

**BCH 471**

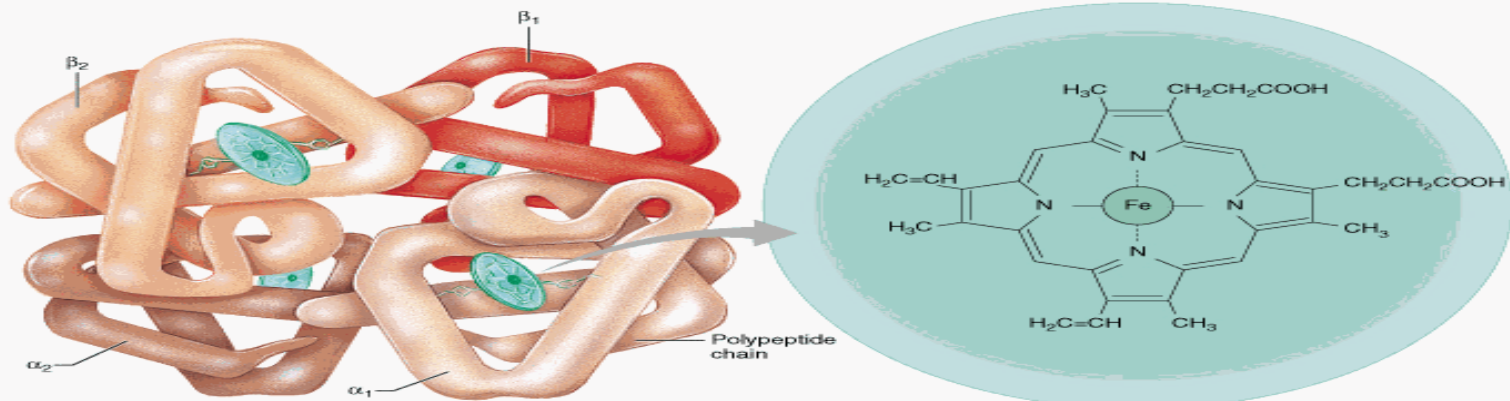
# Hemoglobin and anemia

# Objectives

- **Quantitative** determination of hemoglobin in a blood sample.
- **Qualitative** determination of hemoglobin S (HbS) in blood using a phosphate solubility method.

# 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 heam 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

## 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 anoxia), 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 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 .

