بسم الله الرحمن الرحيم

BIOCHEMISTRY

Lecture 21

Regulation of hemoglobin

﴿ وَإِن تَتَوَلَّوْا۟ يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوٓا۟ أَمْثَٰلَكُم ﴾

اللهم استعملنا لنصرة دينك

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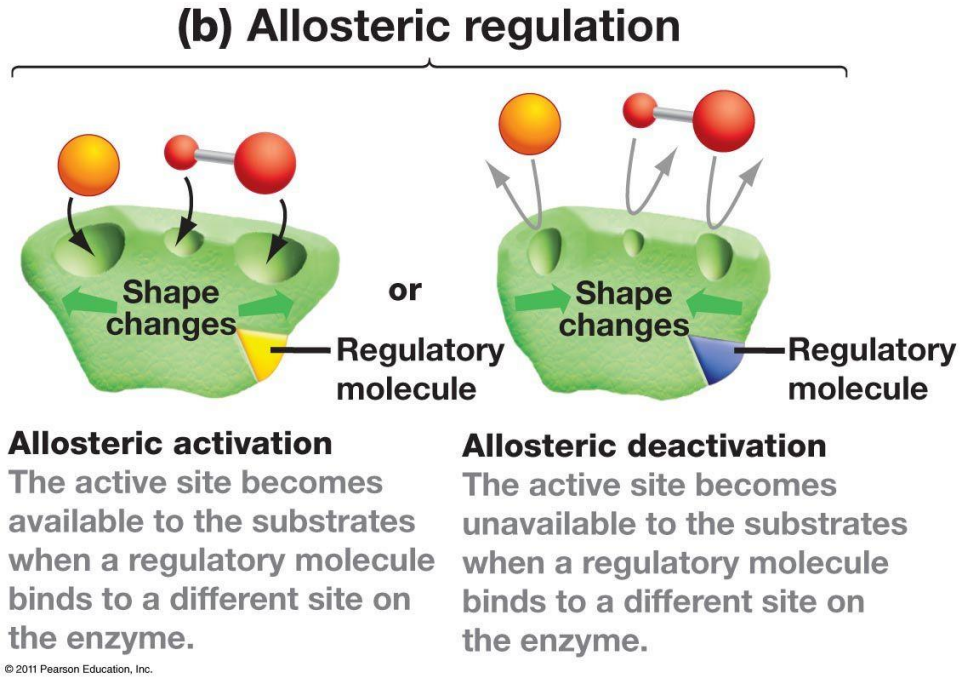
Allosteric regulation

- Ligands that induce conformational changes in allosteric proteins are referred to as allosteric modulators or effectors.
- Modulators may be inhibitors or activators.
  - Homotropic modulators are the same as the ligand itself.
  - Heterotropic modulators are different from the ligand.



- It is OXYGEN for hemoglobin
- It is **positive** allosteric effector because → it makes it easier for the second molecule to bind.

Allosteric effectors

- The major heterotropic effectors of hemoglobin
  - Hydrogen ion,
  - Carbon dioxide
  - 2,3-Bisphosphoglycerate
  - Chloride ions

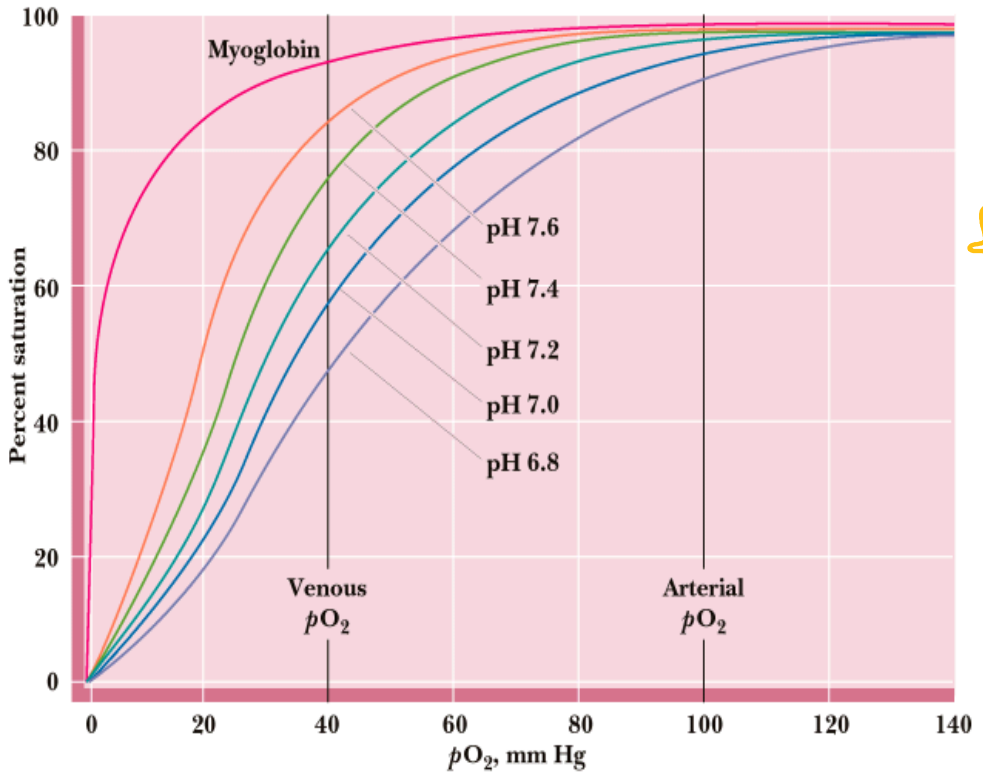
Those all are **negative** allosteric effectors because when they bind to hemoglobin they make it **harder** for oxygen to bind.

- A competitive inhibitor
  - Carbon monoxide

The effect of pH and H^+

The effect of pH

- The binding of H^+ to hemoglobin promotes the release of O_2 from hemoglobin and vice versa.
- This phenomenon is known as the **Bohr effect**.

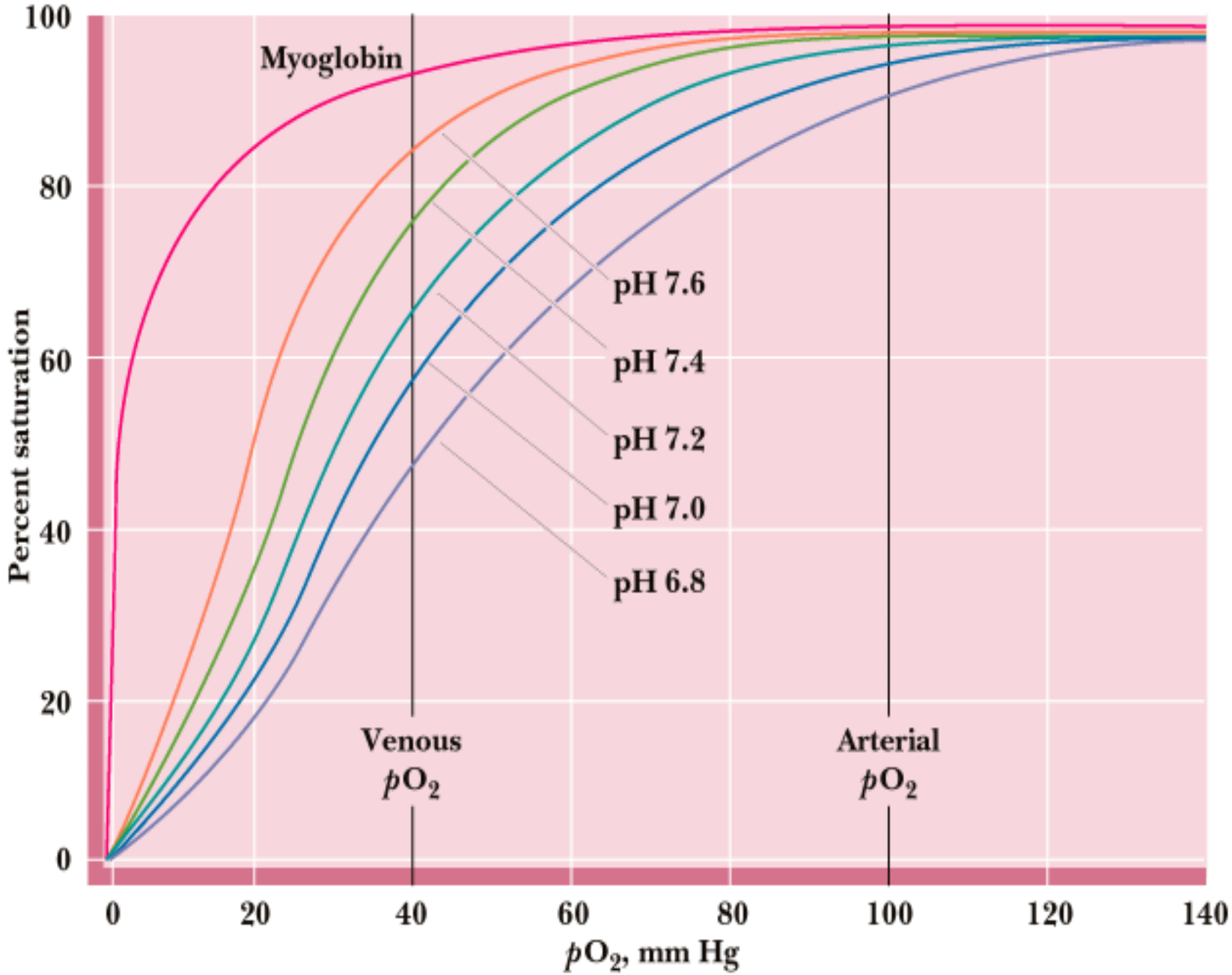


Bohr noticed that every time :

