Doctor.021

no. 2

HLS

BIOCHEMISTRY

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Slides are in bold.

GENETICS OF GLOBIN SYNTHESIS

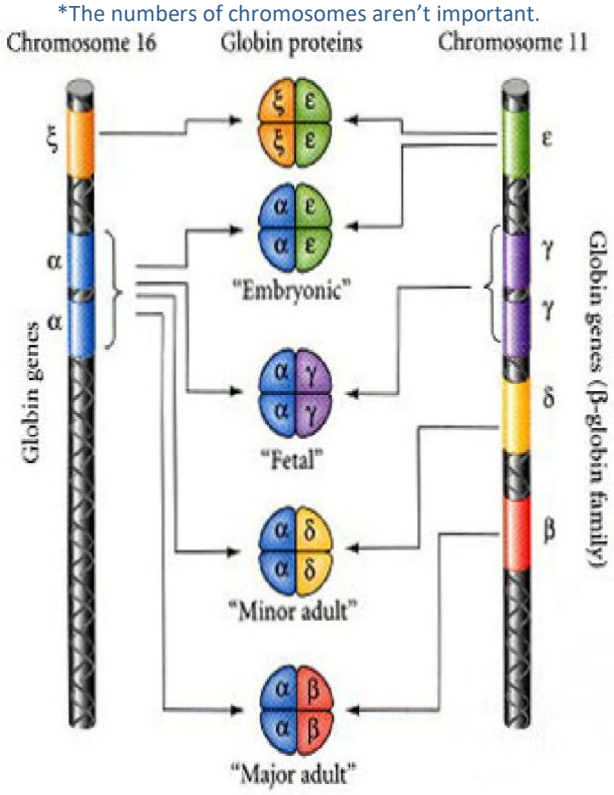
The different forms of hemoglobin are located on chromosomes as clusters of genes.

THE GENES

• **The α gene cluster** (on one chromosome) **contains three genes: two α genes (α1 and α2)** meaning we have 4 α genes as a total in our cells (2 on each chromosome)**, and ζ (zeta) gene.**

• **The β gene cluster contains five genes: ε (epsilon) gene, two γ (gamma) genes, and δ (delta) gene, β gene.** Notice that the order of genes on one chromosome is the same as the order of their expression. **The gene order parallels the order of expression.**

*The numbers of chromosomes aren't important.



1. Embryonic hemoglobin → ε (epsilon) gene.
2. Fetal hemoglobin → two γ (gamma) genes.
3. Adult hemoglobin → δ (delta) and β genes.

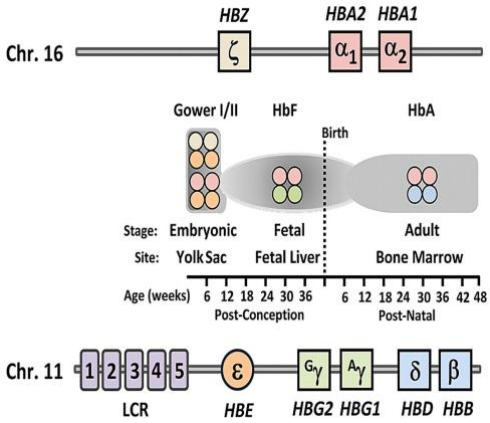
• **Genetic switching is controlled by a transcription factor-dependent developmental clock, independent of the environment.**

It's timed; once the fertilization happens the clock starts, after 6-8 weeks the expression transitions from the embryonic to the fetal gene, before birth it switches again from the fetal γ to the adult β gene.

• **Premature newborns follow their gestational age**/stage, (which is 9 months after the time of fertilization)**.** If a baby was born prematurely, the transition of gene expression from the fetal to the adult genes will wait until the 9-month mark hits (no matter when the birth did happen).

Meaning If a baby was born after 7 months of pregnancy, the transition of expression to the adult form will happen when he is 2 months old (he will have 60% HbF and 40% HbA1) which is what a mature born child would have before he was born.

LOCUS STRUCTURE



• **Each gene has its promoter and regulatory sequences (activators, silencers)** (regulatory elements, DNA sequences) where RNA polymers bind to regulate the expression.

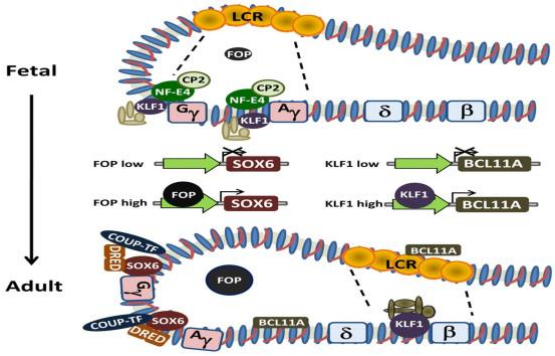
• **The α gene cluster is controlled by the HS40 region** (an enhancer) which is a regulatory element (a part of the DNA sequence) that can be located near the gene or far away from it, and enhancers' positions can be changed according to the next activated gene's location and the enhancer will still be functional because of the DNA looping.