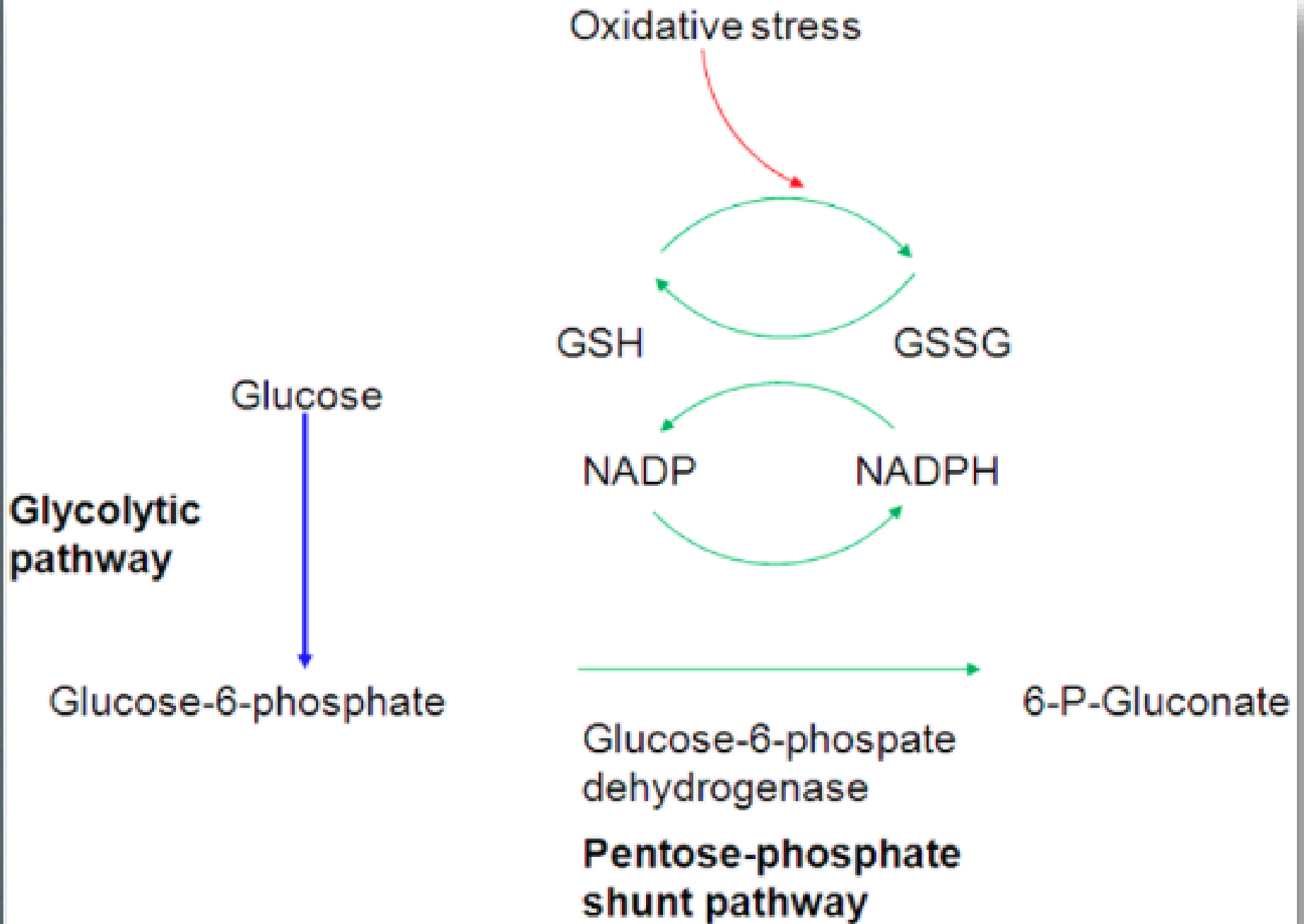
### 2) *Trace metals :*

- 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 vitamine B12 (Cobalamin) )

### 3) *Glucose -6-phosphatase dehydrogenase (G6PD)*

- G6PD is an enzyme required to protect cells from damage by oxidation.
- It is responsible for the conversion glucose in the **pentose phosphate pathway (PPP)** to form 6-phosphogluconate , this pathway provide *NADPH* which is used to produce *reduced glutathione (GSH)*.
- GSH is necessary for cell integrity by neutralizing free radicals that cause oxidative damage.
- Failure to withstand oxidative stress due to G6PD deficiency, leads to decreased level of NADPH ,therefor **Hb is oxidized by free radicals to met-Hb**, which aggregates together causing hemolysis.





## 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 →

- **RBC membrane Defect:** The best-known disorders are **hereditary spherocytosis** and **hereditary elliptocytosis**.

II. Acquired

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

**Iron-Deficiency Anemia**

# 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

# **Qualitative determination of hemoglobin S (HbS) in blood.**

# Introduction

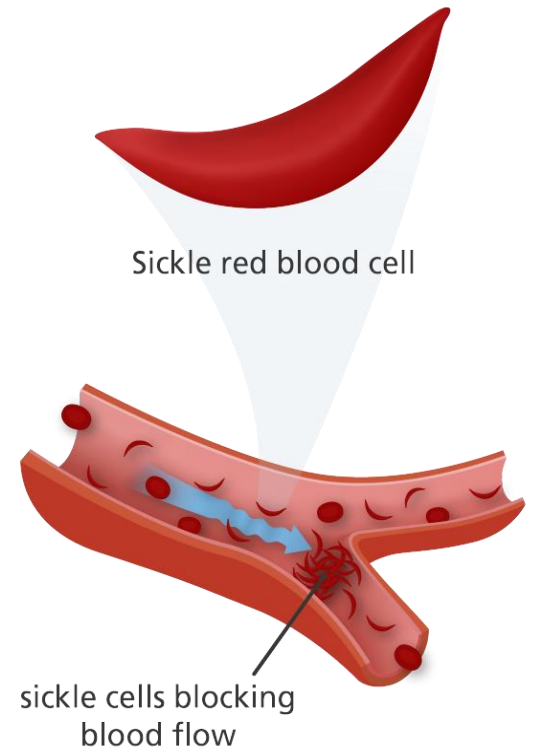
There are hundreds of Hb variants, and the most common are:

