Biochemistry Team 435

جامعة الملك سعود
King Saud University 1957

STRUCTURE AND FUNCTION OF HEMOGLOBIN

* Please check out this link to know if there are any changes or additions.

Revised by
هشام الغفيلي & خولة العماري

Color index: Important | Doctors notes | Further explanation.

Globular proteins

This slide is Extra (from the respiratory block) ..

- **What are globular proteins ?**
  - Proteins which their Amino acid chains are folded into shapes that resemble "spheres".
- **Benefit of that folding :**

That type of folding increases the solubility of proteins in water.

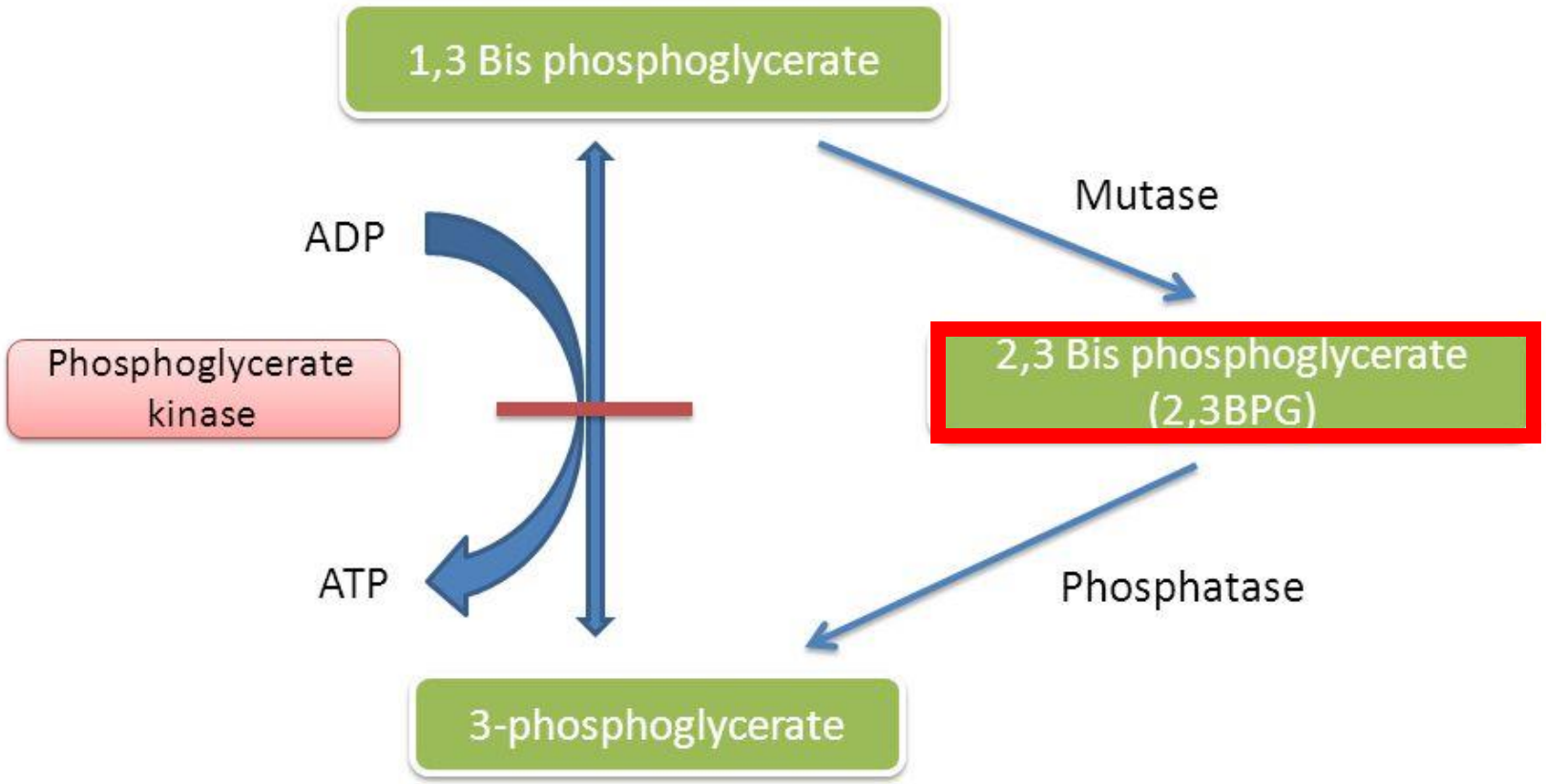
- Polar groups → on the protein's surface
- Hydrophobic groups → in the interior

Examples

- **Hemoglobin**
  - oxygen transport function.
- **Myoglobin**
  - oxygen storage/supply function in heart and muscle.
- **α1, α2, β-globulins**
  - various functions.
- **γ-globulins (immunoglobulins)**
  - immune function.
- **Enzymes**
  - catalysis of biochemical reactions.

This slide is Extra (from the foundation block) ..

Glycolysis in Erythrocytes:



- Net ATP production during production of 2,3 BPG in RBCs = 0 ATPs
- Increase in 2,3 BPG shifts the oxygen dissociation curve to the right

OBJECTIVES:

By the end of this lecture, the students should be able to know:

- the structure and function of hemoglobin.
- the factors affecting oxygen binding to hemoglobin.
- examples of normal and abnormal hemoglobin structures.

A hemeprotein found only in red blood cells.

What is "Hemoglobin"?

Hemoglobin:

Males: 14-16 g/dL

Females: 13-15 g/dL

It Contains heme as **prosthetic** group.

Prosthetic means it acts as a co-factor (it doesn't consume the oxygen itself but it delivers O2 to the tissues).

