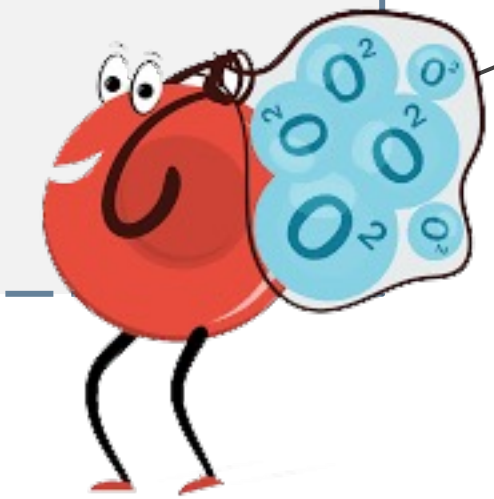
Blood Biochemistry BCH 471 [Practical]

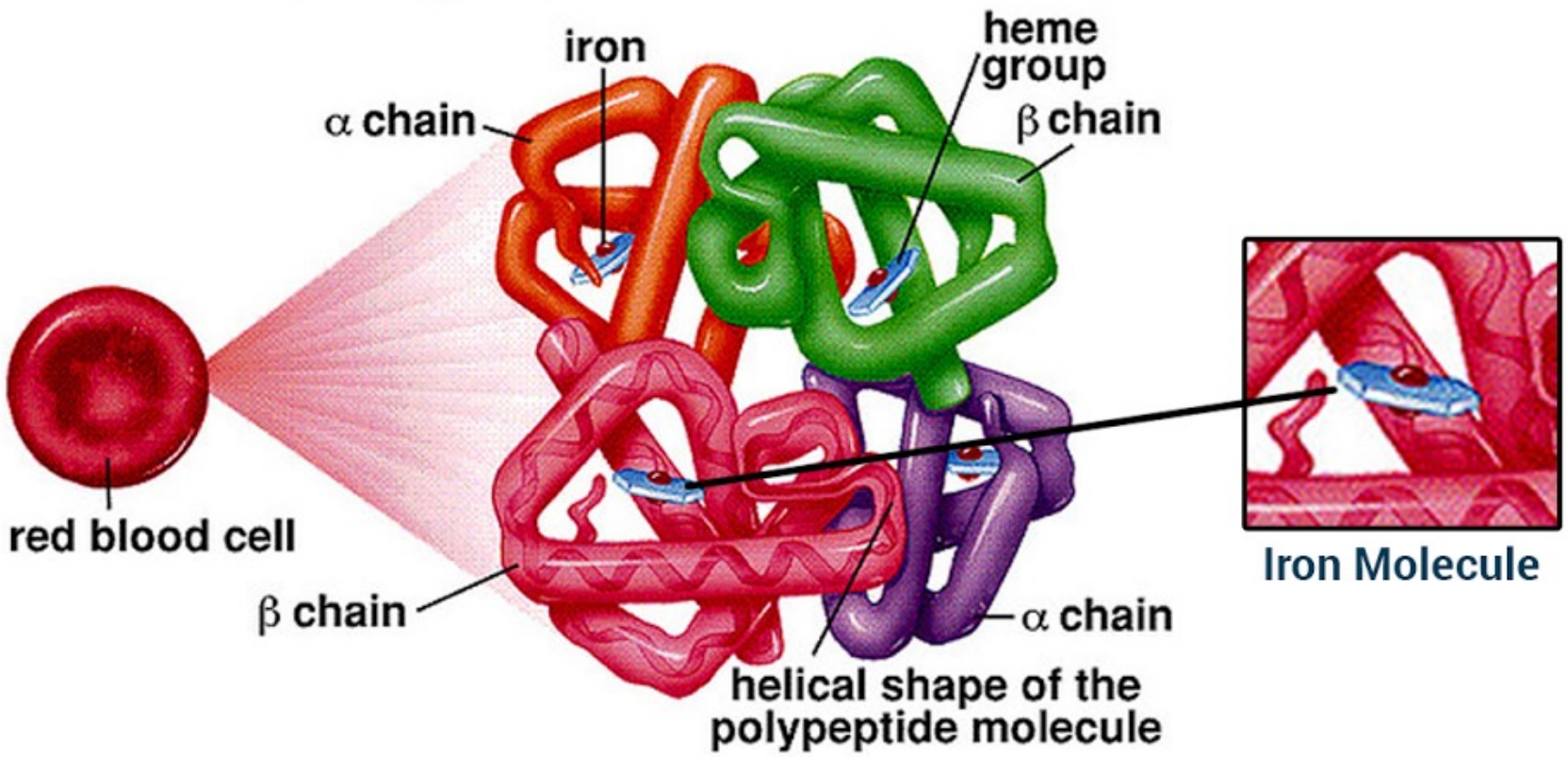
Lab (5) Hemoglobin and Anemia



Part one

Hemoglobin Structure

- **Hemoglobin** is the protein molecule in **red blood cells.**
- **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 pigment).