{PH decreases (H+ increases) → P50 increases → affinity decreases}

– Protons reduce the affinity –

WHY ?? Next slides


100
80
60
40
20
0
Percent saturation
Myoglobin
pH 7.6
pH 7.4
pH 7.2
pH 7.0
pH 6.8
Venous
pO_2
Arterial
pO_2
0
20
40
60
80
100
120
140
pO_2, mm Hg

Mechanism of Bohr effect

- Increasing H^+ (in tissues) causes the protonation of key amino acids, including the last histidine residue of the β chains (**His146**).
- Electrostatic interaction occurs between the carboxylic group of His146 and a lysine of the α chain.
- The protonated histidine also forms a salt bridge to Asp94 within the same chain.
  - The pKa of the imidazole ring of His146 is reduced from 7.7 in the T-state to 7.3 in the R-state, meaning that it is protonated (charged) in the T-state and deprotonated (uncharged) in the R-state.
- This favors the deoxygenated T-form of hemoglobin.

Note
- When pH> pKa, the group is deprotonated.
- When pH < pKa, the group is protonated.

Firstly:

*PH causes protonation or deprotonation of amino acid depending on its changes relative to the Pka of the amino acid (relation is below).

So when H+ increases in tissues, cells or RBCs protonation will happen and charges will increase or decrease depending on the amino acid that will affect electrostatic interactions (+ .. -)

Secondly:

(His) "alone" Pka is 6.16 near physiological ph & affected by surrounding amino acids.

(His146) because it is the last one in its (beta chain) it is gonna make 2 electros interactions: 1. With (**Asp**) in the same chain and 2. With (**Lys**) in the opposite (alpha chain).

Mechanism of Bohr effect

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Thirdly:

Metabolism causes the change in PH

For (His):

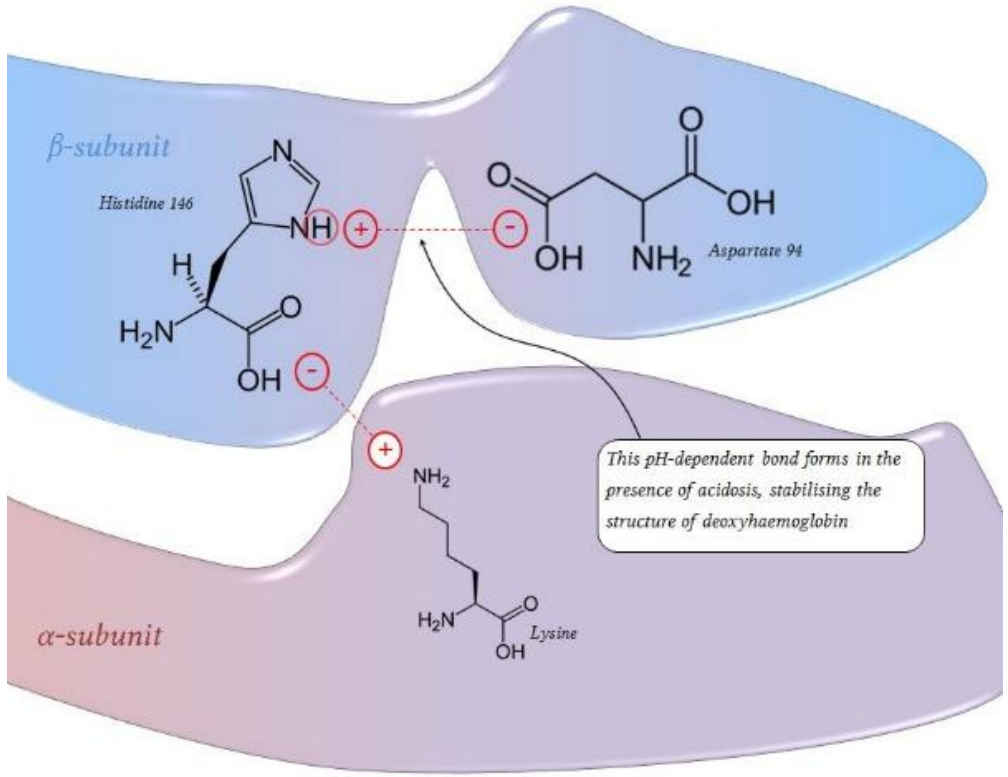
Tissues → PH=7.4 → Pka=7.7 → Protonated group → Electrostatic interactions → tight T-state → Release of o2.

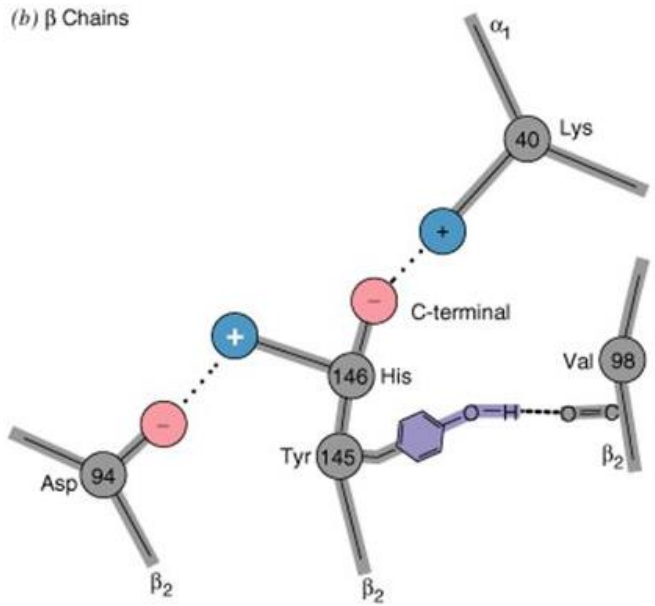
Lungs → PH=7.4 → Pka=7.3 → Unprotonated group → No electrostatic interactions Relaxed → R-state → Strong binding of o2

charge has gone cuz es- interactions are broken and it is easier to lose protons

*Pka of (**His**) reduced from 7.7 into 7.3

Realize the importance of electrostatic interactions ★


β-subunit
Histidine 146
Aspartate 94
This pH-dependent bond forms in the presence of acidosis, stabilising the structure of deoxyhaemoglobin
α-subunit
Lysine


(b) β Chains
α1
Lys
40
C-terminal
146 His
Val 98
Tyr 145
Asp 94
β2
β2
β2

Where do protons come from?

$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$

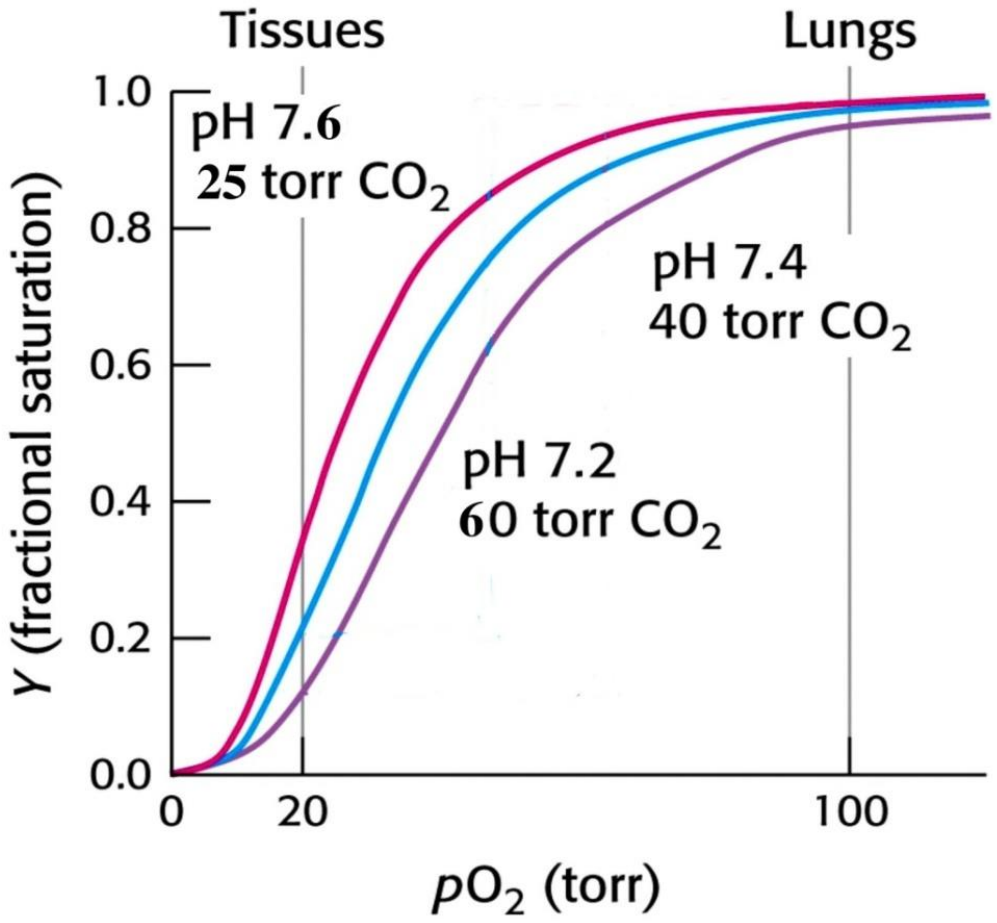
- CO_2 and H^+ are produced at high levels in metabolically active tissues by carbonic anhydrase, facilitating the release of O_2.
- In the lungs, the reverse effect occurs and, also, the high levels of O_2 cause the release of CO_2 from hemoglobin.