Functions:

- Oxygen transportation "from the lungs to tissues"
- Carries carbon dioxide from tissues back to the lungs

Types

Abnormal

- Carboxy Hb
- Met Hb
- Sulf Hb

Normal

النسب هنا هي نسب وجود هذه الأنواع في الإنسان الطبيعي

- HbA (97%)
- HbA_2 (2%)
- HbF (1%)
- HbA_{1c}

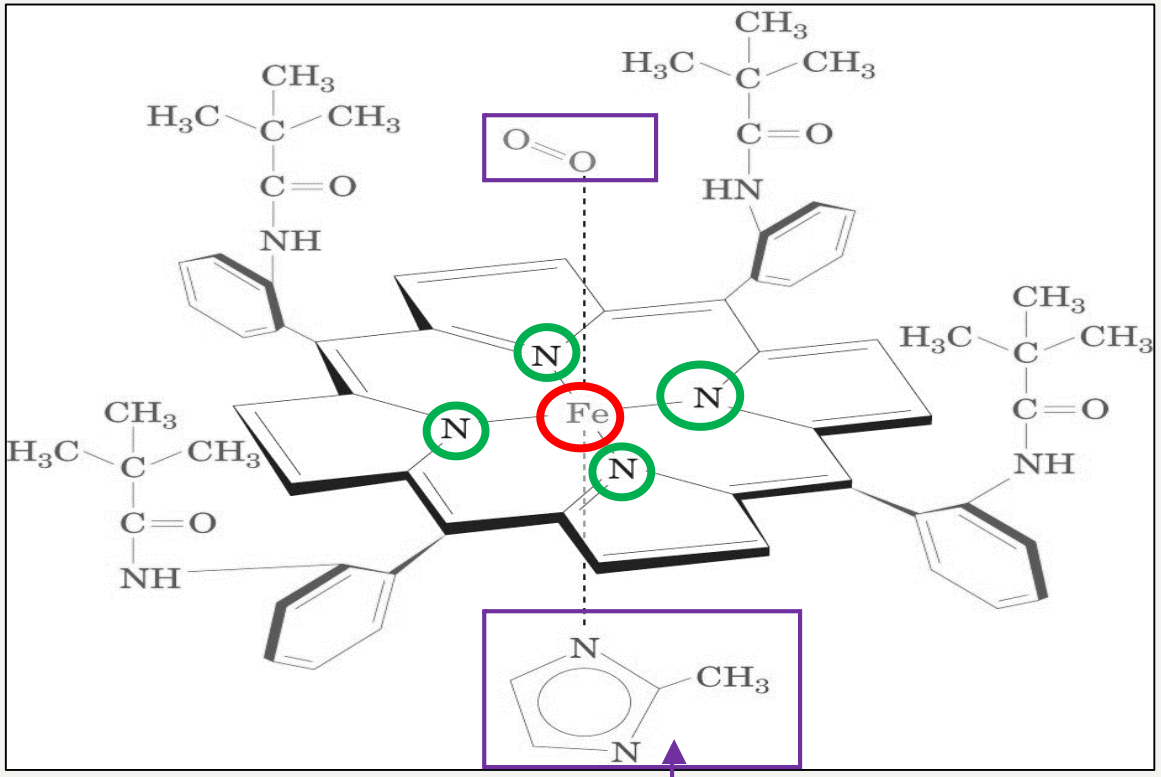
The heme group

❖ **What is "Heme"?**

A complex of **protoporphyrin IX** and **ferrous iron (Fe^{2+}).** Iron has to be in the ferrous state, otherwise it will not bind to Hb

❖ **Structure:**

- Fe^{2+} is present in the **center** of the heme.
- It binds to 4 **nitrogen** atoms of the porphyrin ring.
- It also Forms two additional bonds with:
  1- **Histidine residue** of globin chain.
  2- **Oxygen**. "Molecule not atom"



الحديد يكوّن ٦ روابط ، فلذلك يرتبط بأربع ذرات نيتروجين – التي تعتبر جزء من تركيب البورفرين – ويبقى عنده رابطتين فاضيات:

- يرتبط برابطة مع **الهستدين** الخاص بأحد الـ globin chains.
- ويرتبط بالأخرى مع **الاكسجين** ليكوّن بالنهاية الهيم.

***طيب وش ممكن نستنتج من التركيب؟** ان الهيموجلوبين يحمل ٤ اكسجين.

***كيف؟** الهيموجلوبين يتكون من ٤ هيم.. وبما ان كل هيم يحمل اكسجين فهو يحمل ٤ اكسجين.

فائدة أخرى: المايوجلوبين يحمل جزيء اكسجين واحد "لأنه يتكون من هيم واحد فقط".

Hemoglobin A (HbA)

- **Major Hb in adults.**
- A Hb molecule contains **4** heme groups and carries **4** molecules of O_2.

Structure:

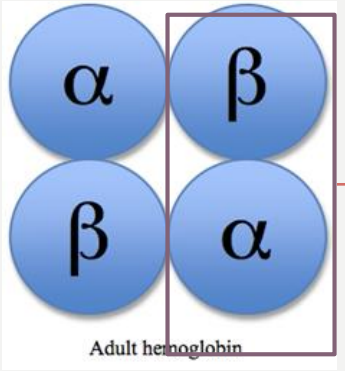
Composed of **four** polypeptide chains:

Two **α** and two **β** chains.

→ These chains form **two dimers** of **αβ subunits**

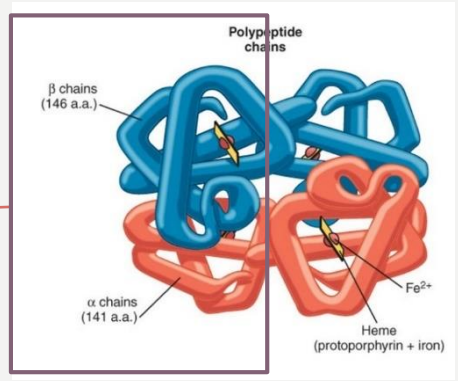
→ The dimers are Held together by **non-covalent interactions** (weak hydrogen and ionic bonds).

→ Each chain (α or β) is a subunit with a heme group in the center that carries oxygen.