Hemoglobin Synthesis

- The circulation blood of normal adult contain about 750g of Hb and of this about 6g are degraded daily.

This amount has to be newly synthesized each day because:

1. The **globin** part of Hb can be reutilized only after catabolism into its constituent amino acid
2. The free **heme** is broken down into bile pigment which is excreted
3. **Iron** alone is reutilized in the synthesis of Hb

The rate of Hb synthesis (Rate of RBC formation) depends on:

1. The amount of oxygen reaching the blood (inversely proportional)
2. Capacity of the blood to carry oxygen, which in turn depend on the amount of circulating hemoglobin

Regulation of Hb Synthesis

- **Hemoglobin** accounts for approximately **90**% of erythrocyte dry weight, therefore its biosynthesis is intimately related to **erythrocyte synthesis**.
- **Erythropoiesis** is the process which produces red blood cells (erythrocytes).
- **Erythropoiesis** is stimulated by anoxia or hypoxia , whether due to oxygen deficiency or due to anemia.
- **Hypoxia** stimulates the bone marrow to produce RBC through the action of **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**

Hypoxia low tissues oxygen.

Anoxia a complete lack of blood oxygen “extreme form of hypoxia”.

Regulation of Erythropoiesis

Tissue hypoxia (low O_2)

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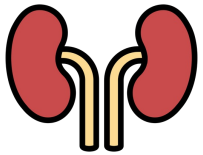
Kidney secrete erythropoietin into blood 

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Increase erythropoiesis in bone marrow

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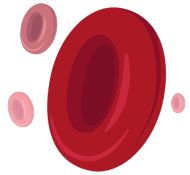
Increase number of RBC 

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Increase oxygen carrying capacity 

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

Factors Affecting Hemoglobin Synthesis

1. **Vitamins:**

Cobalamin (B12), folic acid (B9), ascorbic acid (C) pantothenic acid (B5) and pyridoxine (B6) are essential for hemoglobin synthesis.

2. **Proteins (Amino acid):** Proteins of high biological value are needed in the formation of RBCs.

3. **Metals:**

- **Iron** is part of hemoglobin
- **Copper** plays a role in the absorption of iron.
- **Cobalt** is essential constituent of vitamin B12 (Cobalamin).

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.

Anemias are classified as follows:

1. Hypoproliferative anemias (inadequate production of RBCs).
2. Maturation defect anemias.
3. Hyperproliferative anemias (decreased Hb, increased production of RBCs)
    a. Hemorrhagic: acute blood loss
    b. Hemolytic: a premature, accelerated destruction of RBCs
4. Dilutional anemias.
    a. Pregnancy

Anemia

Depending on the cause, anaemias can be classified as:

- **Caused by a deficiency in haemoglobin synthesis:**

1. microcytic anemias: (iron metabolism, heme synthesis, globin synthesis)
2. normocytic normochromic anemias: (bone marrow response)
3. macrocytic anemias: (B12 deficiency, folate deficiency)

- **Depending on the inheritance :**

1. Genetics:

(G6PD) deficiency. – Sickle cell anemia. – Thalassemia.

2. Acquired:

Megaloblastic anemia. – Iron-deficiency anemia.

In this lab we will focus on: the amount of Hb, Sickle Cell Anemia, and (G6PD) Deficiency

Estimation of Blood Hemoglobin

Objective:

- Quantitative determination of hemoglobin in a blood sample.

Principle:

- The **ferrous Fe(II)** in each heme in RBC is oxidized by **ferricyanide** to **Fe(III)-methemoglobin**.
- By reaction with K^+CN^- a **cyanide** group (CN^-) is then attached to the **iron** atom (because it is positively charged) to give the **brown cyanmethemoglobin** (stable) which can be estimated quantitatively.

Hemoglobin (Fe^{2+}) + Ferricyanide (oxidation) → **Methemoglobin (Fe^{3+})**

Methemoglobin (Fe^{3+}) + K^+CN^- → **Cyanmethemoglobin (HiCN)**

Method

- Pipette into clean dry test tubes

	Test	Blank
Hemoglobin reagent	2 ml	2 ml
Blood sample	0.01 ml (10µl)	_____
Mix, allow to stand at room temperature for **3 min** and read the absorbance at **540 nm** against blank		

Results

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

- **Normal Values**: Males: 14 - 18 g/dl. Females: 12 - 16 g\dl

⬆ Level of Hb is associated with **polycythemia** and **dehydration**

⬇ Level of Hb is associated with **anemia**

Polycythemia: is the term used to describe an abnormal increase in the number of RBCs.

