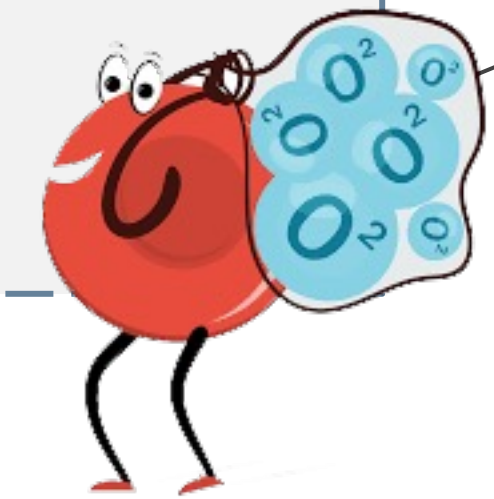
Blood Biochemistry BCH 220 [Practical]

Lab (5) Hemoglobin and Anemia

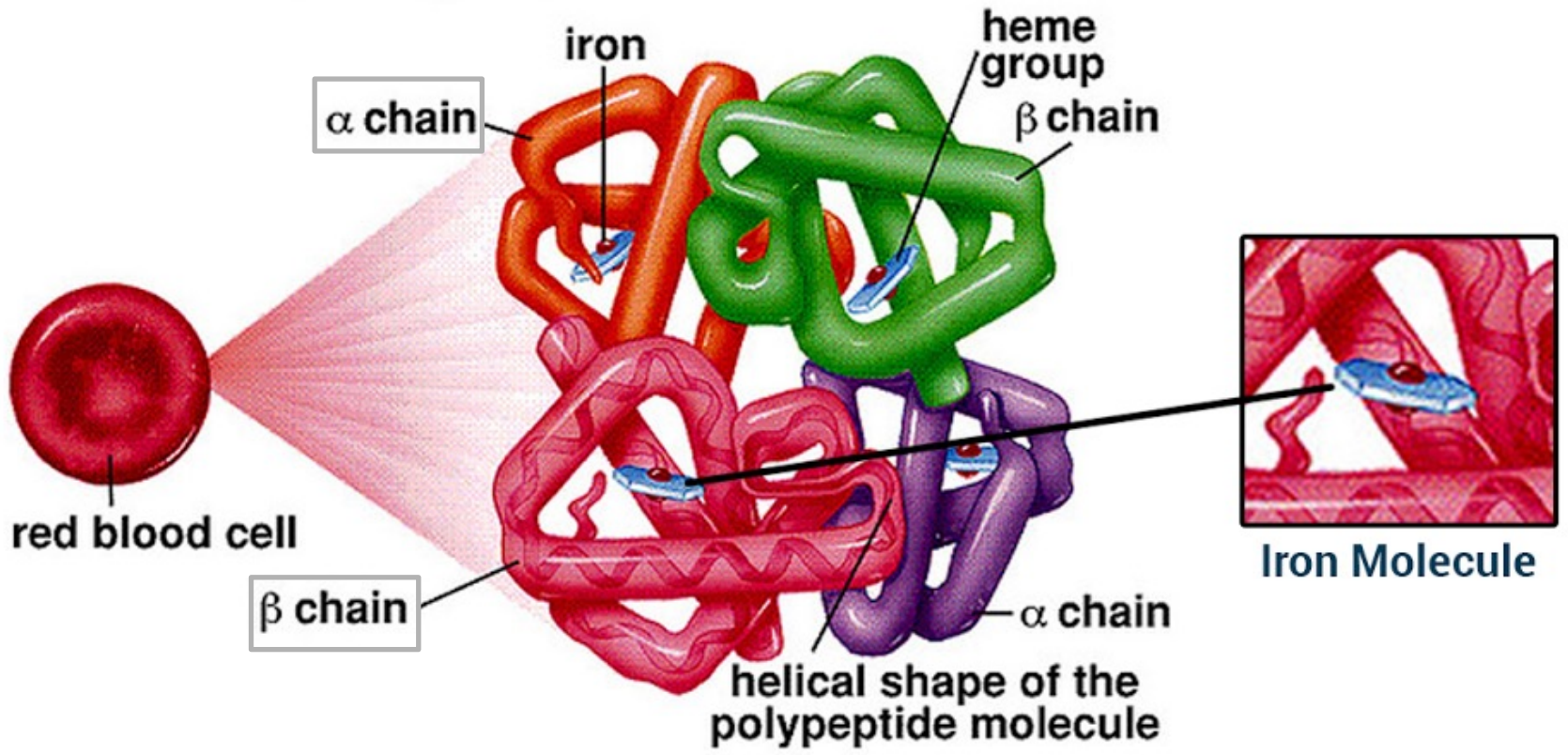


Objectives

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

Hemoglobin Structure

- **Hemoglobin** 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 **erythropoiesis**
- **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 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.