• **The β-globin cluster is controlled by a master enhancer called locus control region (LCR).**

THE MECHANISM OF REGULATION

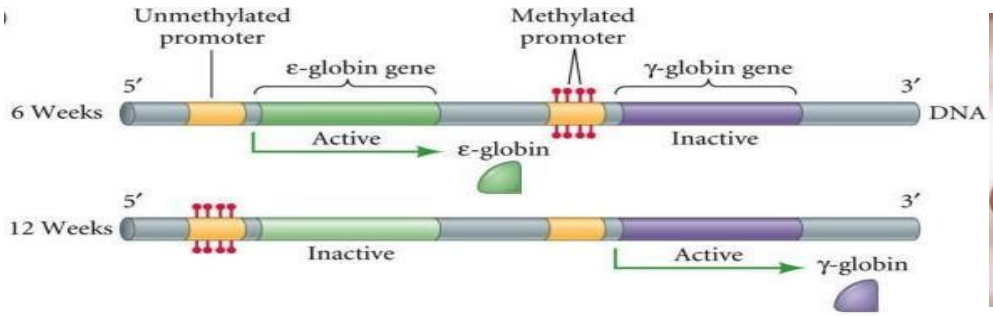
• **The mechanism requires timed expression of regulatory transcription factors for each gene, epigenetic regulation (e.g., acetylation, methylation,** phosphorylation**), chromatin looping, and non-coding RNA (e.g., long non-coding RNA, microRNA, etc.).**

• **Note: treatment!!**



Researchers tried to treat beta thalassemia, which is highly prevalent in our region, by making some epigenetic modifications. This disease is related to abnormal bata globins, so they induced the expression of gamma globin instead of beta (which works similar to beta globins but has more affinity for O2) using CRISPR-Cas9, long noncoding RNAs, and enhancers. Here is recently published research that comes with the finding that there is a factor that induces the expression of gamma globin, so we can use this factor to compensate for the loss of beta globin. (What is in this color is not included in the exam, it's just for entertainment reasons •̀ᴗ•́)

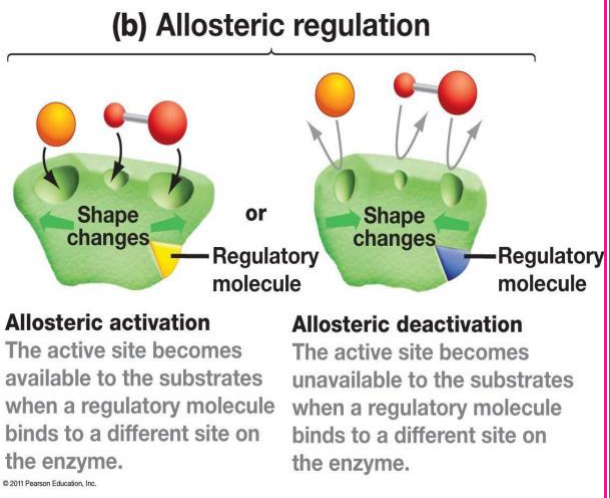
https://www.nature.com/articles/s41586-022-05312-w



REGULATION OF HEMOGLOBIN FUNCTION

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** (O2).
- **Heterotropic modulators are different from the ligand.**



ALLOSTERIC EFFECTORS

The major heterotropic effectors of hemoglobin: (Carbon dioxide, **Hydrogen ion**, **2,3-Bisphosphoglycerate**, **Chloride ions**, carbon monoxide, fluoride ions)**.**

Allosteric effectors are of two types:

1. Positive heterotropic effectors such as **carbon monoxide (a competitive inhibitor).**
2. Negative heterotropic effectors, most of the regulators, they reduce the affinity of O2 to the hemoglobin.

THE EFFECT OF PH AND H+

THE EFFECT OF PH

- **The binding of H+ to hemoglobin promotes the release of O2 from hemoglobin and vice versa.**
- **This phenomenon** (the positive effect of H+ on O2 release from hemoglobin) **is known as the Bohr effect.**