Part two

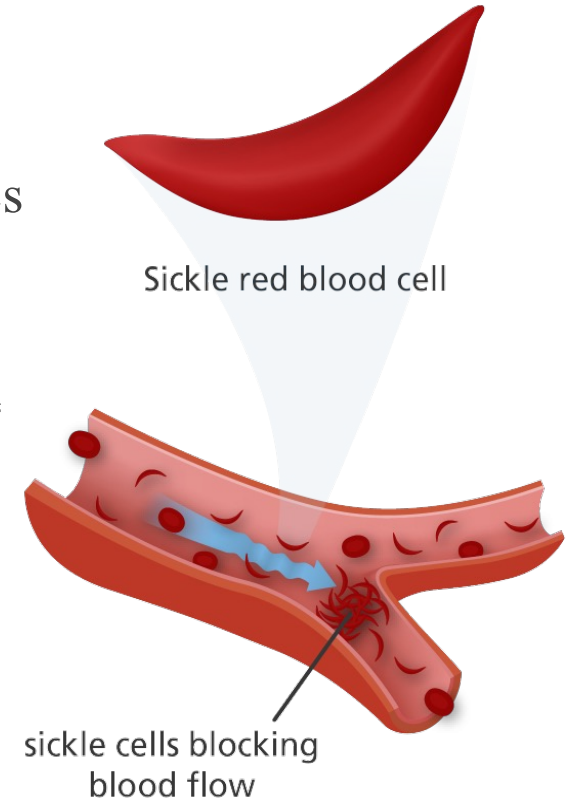
Normal Hb Types

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

- **Hemoglobin A1**
  - It is normal hemoglobin that exists after birth (**A**dult) and consist of **(α2β2).**
  - 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 **(α2δ2).**
  - less than 3% of the total red cell hemoglobin.
- **Hemoglobin F**
  - Hemoglobin F is the predominant hemoglobin during **F**etal development and consists of **(α2γ2).**

Abnormal Hb types

- **Hemoglobin S (HbS)**
  - The **alpha** chain is normal, while the **beta** chain is mutated, giving the molecule the structure, α2βS2.
  - A point mutation in the Hb **β 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 β 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	β subunit	Normal hemoglobin α β α β	Molecules do not associate with one another; each carries oxygen.	10 μm
Sickle-cell hemoglobin	1 Val 2 His 3 Leu 4 Thr 5 Pro 6 Val 7 Glu	Exposed hydrophobic region β subunit	Sickle-cell hemoglobin α β α β	Molecules crystallize into a fiber; capacity to carry oxygen is reduced.	10 μm

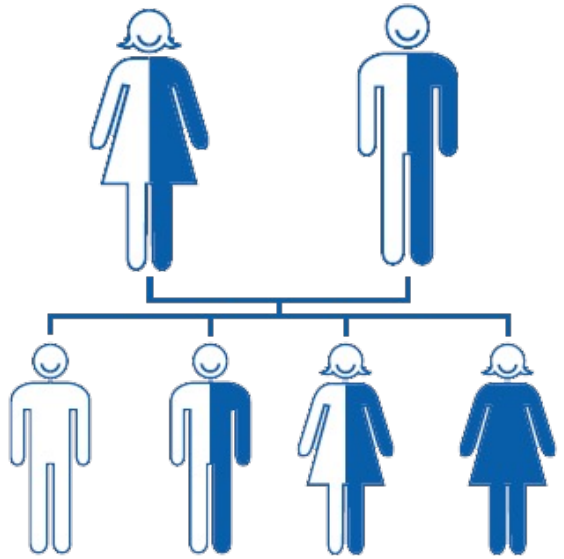
Sickle Cell Anemia

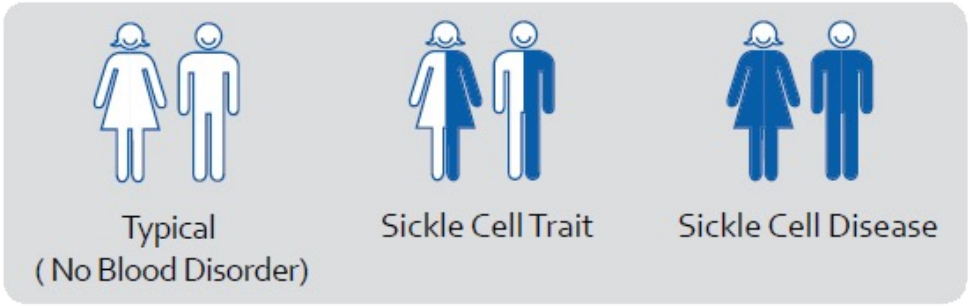
- **Sickle Cell disease** is a genetic disorder that affects erythrocytes (RBC) causing them to become sickle or crescent shaped.
- HbS can be inherited in an **autosomal recessive**