A dimer:
αβ subunits

Also called heterodimer

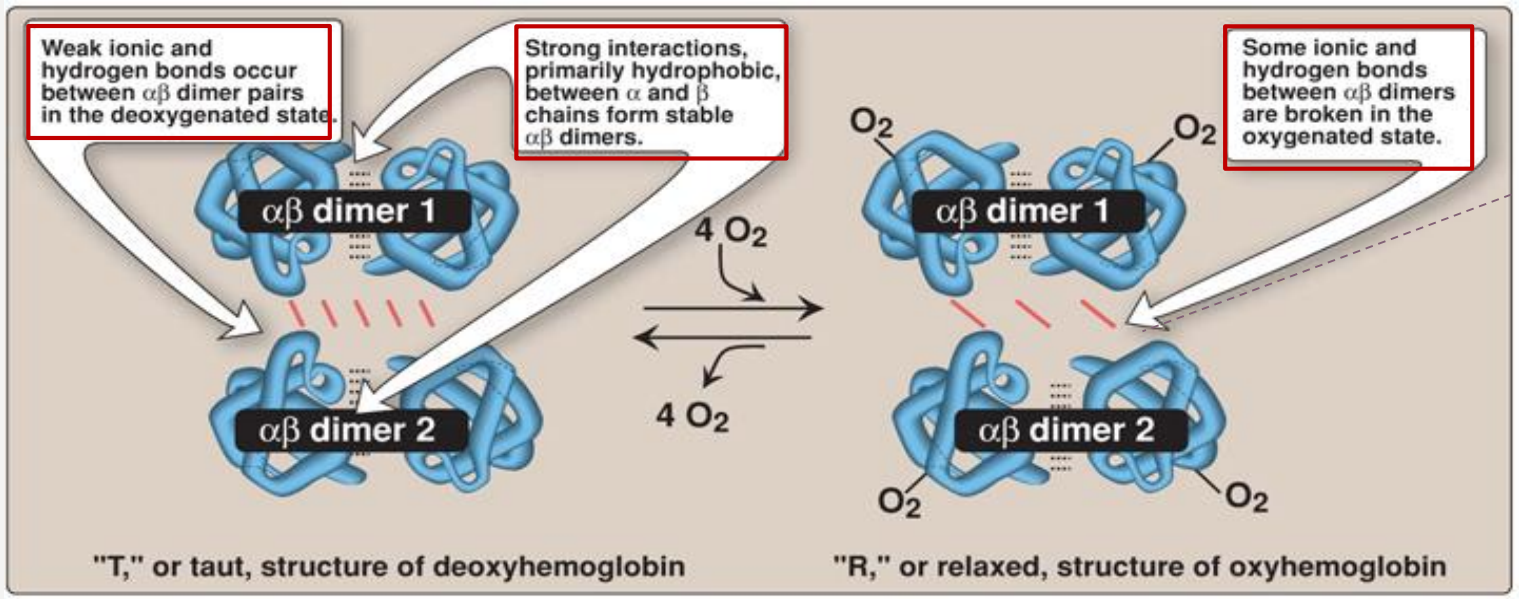


The two polypeptide chains **within** each dimer are held tightly together by hydrophobic interactions . These multiple interchain interactions form **strong** association between α-subunits and β-subunits in the dimers, so there is almost no movement between the subunits "with chains"!

In contrast, **the two dimers** are held together by polar bonds. The weaker interactions between the dimers allow them to move with respect to one other. This movement results in the two dimers occupying different relative positions in deoxyhemoglobin as compared with oxyhemoglobin (see next slide).

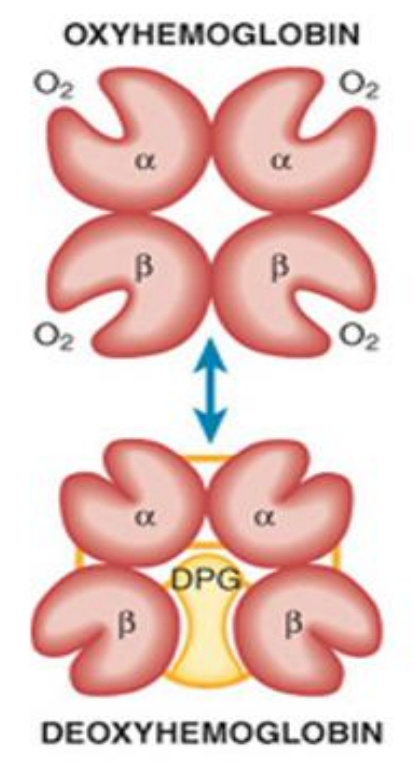
HbA forms:

T-form (Taut form)	R-form (Relaxed form)
Deoxygenated form	The oxygenated form
The movement of dimers is constrained by DPG (will be explained in next slides).	The dimers have more freedom of movement.
Low-oxygen-affinity form	High-oxygen-affinity form.



*الصورة اعادة للشرح اللي بالرمادي اللي بالشريحة السابقة.

لاحظوا ان بعض الروابط الهيدروجينية او الأيونية تكسرت في الريلاكسد فورم، هذا صار عشان توفر مكان للأكسجين لتحمله.

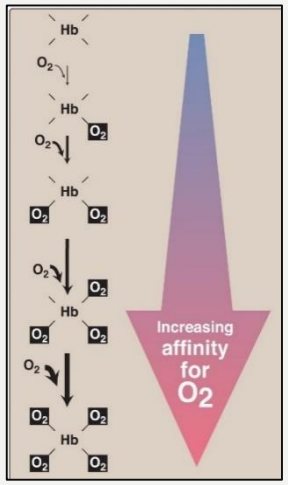


Recall: RBCs use anaerobic pathway (due to lack of mitochondria), so the net ATP for RBCs is 2, but the "shunt" consumes these 2 (to unload O2), this shunt is DPG.

T-State and R-State of Hemoglobin

Oxygen Dissociation Curve

Extra picture



- It is a curve that plots the proportion of hemoglobin in its saturated (oxygen-laden) form on the vertical (X) axis against the prevailing oxygen tension on the horizontal (Y) axis.
- The curves show important differences between hemoglobin and myoglobin (e.g the graph illustrates that myoglobin has a **higher oxygen affinity** at all pO2 values than does hemoglobin).

hemoglobin.