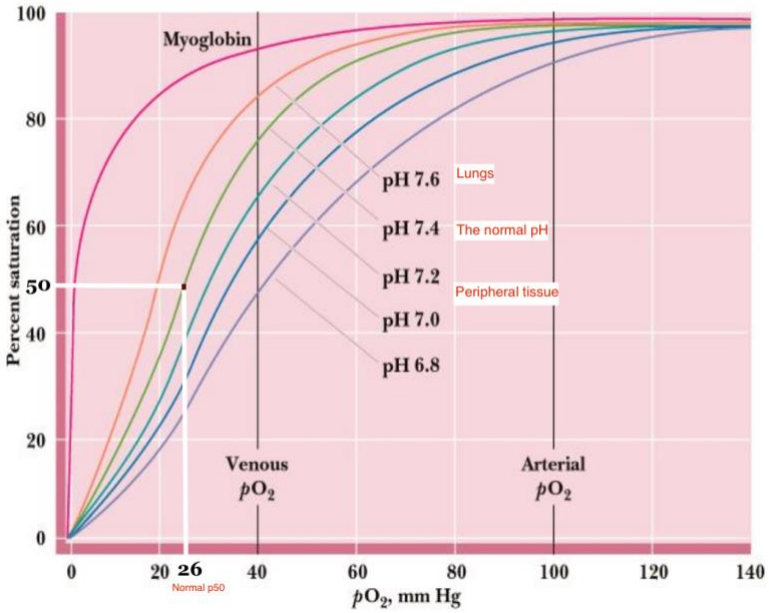
MECHANISM OF BOHR EFFECT

- **Increasing H+ (in tissues) causes the protonation of key amino acids, including the last histidine residue of the β chains (His146).**

Watch this vid to make understanding what is next easier.
https://youtu.be/StpQmmuVnTA?si=Y6huWNUuSQ3NNZuG

▪ Normally the pH of the body is 7.4 and its p50 is 26, see the green blot.

▪ In the **lungs**, where the pH increases 7.6, the blot will shift to the left and the p50 will decrease, see the orange blot.



▪ In the **peripheral tissues**, where the pH decreases 7 or 6.8 (due to the increased metabolic activity), the blot will shift to the right and the p50 will increase, meaning more oxygen is needed to fill 50% of the hemoglobin, less affinity to oxygen, see the purple blot.

- Bohr effect describes the decrease in the oxygen affinity of hemoglobin in the presence of low pH, which increases the proton number, and it's going to protonate some groups; when the amino group gets protonated it will be positively charged, and the carboxylic group will be neutral.This change is going to happen according to the pKa of the hemoglobin.
(pKa is a constant value that describes the pH when the group is half protonated, ionizable, charged, conjugated base and the other half is unprotonated or acidic...)

✔ When the pH is less than the pKa, groups are going to be protonated because there is a lot of protons, so the amino groups (and imidazole group) are going to be positive and carboxylic group neutral. **pH < pKa, the group is protonated.**

✔ When the pH is more than the pKa, groups are going to be deprotonated because there aren't enough protons to protonate other groups, so the amino groups (and imidazole group) are going to be neutral and carboxylic groups negative. **pH> pKa, the group is deprotonated.**

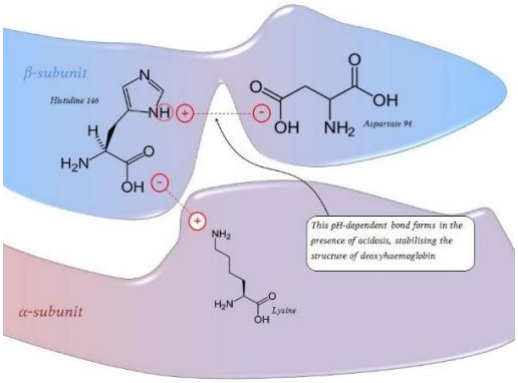
- Remember: His146 is the last AA in the β subunit of hemoglobin, and it has 2 charged groups; the first is a positive charge from its own imidazole ring, the second is a negative charge from the carboxylic group since it's the last AA.

▪ Remember also that there are two states of hemoglobin: T (tense) when there are electrostatic bonds and R (relaxed) when there are no electrostatic bonds.

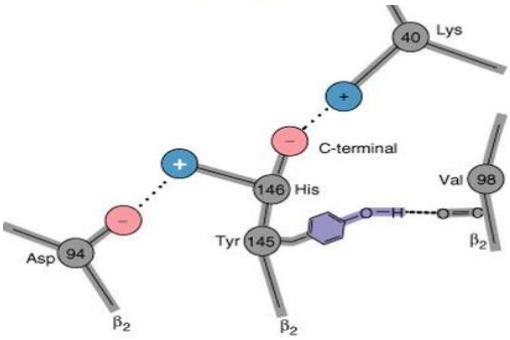
▪ His146 forms two electrostatic interactions when it's in the T form:

1. **Electrostatic interaction occurs between the carboxylic group of His146 and a lysine of the α chain.**
2. **The protonated histidine** (specifically its imidazole ring) **also forms a salt bridge to Asp94 within the same chain.** (Since protonated imidazole is positive and Asp is negative.)

▪ His146 in the R state won't form electrostatic interactions and sliding would happen.



• The pKa of the R group of the histidine alone when it's out of the polypeptide chain is 6, meaning on pH of 6 half of the histidine will be protonated and the other half won't. But when the histidine is a residue as a part of a polypeptide in the hemoglobin, like His146, its pKa will increase to 7.7, because it will be affected by the surrounding amino acids.