a. **Homozygous state (HbSS)** produce sickle cell anemia

b. **Heterozygous (HbAS)**, also called sickle cell trait (carrier), 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 results in serious and fatal clinical complications.





Estimation of Blood Hemoglobin S (HbS)

Objective:

- Qualitative determination of hemoglobin S (HbS) in blood.

Principle

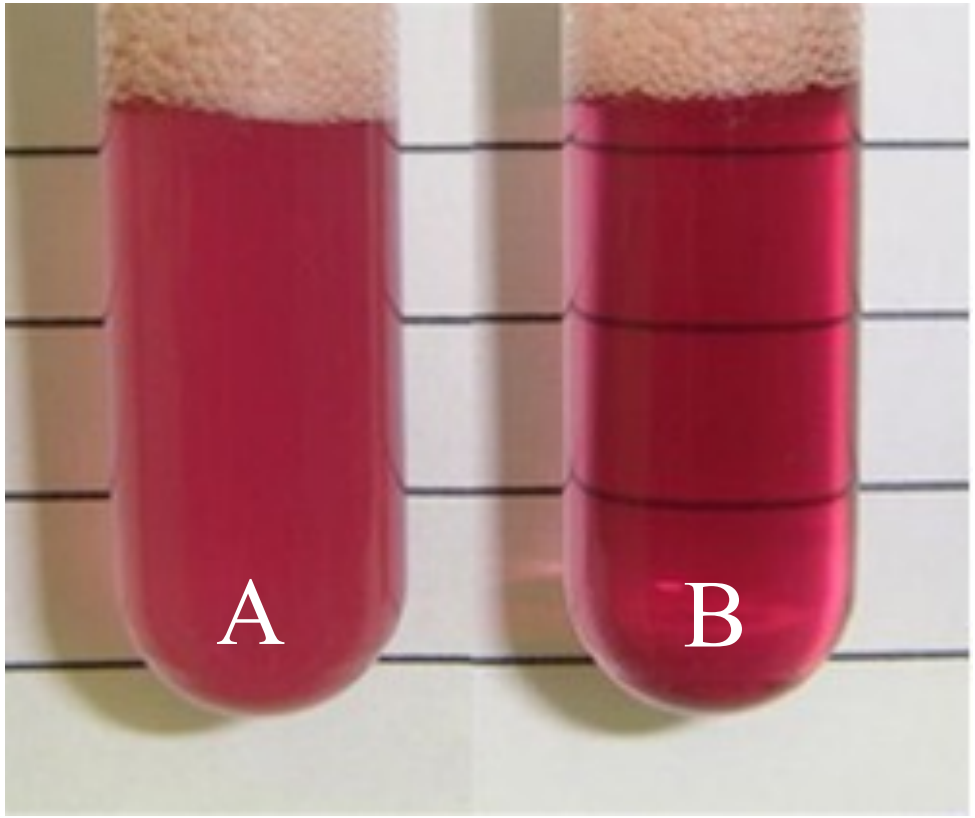
- Erythrocytes are **lysed** (by saponin) and the released hemoglobin is **reduced** (by dithionite) in phosphate buffer.
- ***Reduced HbS is characterized by its very low solubility*** → So that in the presence of HbS, the solution becomes **turbid** and the lines behind the test tube will not be visible,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

Method

- Pipette into clean dry test tubes

Reagent	Volume
Sickling solution	2 ml
Patient sample (whole blood)	0.02 ml (20 µl)
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