Regulation of Erythropoiesis

Tissue hypoxia (↓ Oxygen)

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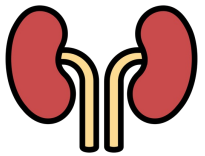
Kidney secrete erythropoietin into blood 

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Increase erythropoiesis in bone marrow

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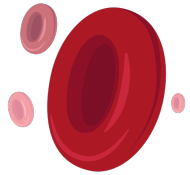
Increase number of RBC 

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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, copper and cobalt.

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

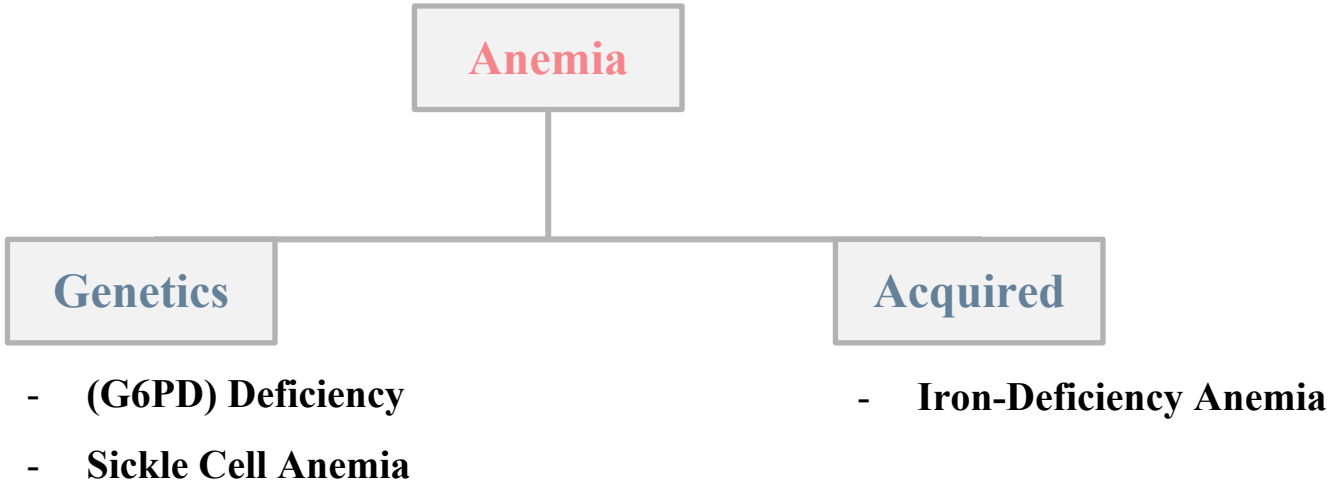
Anemia

- 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.

Causes:

I. Genetics

II. Acquired



Practical Part 💉

Estimation of Blood Hemoglobin

Principle

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

1. **Hemoglobin (Fe^{2+})** + Ferricyanide (oxidation) → Methemoglobin (Fe^{3+})
2. Methemoglobin (Fe^{3+}) + K^+CN^- → **Cyanmethemoglobin (HiCN)**