• **The pKa of the imidazole ring** (the R group) **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.**

- For example, if the pH was 7.4 around the deoxygenated His146 which has a pKa of 7.7, it would be protonated, positive and in the T form, and cause electrostatic interactions with the negative Asp94.
- And if the pH was 7.4 around the oxygenated His146 which has a pKa of 7.3, it would be deprotonated, neutral and in the R state with no electrostatic interactions with other amino acids, which causes sliding of amino acids.

✔ **Deoxygenated** histidine is in the **T** state (**pKa is 7.7**) and have **electrostatic interactions with Asp94** because it's **positive**. This happens in **peripheral tissues** where pH is low because of the high level of metabolism and the production of H+ and CO2.

✔ **Oxygenated** histidine is in the **R** state (**pKa is 7.3**) and have **no electrostatic interactions,** because it's **neutral**. This happens in **lungs** where oxygen pressure is high.

WHERE DO PROTONS COME FROM?

- **CO2 and H+ are produced at high levels in metabolically active tissues by carbonic anhydrase, facilitating the release of O2.**
- **In the lungs, the reverse effect occurs and, also, the high levels of O2 cause the release of CO2 from hemoglobin.**

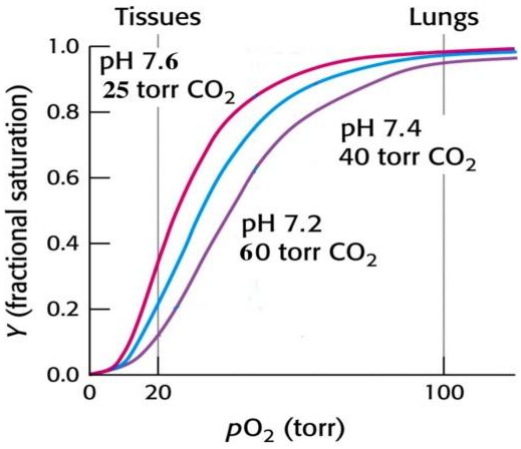
THE EFFECT OF CO2

We have 2 mechanisms in which hemoglobin turn from the R state to T state:

Mechanism #1 - production of protons

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

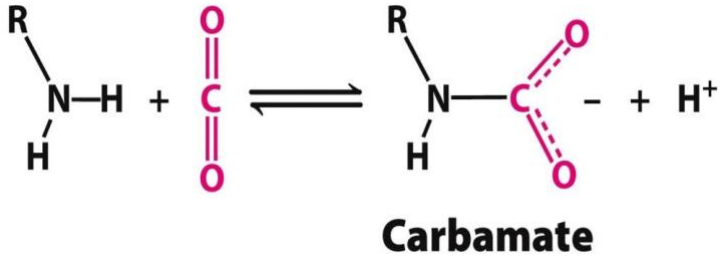
CO2 (produced from metabolic activities, mainly Krebs cycle) leaves tissues and enters RBCs and combine with water by the enzyme carbonic anhydrase to make carbonic acid H2CO3, which disassociate into one proton and bicarbonate HCO3- (the ionizable groups).



(In the peripheral tissue) The protons in the RBC will bind to the hemoglobin in the R state (remember because it's oxygenated and came from the lungs) to make it in the T state and release oxygen which then enters tissues.

Mechanism #2- formation of carbamates

- **Hemoglobin transports some CO2 directly.**
- **When the CO2 concentration is high, it combines with the free α-amino terminal groups to form carbamate and producing negatively-charged groups.**
- **The increased number of negatively-charged residues** (the carbamate is negatively charged) **increase the number of electrostatic interactions that stabilize the T-state of hemoglobin.**



Carbamate

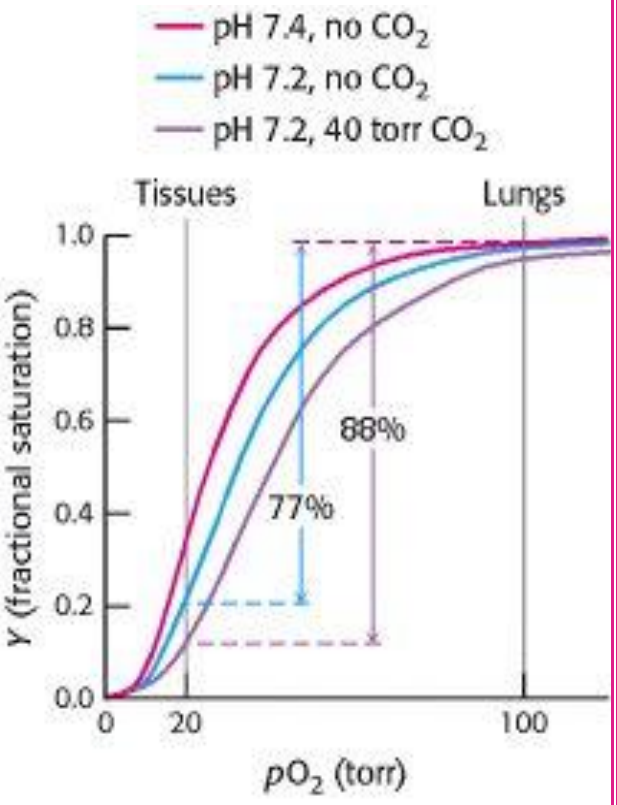
WHICH MECHANISM HAS A STRONGER EFFECT?