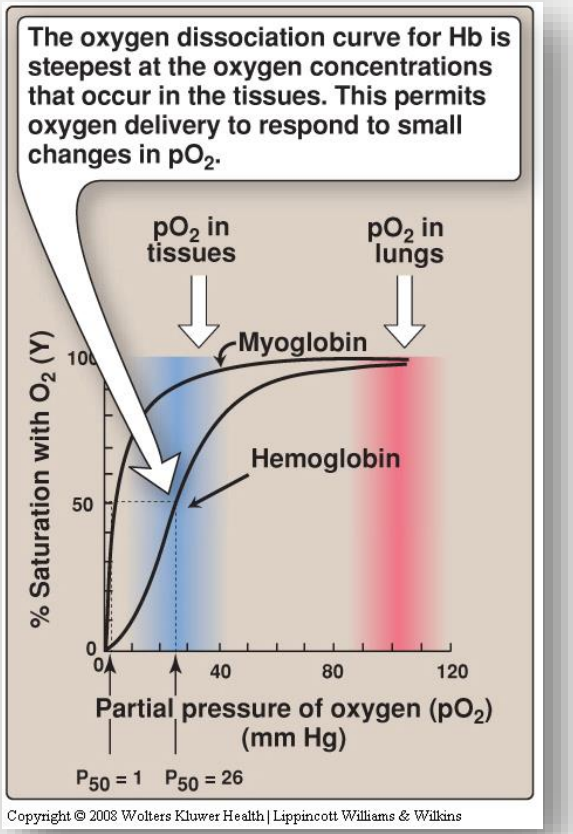
- **Curve's shape: sigmoidal** in shape. We get the Sigmoidal shape because as you slowly start to increase O2 the degree of saturation does not go up directly.
  - Indicates cooperation of subunits in O_2 binding.
- **Cooperative binding of oxygen by 4 four subunits of Hb means:**

Binding of O_2 to one heme group increases O_2 affinity of others.

- **This effect is referred as: Heme-heme interaction**.

Cooperativity is when one O2 binds to Hb it will cause a conformational change that goes to the other subunits and facilitate the binding of the second molecule and so on.

يعني إذا "شبك" أكسجين مع مجموعة هيم راح تزيد قابلية ارتباطه مع المجموعات الأخرى ، كأن مجموعة الهيم هذه تشجع صديقاتها.



Myoglobin.

-Myoglobin curve is called : hyperbolic curve.
- Notice that the p50 of myoglobin is 1 mmHg (because it has one subunit), while the p50 of hemoglobin is 26 mmHg.

Oxygen Binding Curve for Myoglobin and hemoglobin

Factors affecting oxygen binding (allosteric effectors)

We have 3 allosteric factors (pO2 & pCO2) are counted as one.

- pO2 (partial oxygen pressure)
- pH of the environment
- pCO2 (partial carbon dioxide pressure pressure)
- Availability of 2,3-bisphosphoglycerate

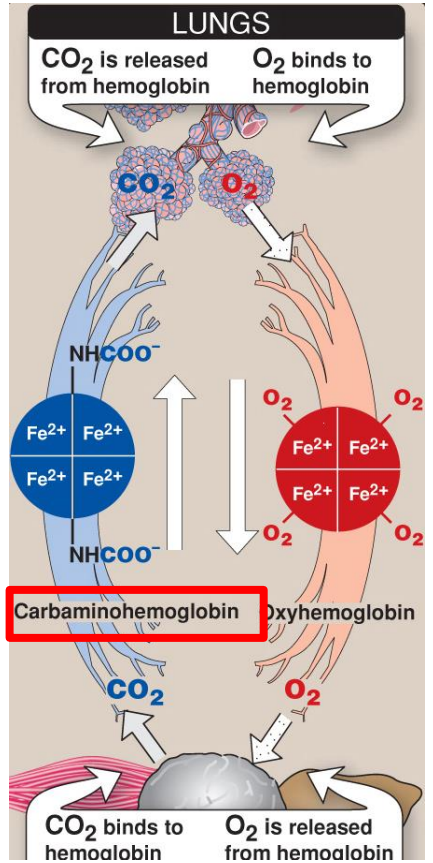
P_{50} :

- It is the pressure at which Hb is 50% saturated with O_2 (mm Hg).
- It indicates affinity of Hb to O_2:
  - **High** affinity → slow unloading of O_2 → low P50 value
  - Low affinity → fast unloading of O_2 → high P50 value.

بإختصار: كلما ازداد الهيموجلوبين بالأكسجين تعلقًا ، كلما ازدادت صعوبة استخلاصه من قبل الانسجة، تخيلوا الهيموجلوبين ماسك مجموعة كرات ، وأنسجة الجسم تحاول تاخذ هذه الكرات منه، فكل ما كان تشبثه بالكرات اقوى كل ما صار أصعب على الأنسجة أنها تأخذ الكرات منه

- Lung pO_2 is 100 mmHg → Hb saturation 100%.
- Tissue pO_2 is 40 mmHg → Hb saturation reduces.
- Hence O_2 is delivered to tissues.

-. ضغط الاكسجين في الانسجة أقل من ضغطه في الرئة ، فيكون اسهل على الهيموجلوبين انه ينزله فيها



The body get rid of CO2 by to ways :

1- by binding to hemoglobin (10%). 2- by Bohr effect (90%).

More explanation for the first method:

You breath in → o2 is bound to hemoglobin forming **oxyhemoglobin**.→ goes to the muscles → oxygen release (due to low o2 amounts in tissues)→ CO2 binds to the N-terminus of the amino groups of each globin, forming **carbaminohemoglobin**, then it travels through the circulation to the lungs, and then got unloaded in the lungs to be excreted.

Factors affecting oxygen binding (allosteric effectors)

- pO2 (partial oxygen pressure)
- pH of the environment
- pCO2 (partial carbon dioxide pressure pressure)
- Availability of 2,3-bisphosphoglycerate

The Bohr effect :

❖ The Bohr effect is a physiological phenomenon stating that Hemoglobin's oxygen binding affinity **is inversely related** both to acidity and to the concentration of carbon dioxide. That is, an increase in blood CO_2 concentration, which leads to a decrease in blood pH, will result in hemoglobin proteins releasing their load of oxygen.