Method

1. 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 hemoglobin reagent		

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

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

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

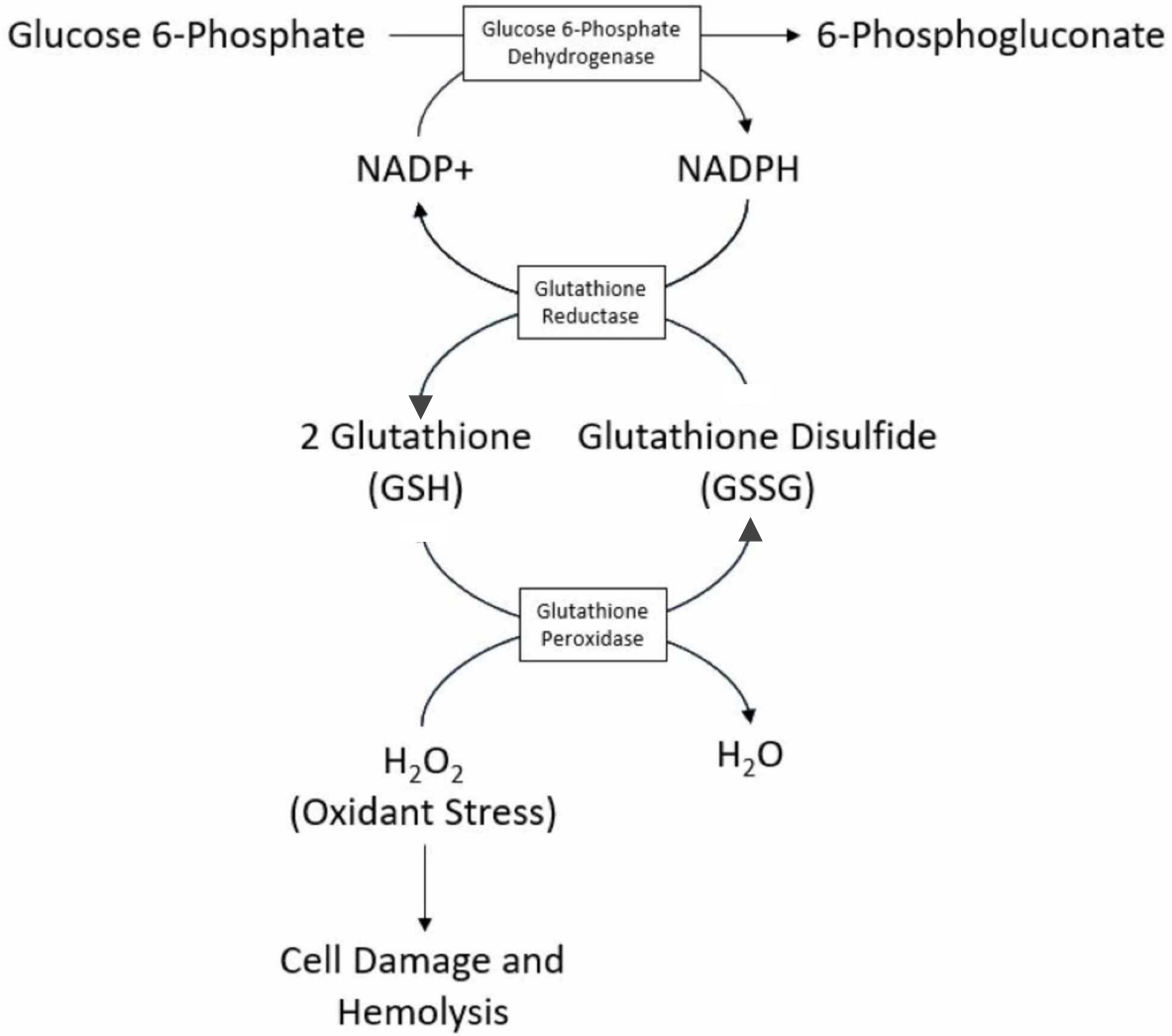
⬇Level of Hb is associated with **anemia**

Glucose -6-phsphate dehydrogenase (G6PD)

Glucose -6-phsphate 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 (*oxidants*) that cause oxidative damage.
- **G6PD deficiency** is an inherited X-linked recessive trait that results in an inadequate amount of (G6PD) in the blood.

Glucose -6-phsphate dehydrogenase (G6PD)



Blood Biochemistry BCH 220 [Practical]

Qualitative Determination of Hemoglobin S (Hbs) in Blood

Objectives

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

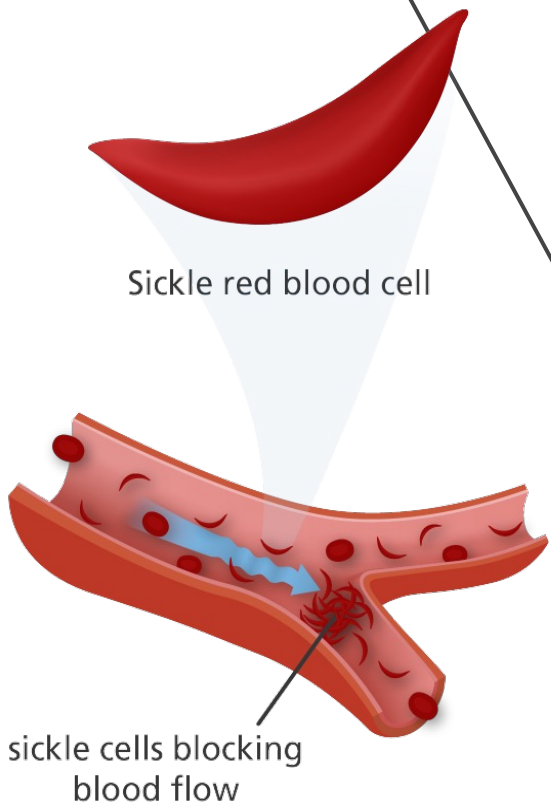
Introduction

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

- **Hemoglobin A**
  - It is normal hemoglobin that exists after birth and consist of **(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 fetal development and consists of **(α2γ2).**