- CO2 is produced by metabolism Glucose + O_2 ⟶ CO_2 + H_2O
- CO2 is converted through carbonic anhydrase (an enzyme) into H+
- H+ produced → protonation → T-state → electrostatic interactions → Low affinity → release of O2

The effect of CO_2

Mechanism #1 - production of protons

$$CO_2 + H_2O \Longleftrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$



One mechanism & not the only one

- increasing the pressure of CO2
  ↓
- PH decreases
  ↓
- P50 increases
  ↓
- Affinity decreases

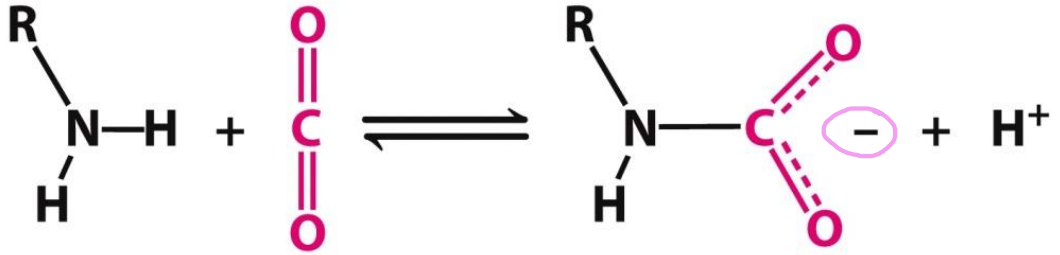
Mechanism #2- formation of carbamates

→ The **Primary** function of hemoglobin = is to transport O2
→ The **secondary** = is to transport CO2 → How ? By this mechanism

- Hemoglobin transports some CO_2 directly.
- When the CO_2 concentration is high, it combines with the free α-amino terminal groups to form carbamate and producing negatively-charged groups

- So rather than having a positively charged group at the end of the peptide

we will have negatively charged group



Carbamate

Co2 is going to bind to the (**free alpha amino terminal group**)

= forming carbamate

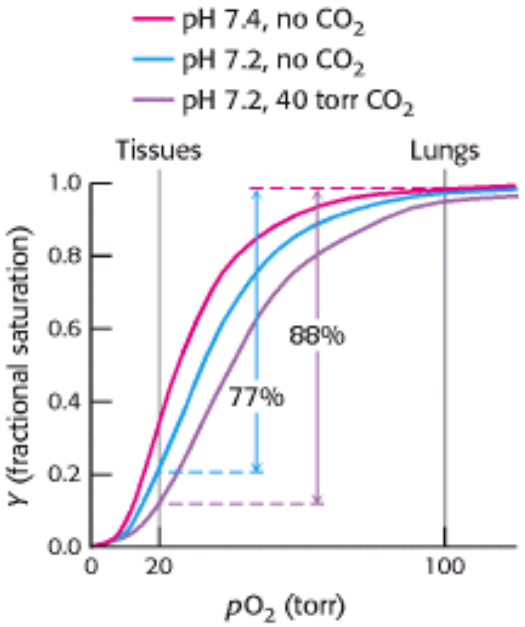
- The increased number of negatively-charged residues increases the number of electrostatic interactions that stabilize the T-state of hemoglobin.

How can we test that? Which one is more important?
Reducing of PH or formation of carbamate?

Which mechanism has a stronger effect?

- About 75% of the shift is caused by H^+.
- About 25% of the effect is due to the formation of the carbamino compounds.

How do we know that?
By changing one factor and keeping the other constant.
An increase in CO_2 tension will shift the oxygen dissociation curve to the right, even when the pH is held constant.



- We will put hemoglobin in different environments:

#1 without CO2

PH=7.4
PH=7.2

Pink VS Blue

P50 ⟶ Increases
Affinity ⟶ Reduced

it happens by reducing the PH (H^+ ↑)

#2 with CO2

PH is unchanged .. How? Using a **Buffer**

PH=7.2

Blue VS Purple

Affinity ⟶ Reduced

CO2 does affect the affinity "negatively"

Transport of CO_2 into lungs

1. Cells will produce CO2 (gas so it diffuses out of cells easily)
2. It gets into the RBCs

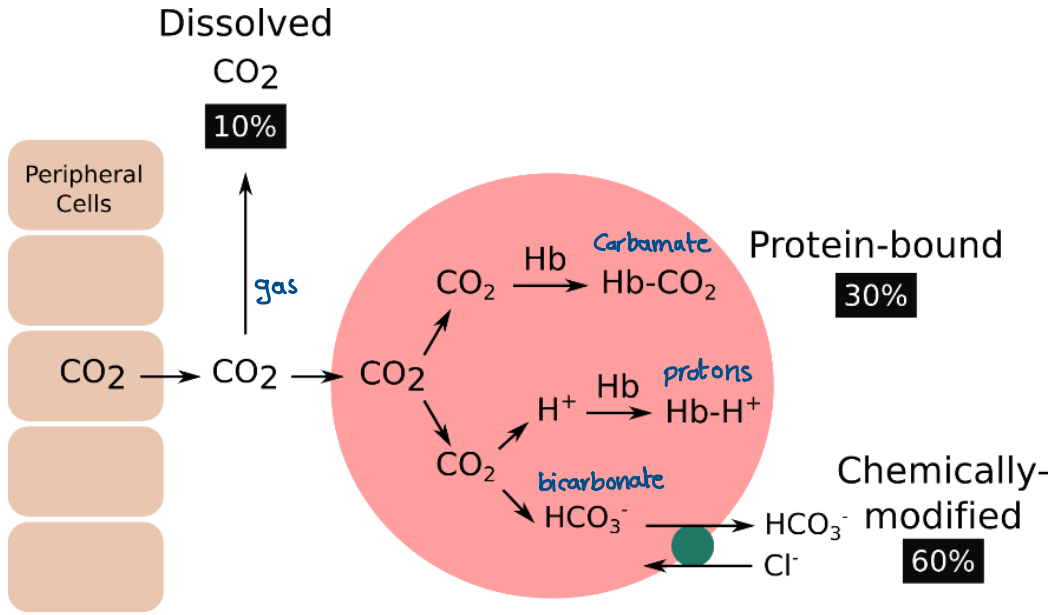
- Approximately 60% of CO_2 is transported as bicarbonate ion, which diffuses out of the RBC.
- About 30% of CO_2 is transported bound to N-terminal amino groups of the T form of hemoglobin .
- A small percentage of CO_2 is transported as a dissolved gas.

The movement of CO_2 in/out of cells does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer.

*PH will not change .. Why?