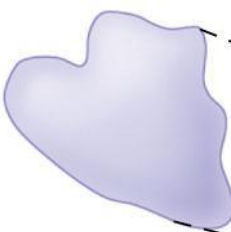
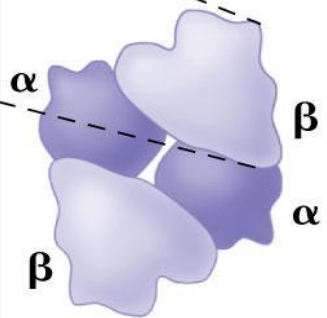
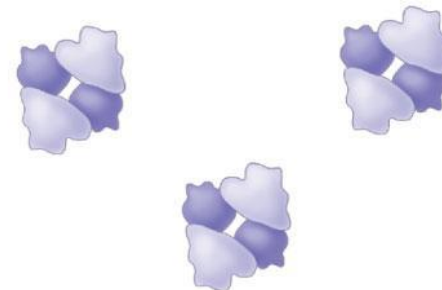
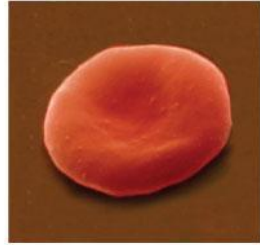
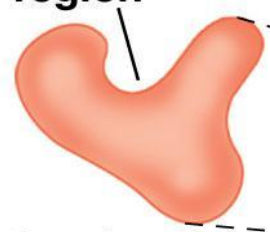
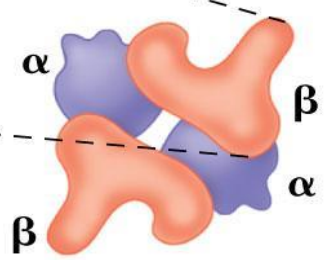
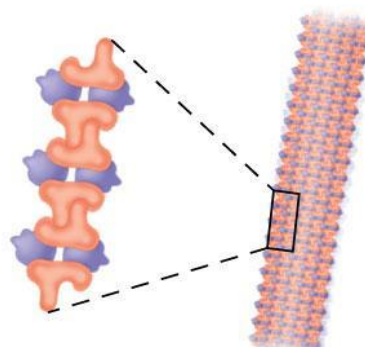

- **Hemoglobin A**
  - It is normal hemoglobin that exists after birth and consist of ( $\alpha_2\beta_2$ ).
  - In normal adult 95% of Hb is present as HbA
- **Hemoglobin A2**
  - It is a minor component of the hemoglobin found in red cells after
  - birth and consists of ( $\alpha_2\delta_2$ )
  - less than 3% of the total red cell hemoglobin.
- **Hemoglobin F**
  - Hemoglobin F is the predominant hemoglobin during fetal development and consists of ( $\alpha_2\gamma_2$ ).

# Example of an abnormal Hb

## Hemoglobin S (HbS)

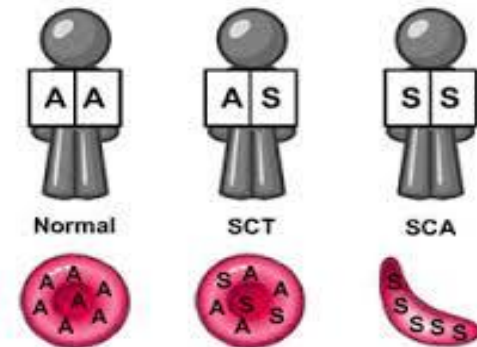
- The alpha chain is normal, while the beta chain is mutated, giving the molecule the structure,  $\alpha_2\beta S_2$ .
- A point mutation in the **Hb  $\beta$  gene** is responsible for the sickling of RBCs seen in sickle cell anemia .The abnormality is due **to Substitution of non polar valine for a charged Glutamic acid in position 6 in the  $\beta$  chain .**



	Primary Structure	Secondary and Tertiary Structures	Quaternary Structure	Function	Red Blood Cell Shape
Normal hemoglobin	1 Val 2 His 3 Leu 4 Thr 5 Pro 6 Glu 7 Glu	 <p><math>\beta</math> subunit</p>	<p>Normal hemoglobin</p> 	<p>Molecules do not associate with one another; each carries oxygen.</p> 	 <p>10 <math>\mu</math>m</p>
Sickle-cell hemoglobin	1 Val 2 His 3 Leu 4 Thr 5 Pro 6 Val 7 Glu	<p>Exposed hydrophobic region</p>  <p><math>\beta</math> subunit</p>	<p>Sickle-cell hemoglobin</p> 	<p>Molecules crystallize into a fiber; capacity to carry oxygen is reduced.</p> 	 <p>10 <math>\mu</math>m</p>



- HbS can be inherited in **the homozygous state (S/S)** **produce sickle cell anemia** , or in heterozygous (A/S) ,also called sickle cell trait, usually don't exhibit symptoms of the sickle cell anemia disease (unless under extreme hypoxia).



- Individuals with HbS will be at high risk when exposed to conditions of **low oxygen tension** such as surgery, high altitude or athletics which may result in serious and fatal clinical complications.

# Principle

- Erythrocytes are lysed (by saponin) and the released hemoglobin is reduced (by dithionite) in phosphate buffer.
- *Reduced HbS is characterized by its very low solubility* → So that in the presence of HbS, the solution become **turbid** and the lines behind the test tube will not be visible while, if no HbS was present the clear solution will permit the lines to be seen through the test tubes.

# Method of HbS

Pipette into clean dry test tube

Reagent	Volume
Sickling solution	2 ml
Patient sample (whole blood)	0.02 ml (20 $\mu$ l)

**Mix by inversion and allow stand at room temperature for 5 to 10 min**

**Read the test by holding the test tube approximately 3 cm in front of a lined scale on the card.**

# Results