Bohr effect

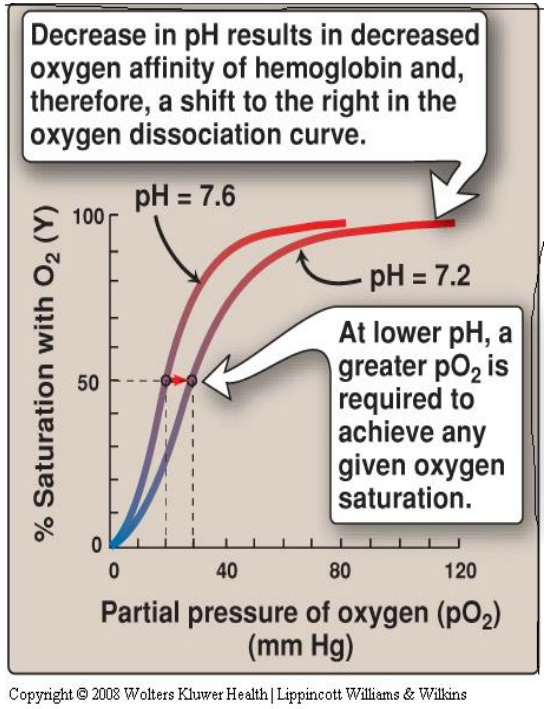
❖ What is "Bohr effect"?

- It is the effect of pH and pCO_2 on:

1-Oxygenation of Hb in the lungs.

2-Deoxygenation in tissues.

- Protons (low PH – High CO2) reduce O2 affinity of Hb → Hb releases its load of oxygen



❖ Source of the protons that lower the pH:

- Tissues have lower pH (acidic) than lungs.
  - ✓ **Due to** proton generation:

$$CO_2 + H_20 \rightarrow H2CO3 \rightarrow HCO_3^- + H^+.$$

In tissues, Co2 is converted by carbonic anhydrase to carbonic acid which spontaneously loses a proton, becoming bicarbonate. The H+ produced by this pair of reactions contributes to the lowering of pH.

Factors affecting oxygen binding (allosteric effectors)

- pO2 (partial oxygen pressure)
- pH of the environment
- pCO2 (partial carbon dioxide pressure pressure)
- Availability of 2,3-bisphosphoglycerate

The Bohr effect :

*الدكتوره شرحت المكانزم بذمة وضمير فركزوا بالمذاكرة

❖ Mechanism of the Bohr effect:

Tissues have **lower pH** (acidic) than lungs.

Due to proton generation:

$CO_2 + H_20 \rightarrow H2CO3 \rightarrow HCO_3^- + H^+$.

Protons (low PH – High CO2) **reduce** O2 affinity of Hb.

البروتونات الناتجة عن التفاعل السابق قللت الافنتي! يعني خلاص الهيموجلوبين ماهو متمسك بالاكسجين ولا بيبيه ☹

Causing **easier O_2 release** into the tissues.

الهيموجلوبين تخلت عن الاكسجين واعطته الأنسجة واصبحت حرة ☹ "كله من البروتونات!"

بعد ماصارت حرة ارتبطت مع البروتونات، كأنها عملية استبدال خوذي الاكسجين وعطيني البروتونات "الهيدروجين الناتج من التفاعل".

The **free Hb** binds to two protons.

Protons are released (in the lungs) and react with HCO^{3-} to form CO_2 gas

$(HCO_3^- + H^+ \rightarrow CO_2 + H_2O)$

لمن وصلت الهيموجلوبين للرئة حنت للأكسجين وقطت البروتونات ورجعت ارتبطت بالاكسجين.
البروتونات بعدين راحت وارتبطت مع البايكاربونايت وعكست التفاعل اللي صار أول بالأنسجة

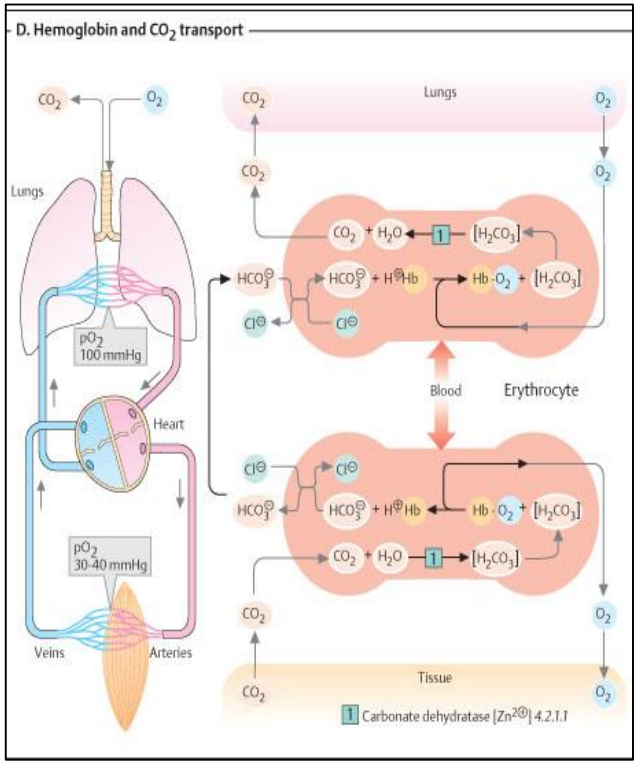
The proton-poor Hb now has **greater** affinity for O_2 (in lungs).

زي ماقلنا قبل شوي حنت وقطت البروتونات وزادت الافنتي وارتبطت بالاكسجين وبتوديهم للأنسجة والقصة بتعيد نفسها

Result: The Bohr effect **removes insoluble CO_2** from blood stream (Produces soluble bicarbonate).

بنهاية القصة بس وده يوصل فكرة ان ثاني اكسيد الكربون طلع "للي ما انتبه لنواتج التفاعل العكسي".