- **About 75% of the shift is caused by H+** from the first mechanism (it is more effective).
- **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.

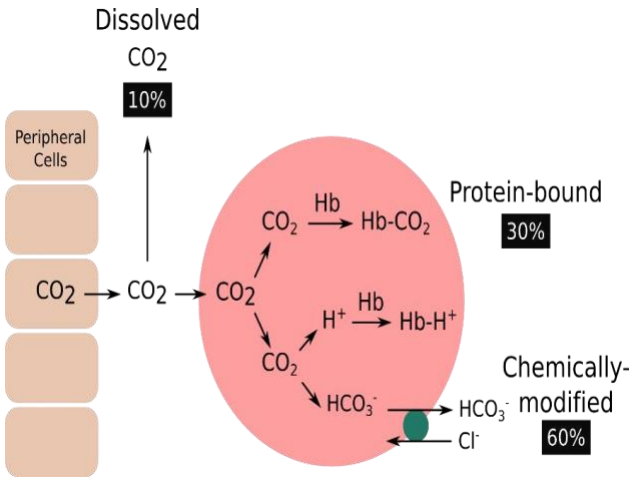
An increase in CO2 tension will shift the oxygen dissociation curve to the right, even when the pH is held constant.



Notice in the figure, when the pressure of CO2 increases, the pH decreases (the number of protons increases), and the curve is shifted to the right, and the affinity decreases.

TRANSPORT OF CO2 INTO LUNGS

- **Approximately 60% of CO2 is transported as bicarbonate ion, which diffuses out of the RBC.**
- **About 30% of CO2 is transported bound to N-terminal amino groups of the T form of hemoglobin.**

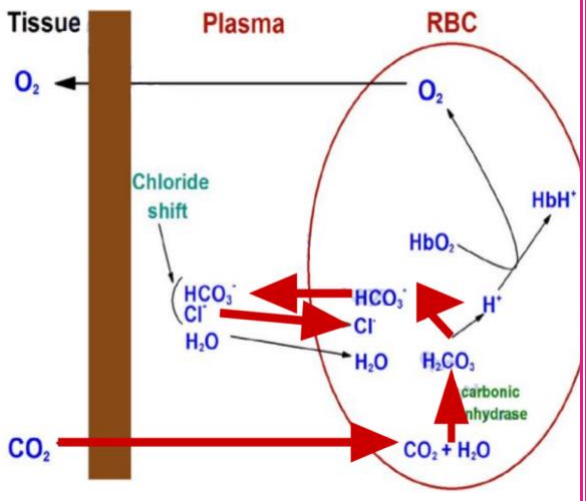


- **A small percentage of CO2 is transported as a dissolved gas. The movement of CO2 in/out of cells does not change the pH, a phenomenon called isohydric shift, which is partially a result of hemoglobin being an effective buffer.**

Because of the bicarbonate buffer system and the hemoglobin molecules which are considered as a major buffer; the CO2 movement into the RBCs won't change the blood pH, and that's called (isohydric shift).

EFFECT OF CHLORIDE ION

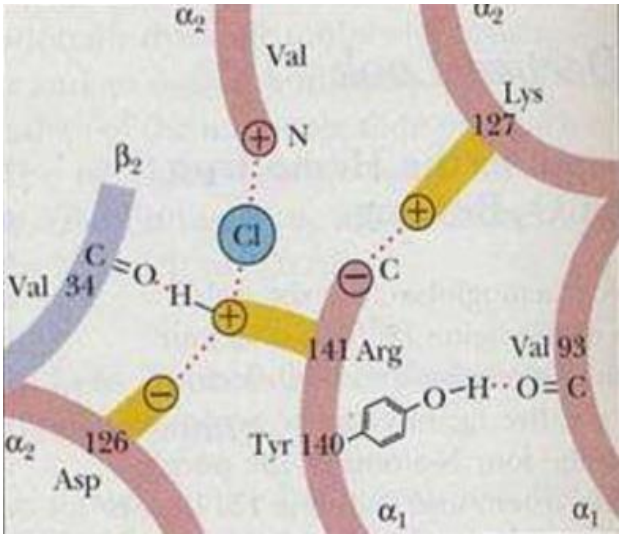
CHLORIDE SHIFT



- **Bicarbonate diffuses out of the red blood cells into the plasma in venous blood and vice versa in arterial blood.**
- **Chloride ion always diffuses in an opposite direction of bicarbonate ion in order to maintain a charge balance,** to compensate the lost negative charge in the RBC from the leaving of bicarbonate.
- **This is referred to as the "chloride shift"** (bicarbonate gets out of RBCs and chloride in).