1) We have the bicarbonate buffer
2) hemoglobin is gonna bind to protons

{Hemoglobin plays a role in buffering blood and stabilizing PH}



It will take CO2 and forms a **carbamate** within the RBCs (30%)

Produced **bicarbonate** will leave out of the cell it can diffuse, travel or carry itself (without RBCs) in blood because it is (negative) (60%)

small amount of **CO2 gas** is dissolved by itself bcz it is not really soluble (hydrophobic) (10%)

It will form **protons** that bind to hemoglobin increasing electrostatic interactions (role of buffering the blood))

Effect of Chloride ion

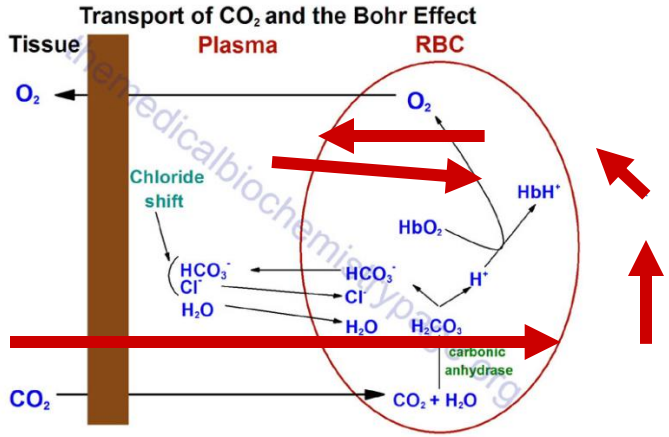
Chloride shift

- Bicarbonate diffuses out of the red blood cells into the plasma in venous blood and visa versa in arterial blood.
- Chloride ion always diffuses in an opposite direction of bicarbonate ion in order to maintain a charge balance.
- This is referred to as the "chloride shift".

*__Bicarbonate__ ion left the cell.. so cells have lost a negatively charged group ☹

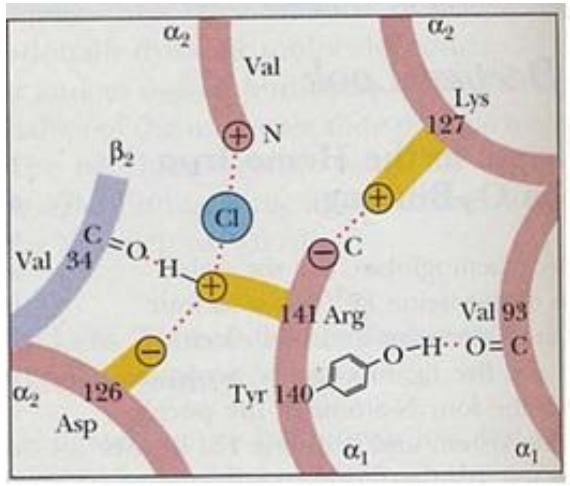
⟶ The electrical gradient will be disturbed

*So it has to compensate that.. so **chloride ions** will enter the cell in place of the bicarbonate



Effect of chloride ions

- Chloride ions interact with both the N-terminus of α2 chain and Arg141 of α1 chain stabilizing the T-state of hemoglobin.
- Increasing the concentration of chloride ions (Cl^-) shifts the oxygen dissociation curve to the right (lower affinity)



Cl- with hemoglobin
-Even without CO2 or change in PH-
It is gonna stabilize the T-state → decrease the affinity → increase the P50 (because of electrostatic interactions) → So release O2

1- Tissues make CO2
2- CO2 gets inside cells Production of

protons & bicarbonate

*Bicarbonate **out** chloride ion **in**

*protons made electrostatic interactions stabilization T-state

Chloride ion bound with hemoglobin further stabilization for T-state

O2 is released more and more from hemoglobin
So .. It diffuses into the peripheral cells inside tissues

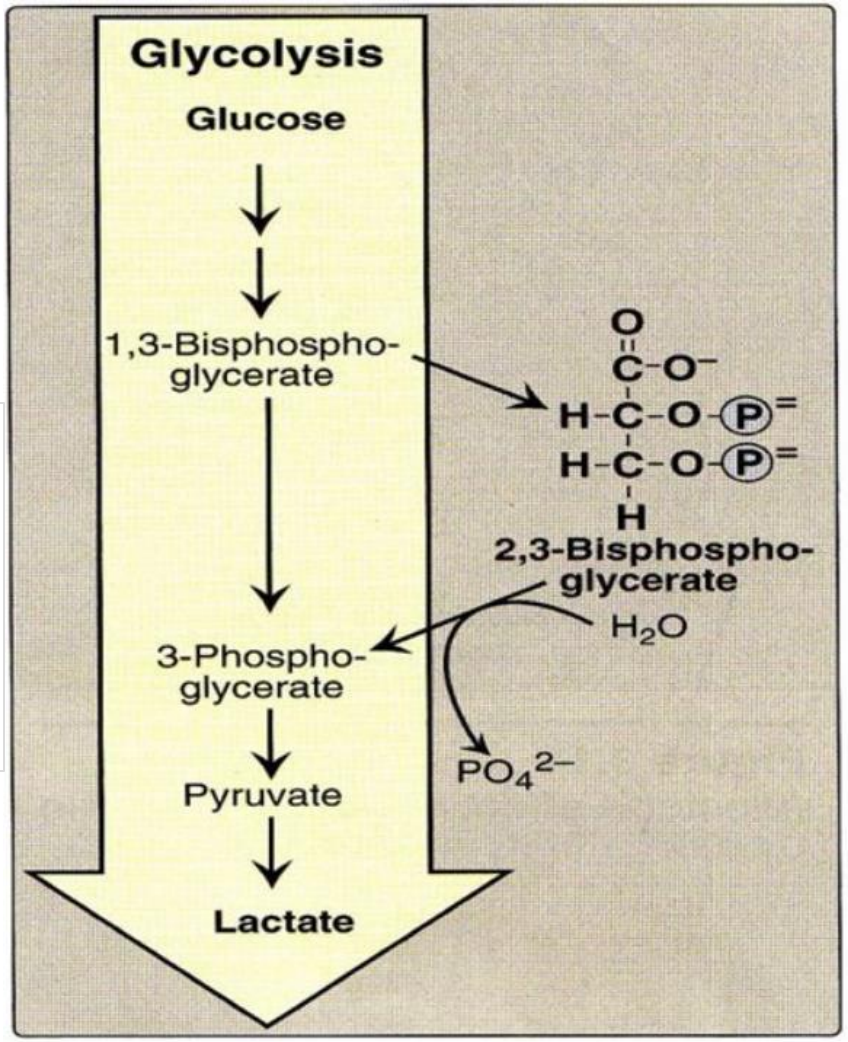
Effect of 2,3-bisphosphoglycerate

2,3-bisphosphoglycerate (2,3-BPG)

- 2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product of glucose metabolism in the red blood cells.
- It binds to hemoglobin and reduces its affinity towards oxygen.

Glycolysis is the metabolism of glucose, the molecule (1,3-Bisphosphoglycerate) is converted into it's isomer which is (2,3-Bisphosphoglycerate) that has an important function in regulating oxygen binding to hb. 2,3-Bisphosphoglycerate can return to the pathway producing pyruvate after doing it's function.

2,3-BPG connects to hB right in the centre to prevent the reconnection of O2 to it[because it's a gas- we can't control gases] -> this stabilizes hB in T-state



note to be smart in front of Dr. Diala next year: Diphosphate -> po4 are attached to each other(ATP) Bisphosphate-> po4 are connected to two different atoms on the molecule.

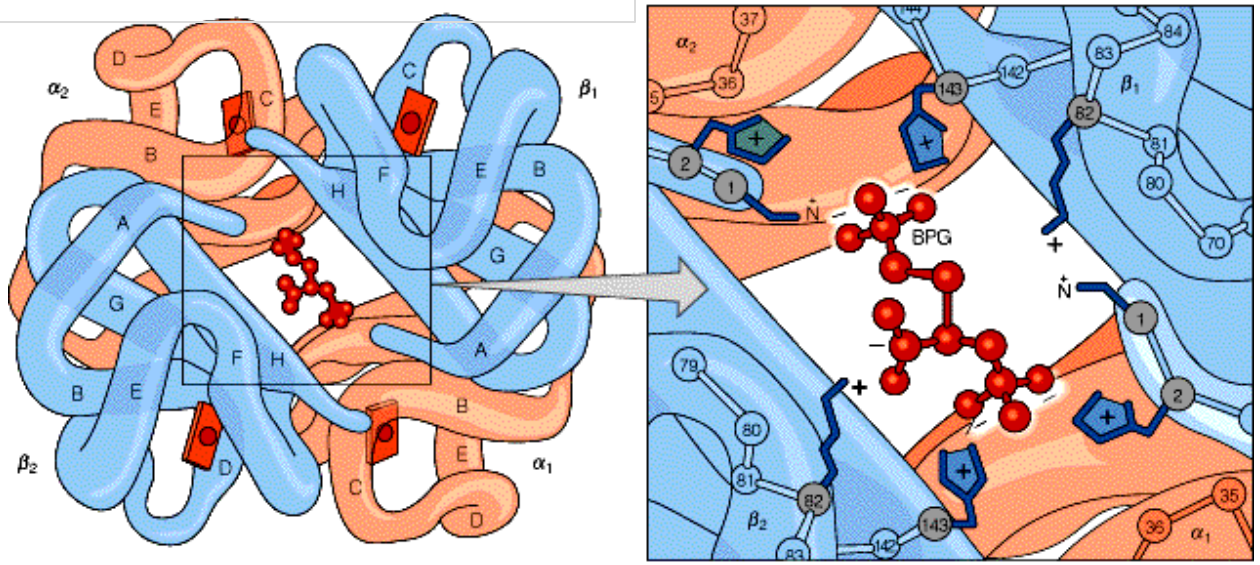
2,3-BPG –hemoglobin interaction

It's larger in the T-state than R-> 2,3-GPB doesn't connect on R

- 2,3-BPG binds in the central cavity of deoxyhemoglobin only in a ratio of 1 2,3-BPG/hemoglobin tetramer.
- This binding stabilizes the T-state hemoglobin reducing the binding of oxygen to hemoglobin and facilitating oxygen release.