Example of an Abnormal Hb

- **Hemoglobin S (HbS)**
  - The alpha chain is normal, while the beta chain is mutated, giving the molecule the structure, α2βS2.
  - A mutation in the Hb ***β gene*** is responsible for the sickling of RBCs → **substitution** of non polar valine for a charged glutamic acid in position 6 in the β chain.
  - **Sickle Cell disease** is an autosomal recessive disorder that affects erythrocytes (RBC) causing them to become sickle or crescent shaped.
  - Individuals with **sickle cell** will be at high risk when exposed to conditions of low oxygen tension such as surgery, high altitude or athletics.
  - **Sickle cell trait** (carrier), usually don't exhibit symptoms of the sickle cell anemia disease (unless under extreme hypoxia).



	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

Principle

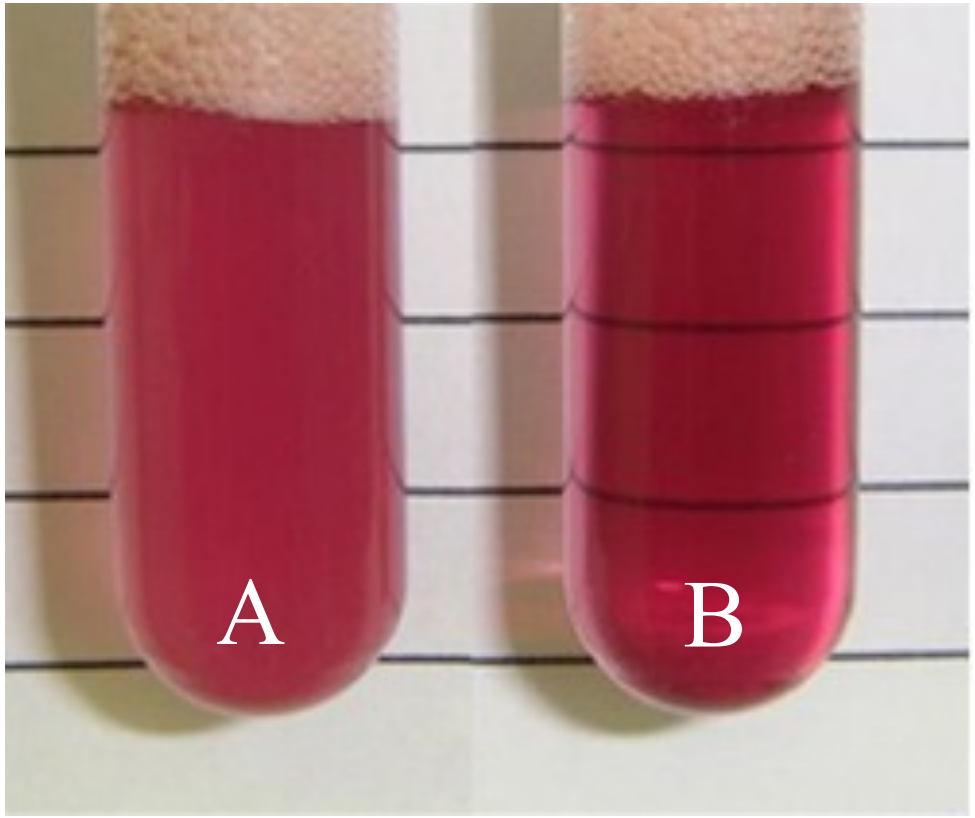
- Erythrocytes are **lysed** (by saponin) and the released hemoglobin is **reduced** (by dithionite) in phosphate buffer.
- ***Reduced HbS is characterized by its very low solubility*** → So that in the presence of HbS, the solution become **turbid** and the lines behind the test tube will not be visible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

Method of HbS

1. Pipette into clean dry test tube

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

Homework:

1. What are the different types of hemoglobin?

2. How is sickle cell anemia related to malaria?