EFFECTS OF CHLORIDE IONS:

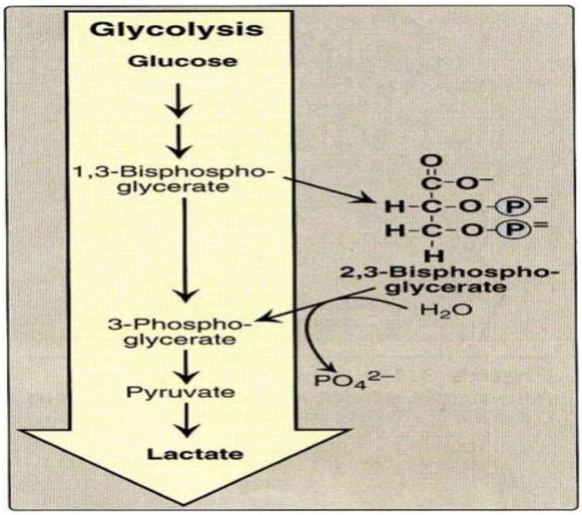
1. It stabilizes the pH in the RBC.
2. **Chloride ions interact with both the N-terminus of α2 chain and Arg141 of α1 chain stabilizing the T-state of hemoglobin.**



- **Increasing the concentration of chloride ions (Cl-) shifts the oxygen dissociation curve to the right (lower affinity)** because it stabilizes the T state and makes more electrostatic interactions.

EFFECT OF 2,3-BISPHOSPHOGLYCERATE

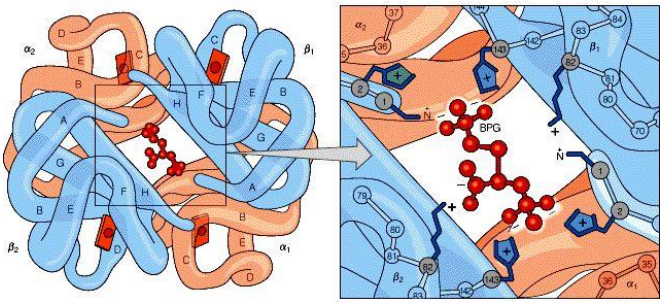
- **2,3-Bisphosphoglycerate (2,3-BPG) is produced as a by-product** (secondary product) **of glucose metabolism in the red blood cells,** by changing the position of phosphate from carbon 1 in 1,3bisphosphoglycerate to carbon 2.
- **It binds to hemoglobin and reduces its affinity towards oxygen.**



- BPG has a lot of negative charges because it has two phosphate groups that allow it to interact with hemoglobin right in the core of the whole molecule (with histidine and lysine), creating a lot of electrostatic interactions that stabilizes the T-state of hemoglobin and releases O2.

2,3-BPG –HEMOGLOBIN INTERACTION

• 2,3-BPG binds in the central cavity of deoxyhemoglobin only in a ratio of one 2,3-BPG/hemoglobin tetramer, (one 2-3BPG binds to one hemoglobin in its center).



• This binding stabilizes the T-state hemoglobin reducing the binding of oxygen to hemoglobin and facilitating oxygen release.

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 O2) and in certain metabolic conditions making hemoglobin more efficient at delivering oxygen to tissues.