This is an imp ratio, 4O2/ 1 hB, the CO2 ratio however we don't really know (could be four too cuz 4 chains , 4 N-terminus , but we don't really know)

2,3-BPG forms salt bridges with the terminal amino groups of both β chains and with a lysine and His143.



Effect of 2,3-BPG on oxygen binding

- In the presence of 2,3-BPG, the p50 of oxyhemoglobin is 26 torr.
- If 2,3-BPG were not present, p50 is close to 1 torr.

- The concentration of 2,3-BPG increases at high altitudes (low O_2) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.

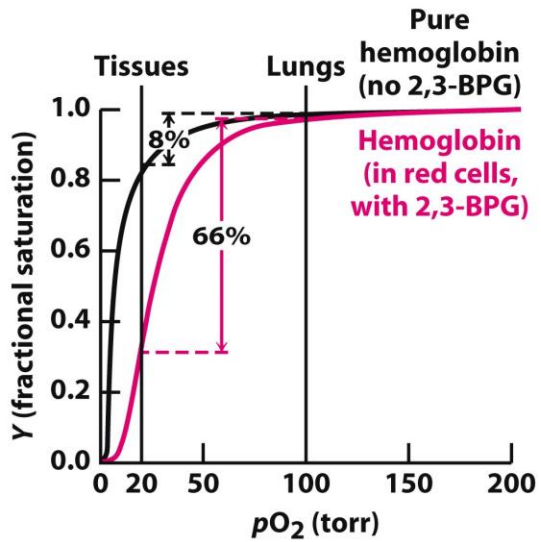
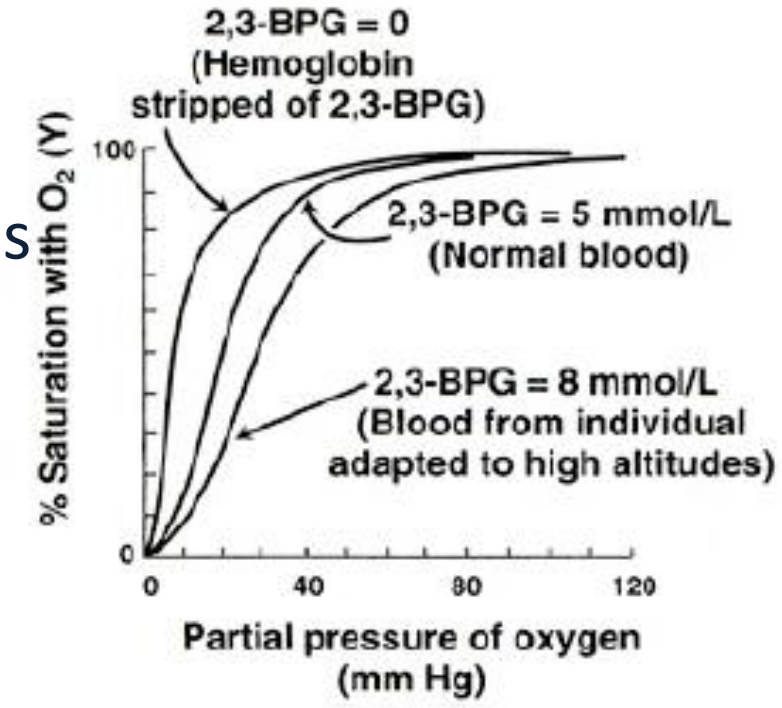


Figure 7.16
Biochemistry, Seventh Edition



The graph in pink shows how grand the effect of 2,3-BPG is, without it, the graph is like the myoglobin graph(but it's till sigmoidal!) showing how 2,3-BPG decreases the affinity .

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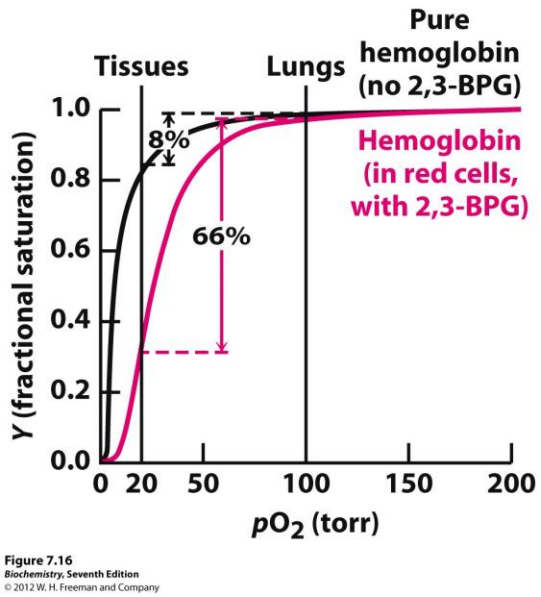

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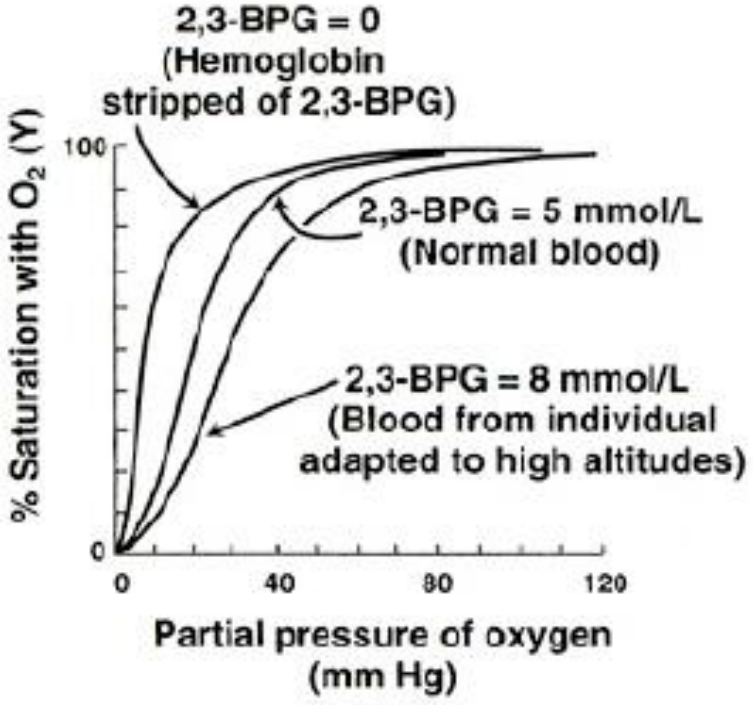


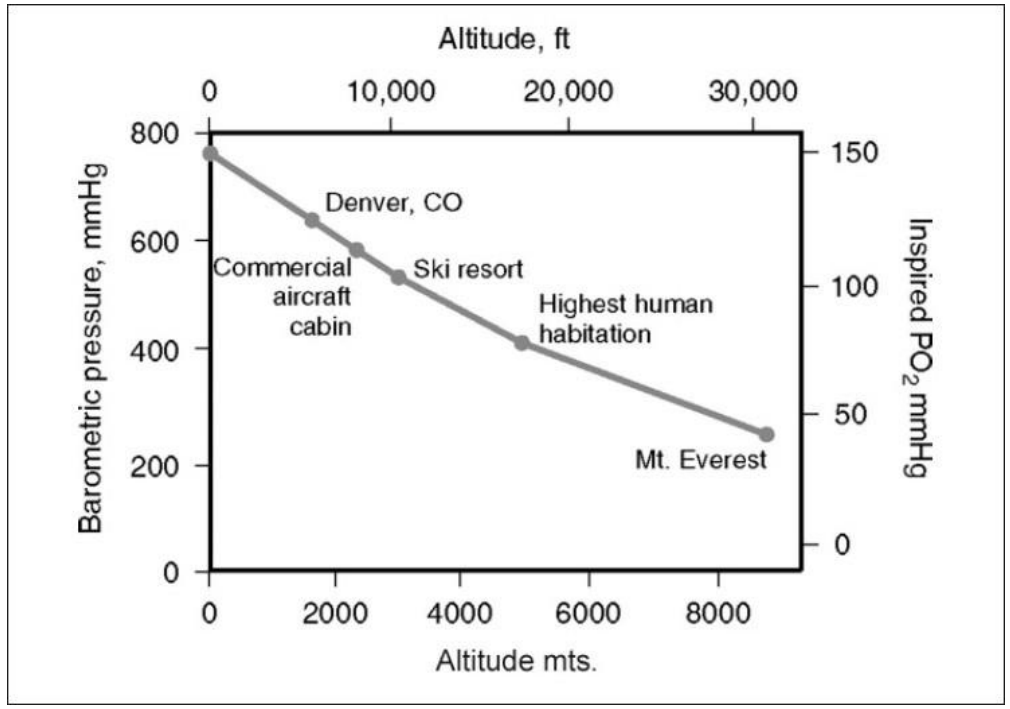
In the mountains your body will need time to readjust and adapt to the higher altitudes(lower O2) , thus, increasing the 2,3-BPG levels in hB(to promote more release of O2 = T-state)

Ok so the O2 is already low and when it goes to lungs how will it be saturated? Will it go to the tissues" empty handed"(خالي الوفاض على رأي الدكتور)?
No why? Go back to the first slide what did we say about hB it's **allosteric**.