Extra picture



Factors affecting oxygen binding (allosteric effectors)

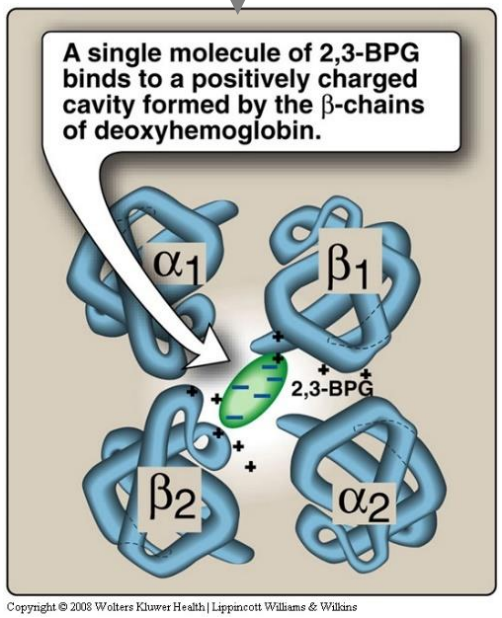
- pO2 (partial oxygen pressure)
- pH of the environment
- pCO2 (partial carbon dioxide pressure pressure)
- Availability of 2,3-bisphosphoglycerate

Effects of 2,3-bisphosphoglycerate on oxygen affinity:

❖ **Binding of 2,3-BPG to deoxyhemoglobin:**

- It Binds to deoxy-hb and stabilizes the T-form.
- When oxygen binds to Hb, BPG is released.

لاحظوا أن البي بي جي مرتبط بوحدات البيتا

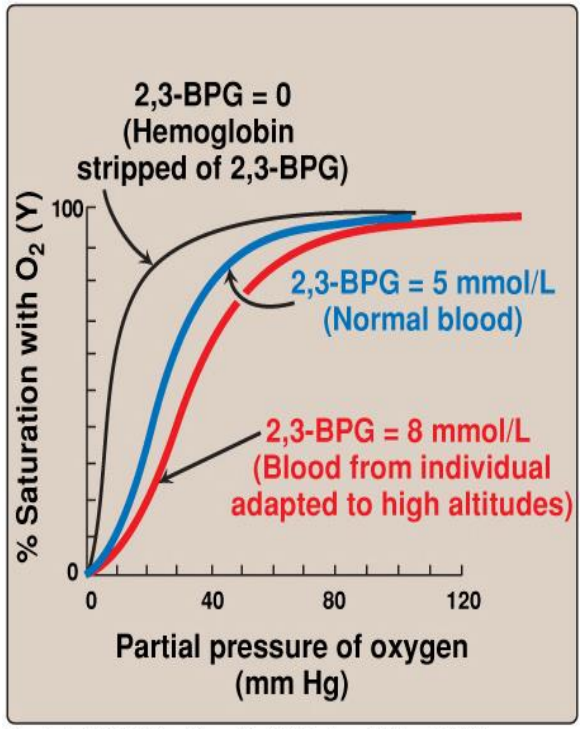


❖ **At high altitudes & hypoxia :**

RBC number increases → Hb conc. Increases → BPG increases

2,3 BPG levels rise

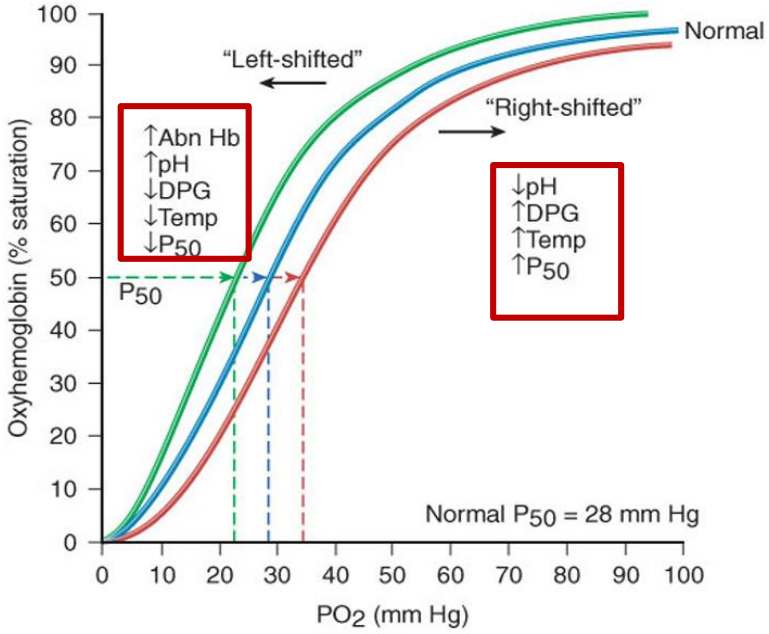
This decreases O_2 affinity of Hb → Thus increases O_2 delivery to tissues.



*يعني الجسم يزيد عدد خلايا الدم الحمراء وتركيز الهيموجلوبين عشان بالنهاية يزيد البي بي جي فيأخذ مكان الاكسجين مما يؤدي الى زيادة كمية الاكسجين في الانسجة علشان يقاوم الجسم الهايبوكسيا.

High O2 affinity

Affinity:		High O2 affinity:	Low O2affinity
Shift:		**Left** shift	**Right** shift
P50:		**Low**	**High**
Factors:	**PH**	**High** pH (alkalosis – low pCO2 – Low H+)	**Low** pH (acidity – high PCO2 – High H+)
	DPG	**Low** DPG: Multiple transfusion of 2,3 DPG-depleted blood.	**High** DPG
	Temp.	**Low** temperature	**High** temperature
		Abnormal Hb (e.g High levels of Hb F)	-



وش دخل الحرارة؟ في حالة الهايبوكسيا والأماكن المرتفعة، تكون حركة الدم في الجسم أسرع علشان تعوض نقص الأكسجين في بعض الأماكن وتحاول توصله بسرعة، فهذي السرعة تولد حرارة ، والحرارة بالتالي تنقص الافنتي للأكسجين في الانسجة.

هذي العوامل حفظ! !

Don't forget :
Shifting to left = high affinity of O2.
Shifting to right = low affinity of O2.