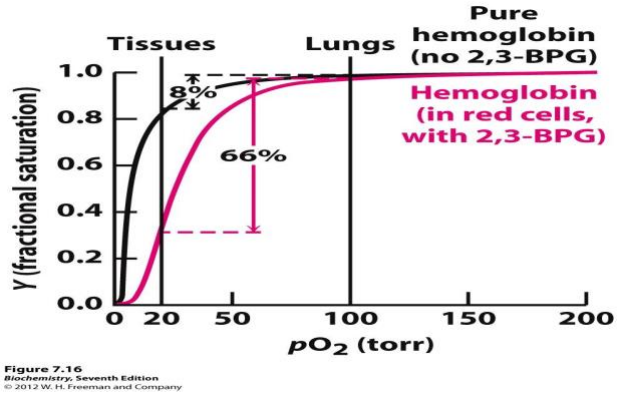


Figure 7.16
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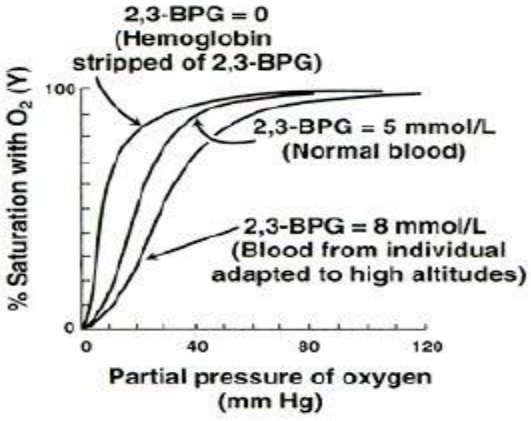
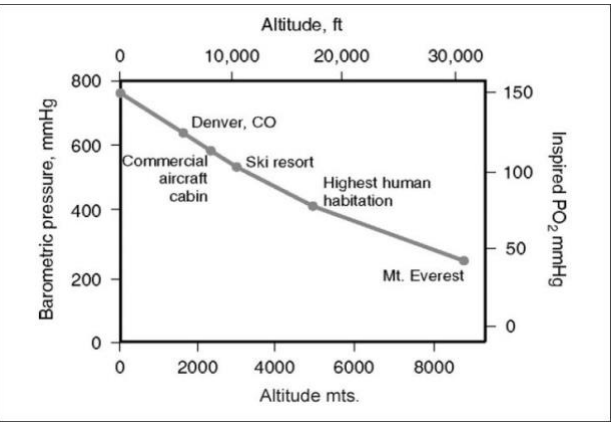


BUT PO2 IS LOW AT HIGH ALTITUDES!!!

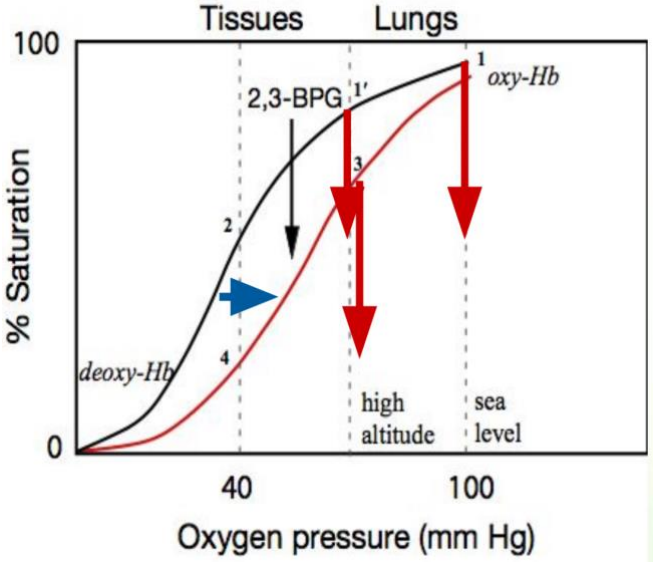
Respiration is difficult at high altitude, because of the low atmospheric pressure of oxygen, and the body needs 2-3 days to adapt because it needs time to build up more 2,3-BPG to bind with the hemoglobin and release more oxygen by lowering its affinity.

However, this can cause an issue in the lungs, because lowering the affinity of O2 will decrease the level of saturation of hemoglobin! So, it is a balancing act; to be able to release more oxygen in the tissues, it would have to also reduce its saturation levels in the lungs, and this will still give us more oxygen.

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



BETTER EXPLANATION OF ROLE OF 2,3-BPG



• 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.

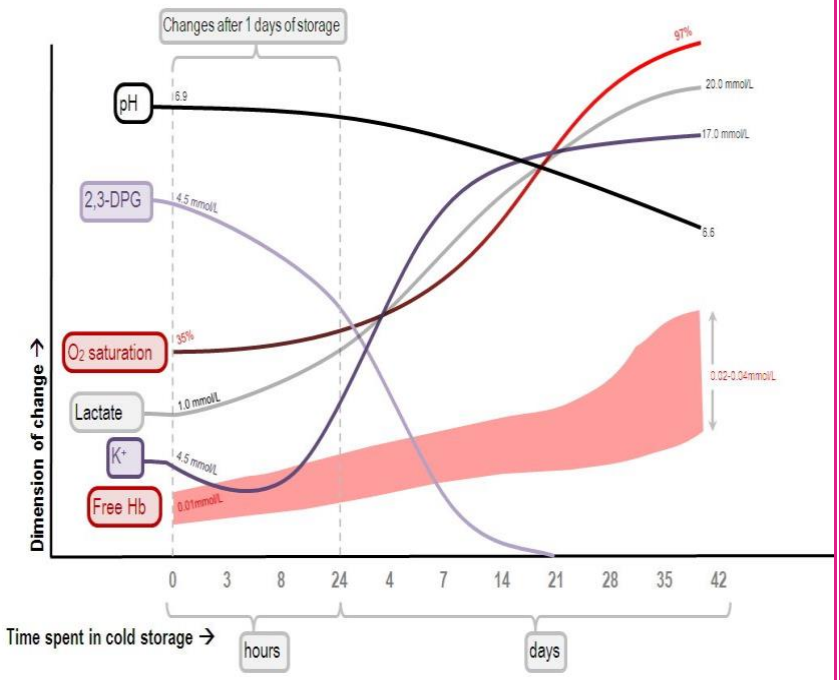
• At high altitudes (in case of no adaptation), Hb is only 80% saturated (1'). Thus at 40 mm Hg in the tissues (2) when Hb is only 55% saturated, it will only have released 25% of its oxygen.