But pO_2 is low at high altitudes!!!

Altitude (feet)	Atmospheric Pressure (mm/Hg)	PAO_2 (mm/Hg)	PVO_2 (mm/Hg)	Pressure Differential (mm/Hg)	Blood Saturation (%)
Sea Level	760	100	40	60	98
10,000	523	60	31	29	87
18,000	380	38	26	12	72
22,000	321	30	22	8	60
25,000	282	7	4	3	9
35,000	179	0	0	0	0

Which do you suppose is better?



More binding
Less release

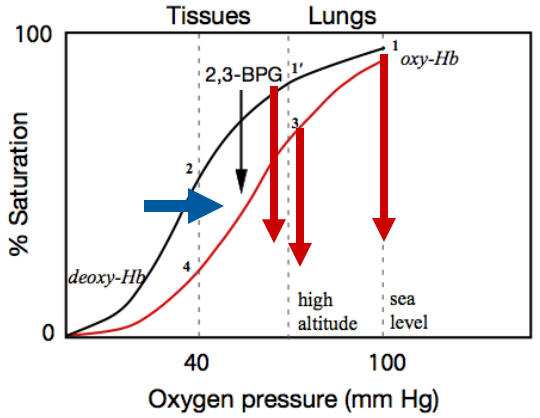
Less binding
More release

This one actually; read the next slide

Better explanation of role of 2,3-BPG

أنك تحمل عشرين تفاحة :مثال التفاح
وتوصل خمسة
more binding
أسوء من إنك تحمل سبعة وتوصل خمسة
Less binding

- At sea level the lungs pick up oxygen with 100% saturation of Hb (1) and when the oxygen pressure drops to 40 mm Hg in the tissues (2) the Hb will be 55% saturated.
  - They have released 45% of bound oxygen.
- At high altitudes (in case of no adaptation), Hb is only 80% saturated (1'). Thus at 40 mm Hg in the tissues (2) when Hb is only 55% saturated, it will only have released 25% of its oxygen.
- At high altitude (with increased 2,3-BPG production- in red), At the lungs (3) the Hb will be less bound with oxygen — only 70% saturation — but at 40mm Hg in the tissues (4) it will be much less saturated than on the black curve — 30%. Thus, it will have made available 40% of its oxygen.



- This is not a perfect solution, but over time there is increased production of red blood cells to provide more hemoglobin to compensate for the smaller amount of oxygen it can bind.

This all proves why less binding, and more release is better
Compensation

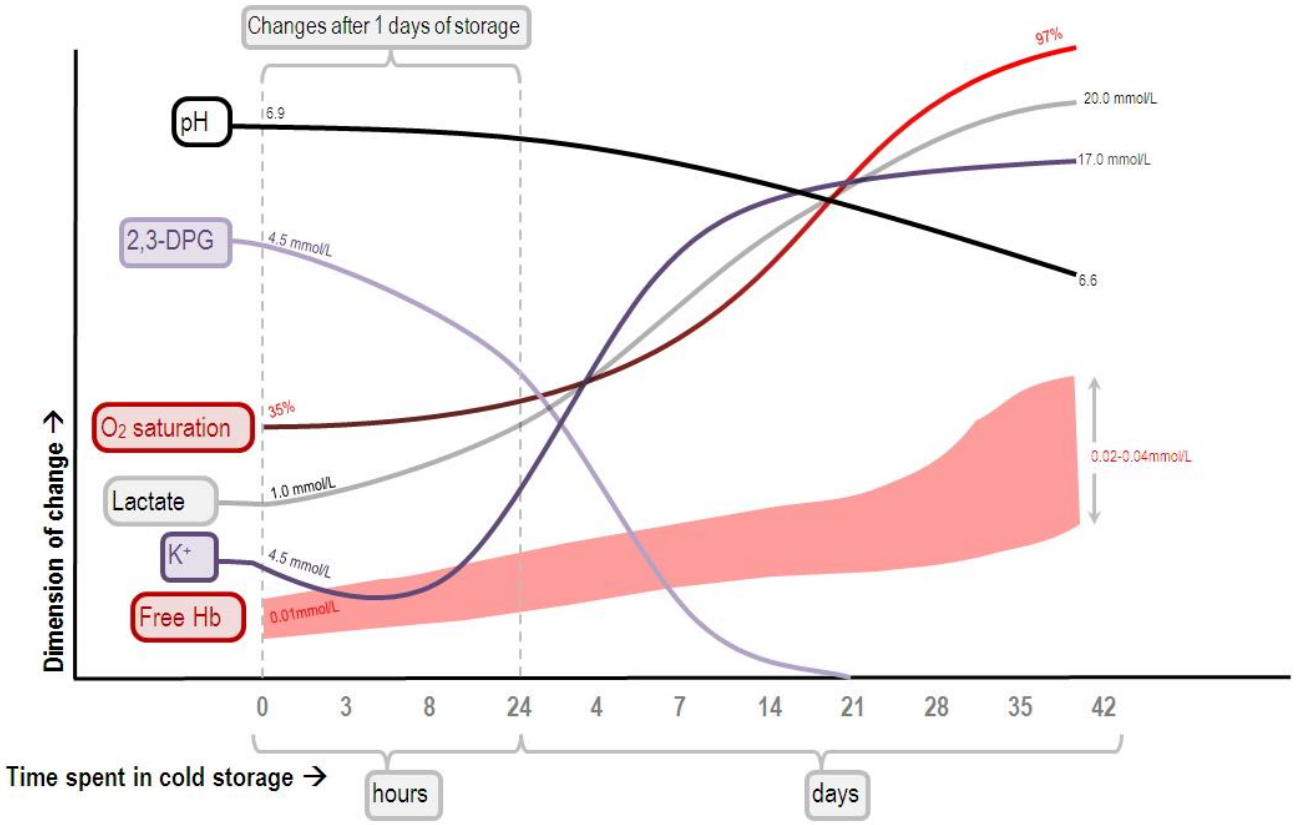
In normal circumstances the release of oxygen is 40% ,
In high altitudes, the saturation will decrease , but in the presence of 2,3-BPG(shift to the right) , the release will remain the same ~ 40%.
But high altitudes with no adaptation/no 2,3-BPG , you'll find that the saturation is higher but the release is lower, which is worse.

2,3-BPG in transfused blood

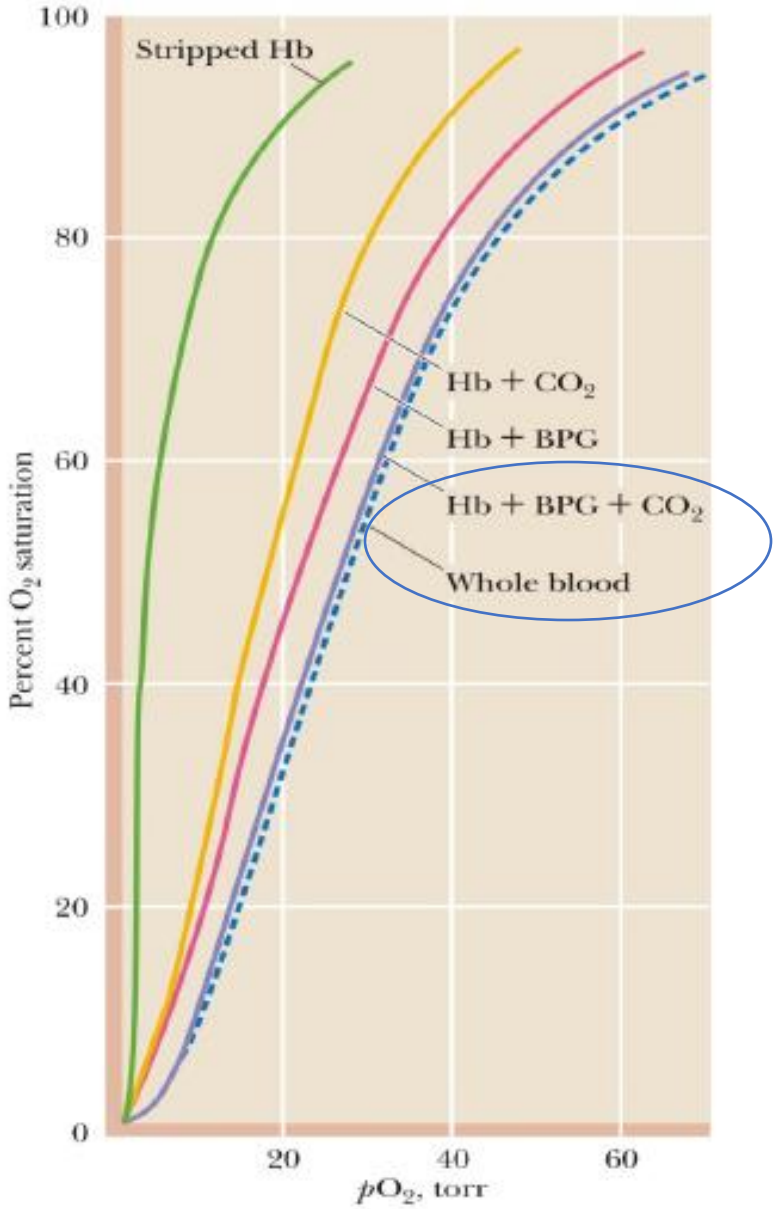
- Storing blood results in a decrease in 2,3-PBG (and ATP), hence hemoglobin acts as an oxygen “trap”, not an oxygen transporter.
- Transfused RBCs are able to restore the depleted supplies of 2,3-BPG in 6–24 hours.