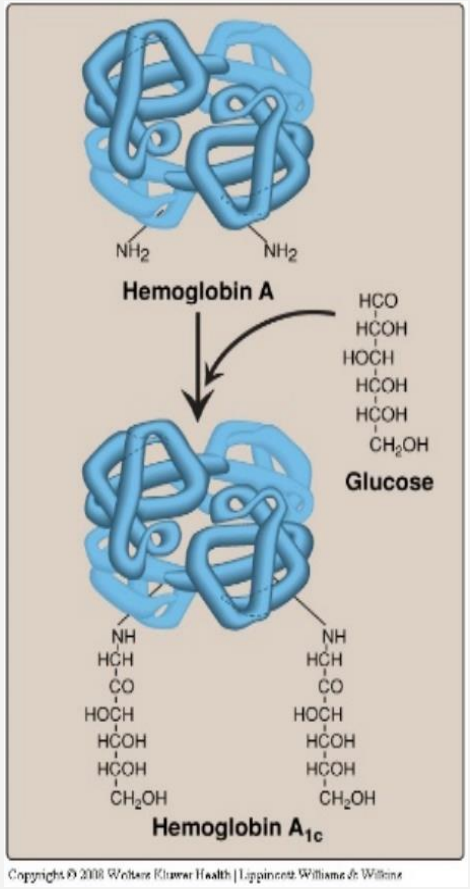
لتسهيل الحفظ: Right shunt = Reduced "Low" affinity.
وبما انه تحرك لليمين فالقيم الموجودة على محور اكس بتزيد "اللي تمثل ضغط الاكسجين" ومعها بيزيد البي ٥٠.
وبالنسبة للعوامل كلها تتبع الليدر ضغط الاكسجين "زيادة" إلا البي تش مايحب يتبع احد فصار "نقصان".

Hemoglobin & Oxygen Dissociation Curves

مقطع قصير وبسيط حلو لترتيب الأفكار

Other normal forms of Hb

Fetal Hemoglobin (HbF)	HbA_2	HbA_{1c}
two α and two γ chains.	Two A and two **δ**	**Two α and two β -Glucose**
The major hemoglobin found in the **fetus** and **newborn**.	Appears ~12 weeks after birth	High in patients with **diabetes mellitus**.
- It has Higher affinity for O2 than HbA. - Transfers O2 from maternal to fetal circulation across placenta الافنتي عالية بهالنوع علشان الجنين يقدر انه يسرق الأكسجين من امه.	Constitutes ~2% of total Hb	An HbA undergoes **non-enzymatic glycosylation**. - Glycosylation depends on plasma glucose levels. (علاقة طردية) يكون موجود عند غالبية الناس - اللي ما عندهم سكري – لكن بكمية قليلة - It indicates a previous history of diabetes (because it takes 2 to 3 months to change or to get back to normal).



Abnormal forms of hemoglobin

Carboxy-Hb

- Hemoglobin that has carbon mono-oxide instead of the normal oxygen bound to it.

- In smokers.

- CO replaces O_2 and binds 220X tighter than O_2.

Carboxyhemoglobin is formed in carbon monoxide poisoning and leads to oxygen deficiency in the body. The source of the carbon monoxide may be exhaust (such as from a car, truck, boat, or generator), smoke from a fire, or tobacco smoke. The level of carboxyhemoglobin is a measure of the degree of carbon monoxide exposure.

Met-Hb

Contains **oxidized** (ferric) **Fe^{3+}** (~2%) that cannot carry **O_2**

Brownish bluish color of blood

Sulf-HB

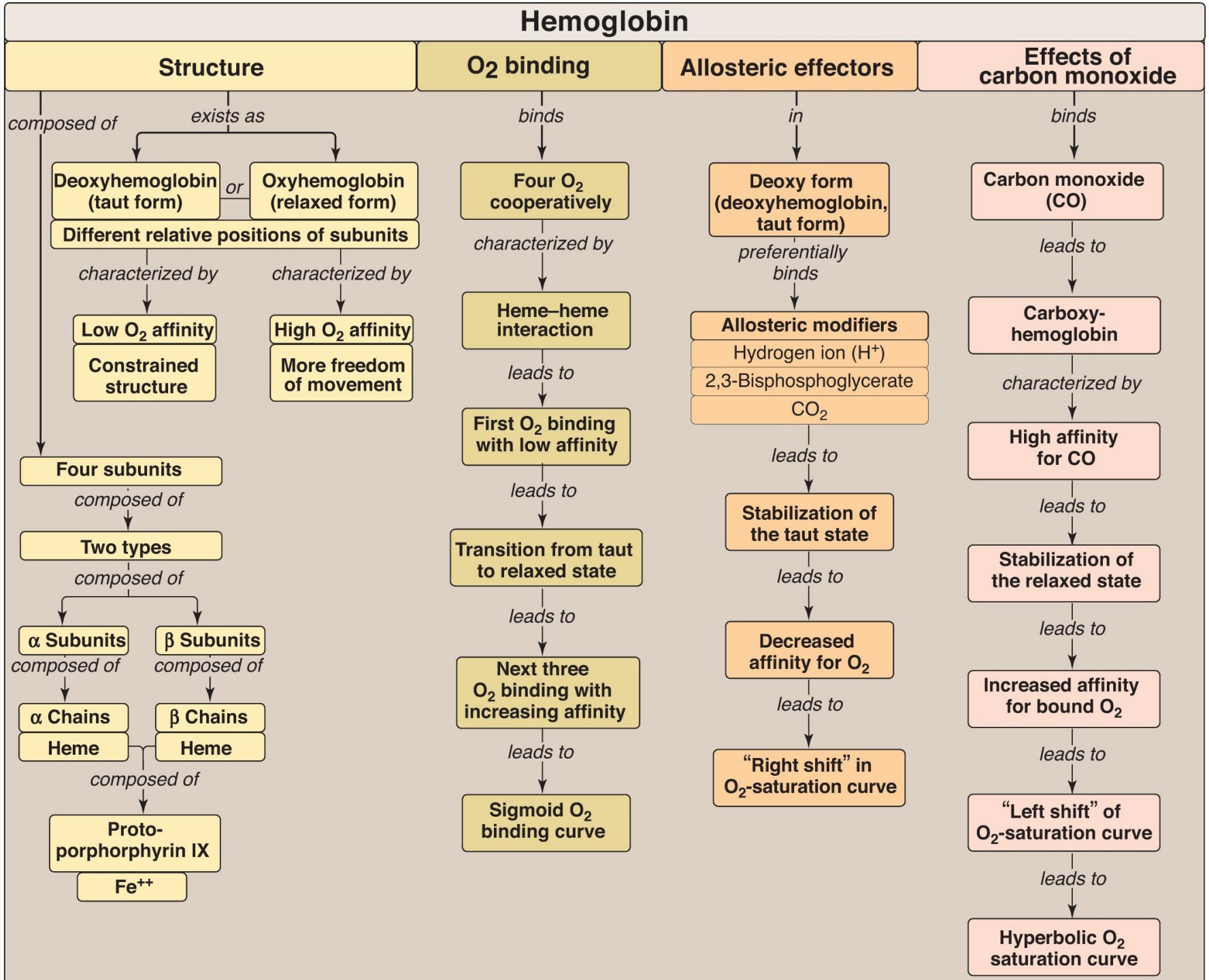
- Forms when sulfur levels are high in blood.
- (irreversible reaction)