A Positive results

B Negative results

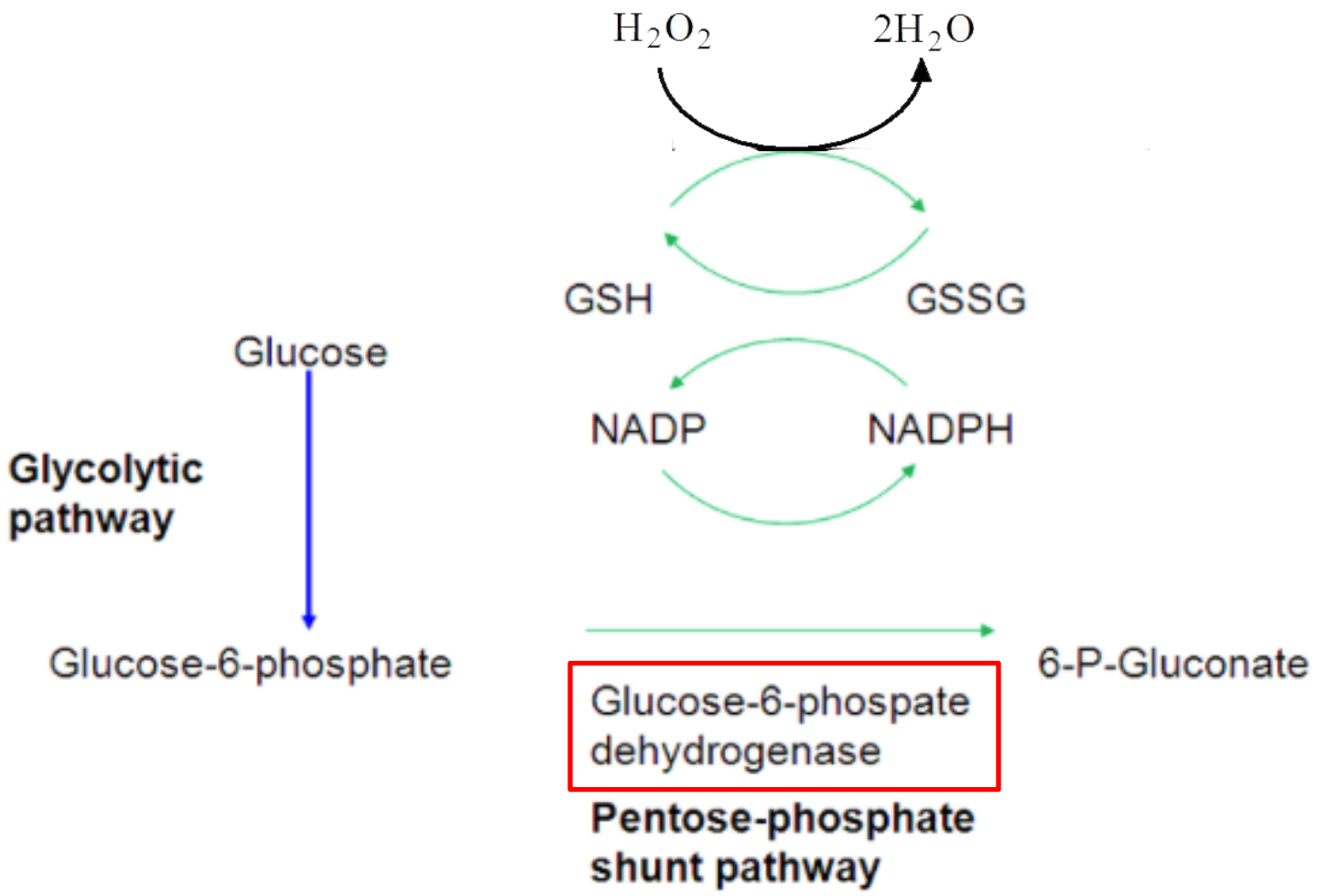
Part three

Glucose -6-phsphate dehydrogenase (G6PD)

Glucose -6-phsphate dehydrogenase (G6PD).

- **G6PD** is an enzyme required to protect cells (e.g. RBC) 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 convert oxidized glutathione (GSSG) to *reduced glutathione (GSH).*
- GSH is necessary for cell integrity by neutralizing free radicals that cause oxidative damage.

Glucose -6-phsphate dehydrogenase (G6PD)



Glucose -6-phsphate dehydrogenase deficiency

- **G6PD deficiency** is an inherited X-linked recessive trait that results in an inadequate amount of (G6PD) in the blood.
- **Normal RBCs** can increase generation of NADPH in response **to oxidative stress**; this capacity is impaired in patients with G6PD deficiency.
- Failure to withstand oxidative stress due to G6PD deficiency, leads to decreased level of NADPH, therefore Hb is oxidized by free radicals to met-Hb and denatures globin, which precipitates forming "*Heinz bodies*" leading to hemolysis.
- **Oxidative stress** can result from infection and from chemical exposure to medication e.g. antimalarial drug and certain foods e.g., fava beans

↓ G6PD ↓ NADPH ↓ GSH ↑ Oxidative Stress

Hemoglobin + Oxidative Stress → Methemoglobin + denatured globin → Heinz bodies → hemolysis