- Severely ill patients may be compromised.
- Both 2,3-PBG and ATP are rejuvenated.

With time , 2,3-BPG is degraded as well as ATP, so when this blood is transfused to somebody in urgent need , it won't be effective because it won’t release O2 (R-state), and the 2,3-BPG takes time to be synthesized , that's why blood banks add it and add ATP before giving the blood unit to a person.



2,3-BPG and CO2 are important players

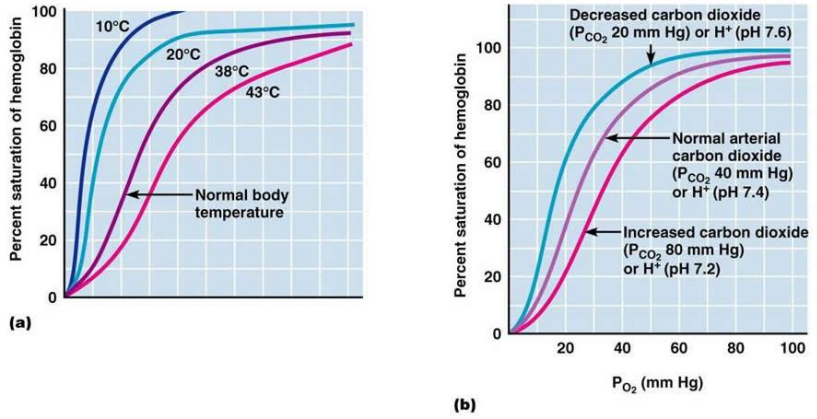


Just notice how imp this combo is-> the graph is very similar to the normal blood graph .
The combination of CO2 and 2,3-Bisphosphoglycerate is a more effective player than decreasing pH or adding chloride ions.

Effect of temperature

Effect of temperature

- An increase in temperature decreases oxygen affinity and therefore increases the P50.
- Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from HbO_2.



If you're exercising or you have a fever , your temp is in increase , now this shifts the graph to the right=> low affinity, high p50, more O2 release, more O2 required(obv!) So generally, temperature increases the release of oxygen .
Dr. Diala: the O2 is needed to generate ATP and synthesize proteins and any other factors needed for homeostasis to overcome this emergency state

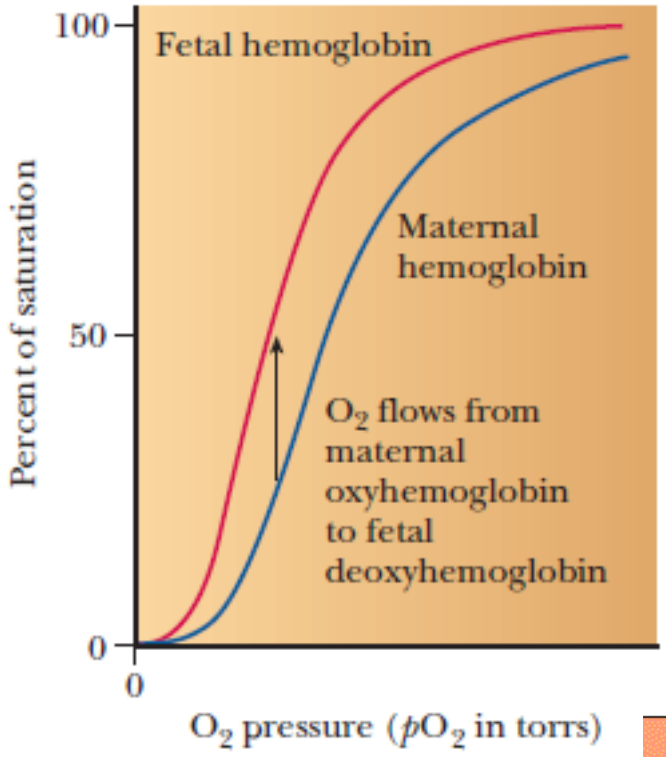
Other considerations

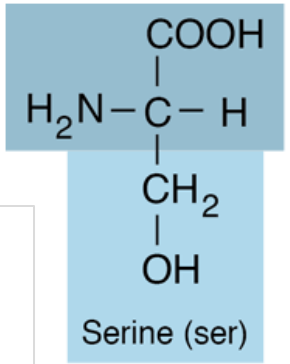
Fetal hemoglobin

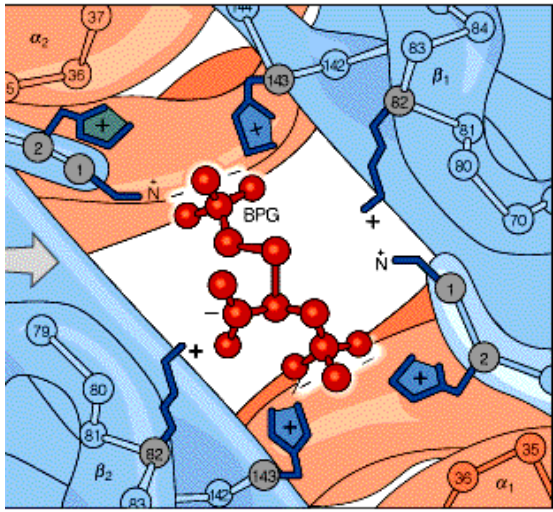
- Fetal Hb (HbF) has <u>higher affinity</u>(present in R-state- it takes/steals O2 from the mother cuz of the fewer interaction with 2,3-BPG) towards oxygen than adult hemoglobin (HBA).
  - HbA = $\alpha 2 \beta 2$
  - HbF = $\alpha 2 \gamma 2$

  They differ in the primary structure
- His143 residue in the β subunit is replaced by a serine residue in the γ subunit of HbF.(Ser is the major change but we do have different amino acids too between adult and fetal)
- Since serine cannot form a salt bridge with 2,3-BPG, it binds weaker to HbF than to HbA.

As serine can't form a salt bridge with 2,3-BPG -as what His143 does in adults- this will make the binding of 2,3-BPG to hb weaker , so low P50 and high affinity.