Caused by some mistakenly prescribed medications, or by children who drank some detergents.

Biochemistry Team

Helpful summary from Lippincott

Click here to check out team's summary



Check your understanding!

Q1: : the heme group is a complex of protoporphyrin IX and …:

A. (Fe2+) ferrous iron.
B. (Fe3+) ferric iron.
C. ferritin.
D. non of the above.

Q2: : which of the following is a form of Hb that is unable to transport the O2:

A. HbA2.
B. HbA1c
C. HbF.
D. Met Hb.

Q3: : at the level of the one dimer αβ subunits are held by:

A. Ionic bond.
B. hydrogen bond.
C. hydrophobic bond.

Q8: : P50 indicate that low affinity = slow unloading of O2 :

A. Fault.
B. True.

Q5: : T-form of Hb is :

A. taut form.
B. has Low-oxygen-affinity.
C. the dimers have more freedom of movement.
D. A+B.

Q6: : one of the 3 allosteric effectors that affecting the oxygen binding is:

A. partial pressure of the H ions
B.pO2.
C.Availability of bicarbonate.

Q7: : from the Oxygen Dissociation Curve tells you that there is a…:

A. Cooperation in the O2 binding to the heme group.
B. Cooperation in the Co2 binding to the heme group.
C. Heme-heme interaction.
D. A+C.

1.A, 2.D, 3.C, 4.A, 5,D. 6.B, 7.D.

Check your understanding!

Q8: : depending on Bohr effect which of the following will reduce the affinity of the Hb to the O2 and cause it release ..:

A. low pH.
B. acidity.
C. Protons.
D. all of the above.

Q9: : Carboxy-Hb is found in …:

A. fetus+ newborn.
B. healthy adult.
C.smoker.
D. sulfa users.

Q10: : which of the following form of Hb has a high affinity to the O2 (in lung) :

A. proton-poor Hb
B. Carboxy-Hb.
C. deoxygenated form of Hb

Q11: : which of the following is true about a person who lives in a 2,400 meters altitude:

A. his RBC number decreases
B. his Hb conc. decreases
C. the availability of the BPG increases.

Q12: : Fetal Hemoglobin has a higher affinity to O2 than:

A. HbA.
B. HbA2.
C. all of the above.

8.D, 9.C, 10.A, 11. C, 12. C.

Done by:

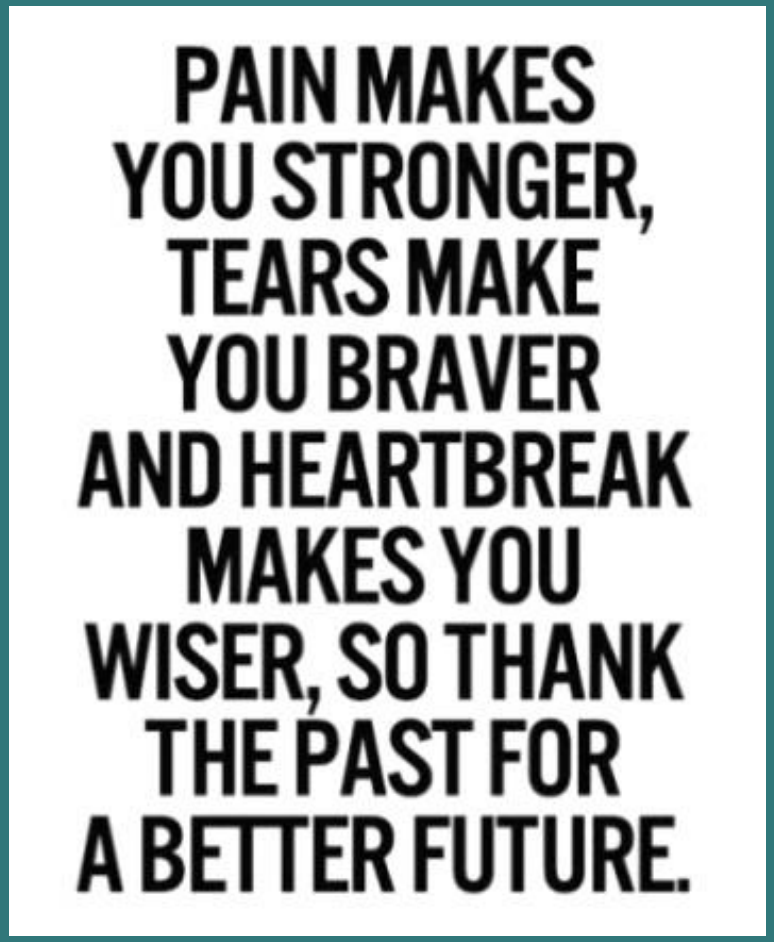
- شهد العنزي.
- عبدالله الغزي.
- نورة الطويل.
- - عبدالله الشنيفي.
- دلال الحزيمي.
- رغد المنصور.
- احمد الرويلي

Revised by:

- فارس المطيري.

Resources:

- 435's slides and notes.
- Lippincott's illustrated reviews: Biochemistry – sixth edition.
- The European Respiratory Journal: Relating oxygen partial pressure, saturation and content: the hemoglobin–oxygen dissociation curve.



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