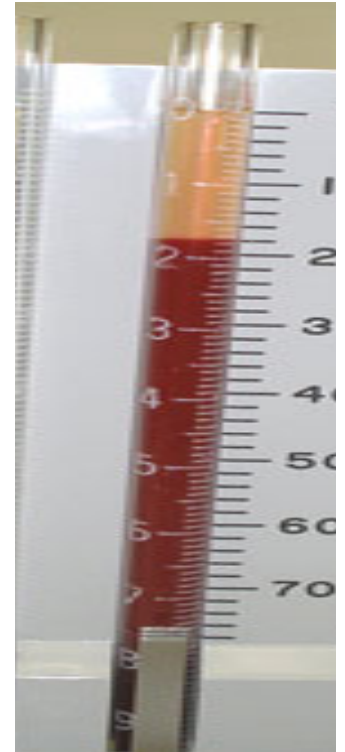
# ERYTHROCYTE SEDIMENTATION RATE (ESR)

- ESR is the rate at which erythrocytes settle out of anticoagulated blood in 1 hour.
- It is used clinically as a non-specific screening test to:
  - detect the presence of infection in the body in general.
  - monitor the status of chronic inflammatory disease such as rheumatoid arthritis.
- ESR is not diagnostic of any particular disease, but rather is an indication that a disease process is ongoing and must be investigated.
- This test is based on the fact that inflammatory and necrotic processes cause an alteration in blood proteins, resulting in an aggregation of red cells, which make them heavier and more likely to fall rapidly when placed in a special vertical tube.



# PRINCIPLE:

- In this technique, anticoagulated whole blood are allowed to sediment under the effect of gravity, using a narrow vertical tube called Westergren's tube.
- The length of the column of clear plasma at the top is noted at the end of 1 hour.



## Normal Range

	After 1 hour	After 2 hours
Men	0 – 5 mm	7 – 15 mm
Women	0 – 10 mm	10 – 20 mm

# HEMATOCRIT (HCT)

- *HCT or packed cell volume (PCV)* is the volume percentage (%) of RBCs in blood
- It is used as a simple screening test for anemia.
- Blood is collected in heparinised *capillary tube*, which is then sealed, centrifuged and the red cell volume expressed as a percentage of the whole blood.

## Calculation :

$$\text{HCT} = \frac{\text{Length of column of RBC}}{\text{Total length of blood component}} \times 100$$

- A normal hematocrit percentage depends on age and gender.

## Normal ranges

Male: 40.7 - 50.3%      Female: 36.1 - 44.3%

## Interpretation :

Low HCT cause of anemia

High HCT cause of Polycythemia

**Blood sample**