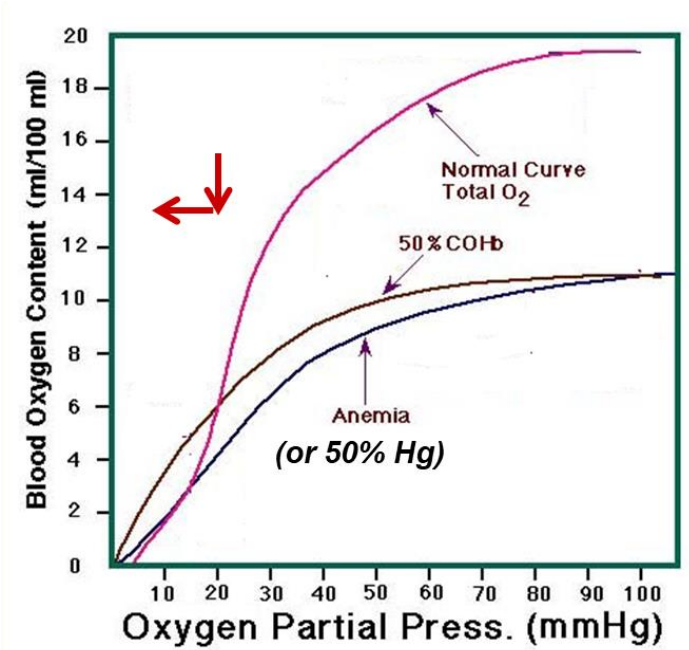


Effect of CO

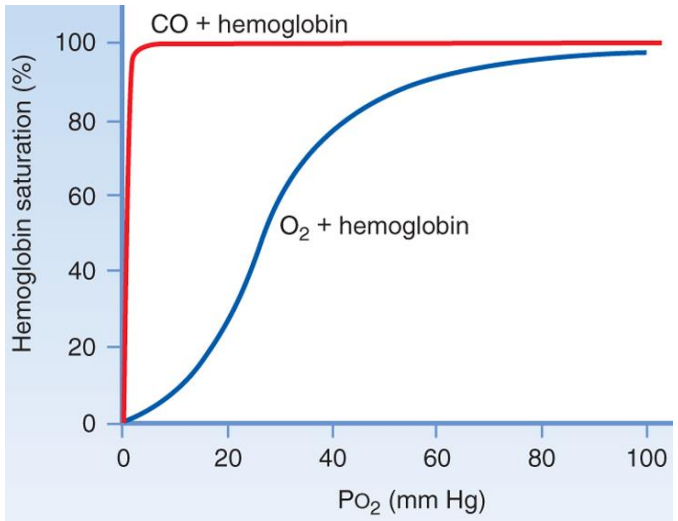
Effect of CO

- In addition to competing with oxygen in binding to hemoglobin, the affinity of Hb-CO towards oxygen increases resulting in less oxygen unloading in peripheral tissues.

$(Hb + O_2)$ versus $(Hb + O_2 + CO)$



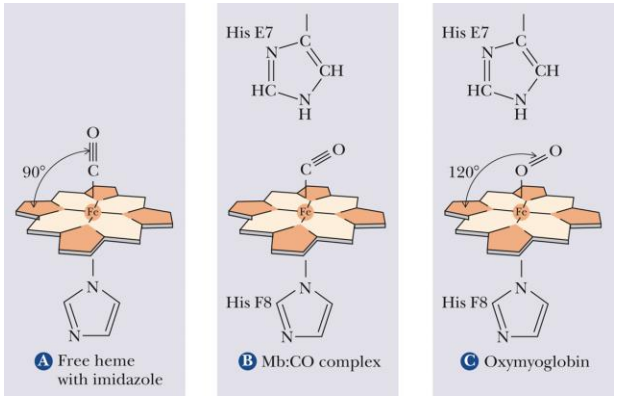
$(Hb + O_2)$ versus $(Hb + CO)$



When we have CO , it binds to the heme and keeps it in the R- state all the time , so oxygen binding sites to hb are really few.

Also , CO shifts the oxygen that's bound to hb from the T-state to the R-state , so it's not released in tissues!

So , two factors :increasing affinity and decreasing binding sites.



It's compared to anemia because CO binding is irreversible (it doesn't move from R to T) so that's a lost place for O2 to bind to hb.

Relevant information

- Increasing the amount of CO in inspired air to 1% and above would be fatal in minutes.
- Due to pollutants, the concentration of CO-Hb in the blood is usually 1% in a non-smoker.
- In smokers, CO-Hb can reach up t"
- " o 10% in smokers.
- If this concentration of CO-Hb in the blood reaches 40% (as is caused by 1% of CO in inspired air), it would cause unconsciousness initially, followed by death.

A word of advice: you're a role model people, respect your self and look up to it . People will listen when you talk, and take your health advice, so don't do stupid and selfish things such as smoking, people will disregard what you say" أي صحة إلي بتحكي فيها و أنت بتدخن"

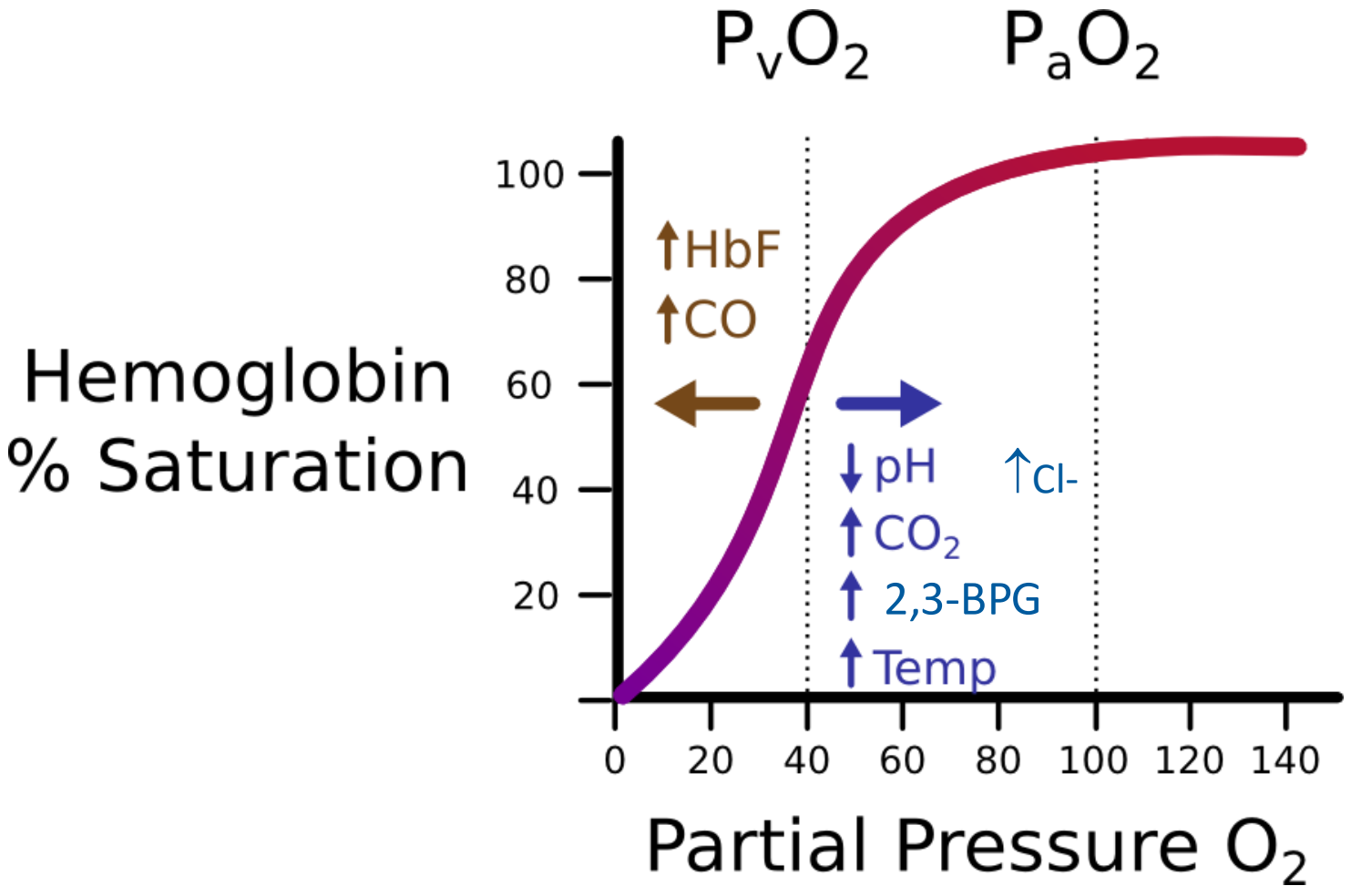
"و إذا بتفكروا إنكم بتحرروا فلسطين ما رح تحرروها و أنتوا بتدخنوا مش رح تقدروا "

أنت إلي بتشرف لقب الطبيب"دكتورة ديالا حكت كلمة حلوة هداك اليوم

So think twice before picking up a habit that may ruin your health and your image.

Be an extraordinary doctor :)

Summary


P_vO_2
P_aO_2
Hemoglobin
% Saturation
100
80
60
40
20
↑HbF
↑CO
↓pH
↑CO_2
↑2,3-BPG
↑Temp
↑Cl-
0
20
40
60
80
100
120
140
Partial Pressure O_2

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	Slide 26	won't have enough O2 to make a difference	Won't release
V2 → V3	Slide 7 below	In the opposite (Beta chain)	In the opposite (alpha chain)
V3 → V4	Slide 26	"release"	"won"t release" خطأ مطبعي

Additional Resources Used:

1. https://youtu.be/Qv-KExGKAYw?si=SuHK63jkUFC-g5bH
2. https://youtu.be/wQ2eCRN02f4?si=N_LN0yqNIc3nyxML

رسالة من الفريق العلمي:

عسى الخفايا من الأقدار تبهجنا, و عسى الجديد من الأيام يحيينا, عسى أن يمسح الله على قلوبنا و يسكن في أرواحنا من لدنه الطمأنينة, و يعطينا لحد الرضا و الغنى, عسى رب الفؤاد يقر أعيننا, و يجمعنا بآمالنا ☺

"صلّوا على الحبيب تغنَموا"