• 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.

2,3-BPG IN TRANSFUSED BLOOD

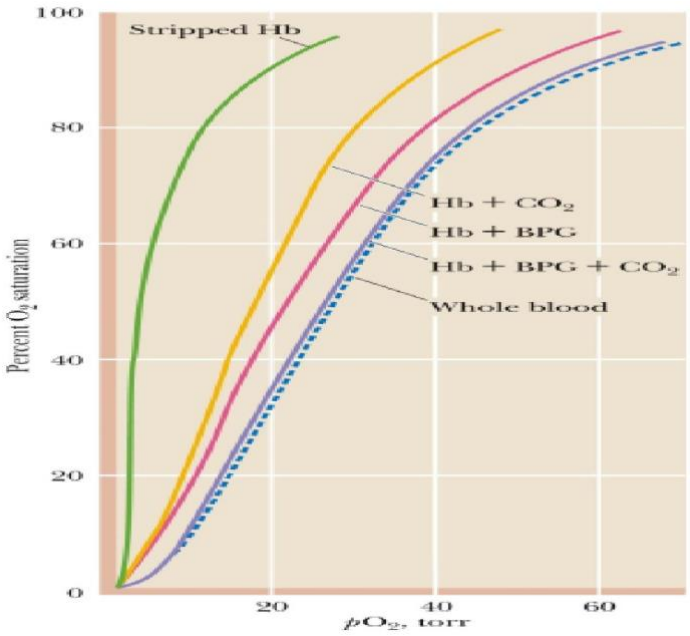
- **Storing blood results in a decrease in 2,3-PBG (and ATP), hence hemoglobin acts as an oxygen "trap", not an oxygen transporter.**
- **Transfused RBCs are able to restore the depleted supplies of 2,3-BPG in 6–24 hours.**
- **Severely ill patients may be compromised** because they won't be able to handle the adaptation time and when they receive the stored blood the hemoglobin will go to the peripheral tissue and won't release the oxygen because it doesn't have 2,3-BPG, because of that blood banks put 2,3-BPG and ADB into the stored blood before giving it to the patients and this is called rejuvenation. إعادة الحيوية



- **Both 2,3-PBG and ATP are rejuvenated.**

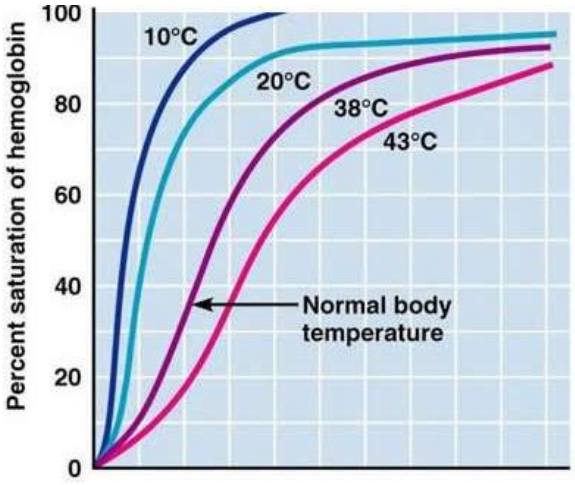
2,3-BPG AND CO2 ARE IMPORTANT PLAYERS

Both CO2 and 2,3-BPG are important and if we only added one of them, we won't be able to reach the full efficiency, but when both BPG and CO2 are with Hb here is the most shifted curve to the right and nearly the normal physiological situation and we can't say that one effector is more important than the others. See this figure.



EFFECT OF TEMPERATURE

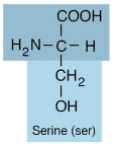
- **An increase in temperature decreases oxygen affinity and therefore increases the P50.**
- **Increased temperature also increases the metabolic rate of RBCs, increasing the production of 2,3-BPG, which also facilitates oxygen unloading from HbO2.**

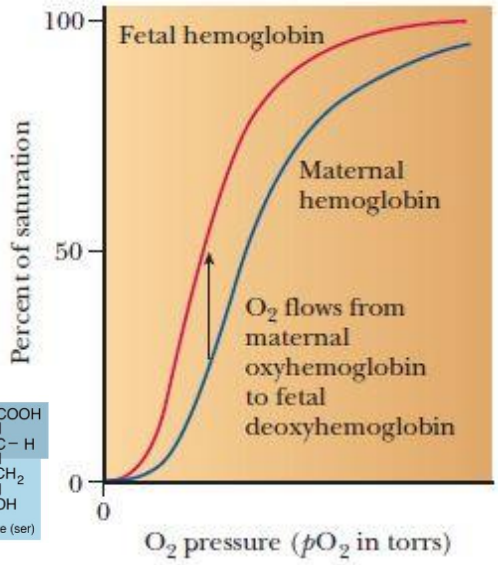


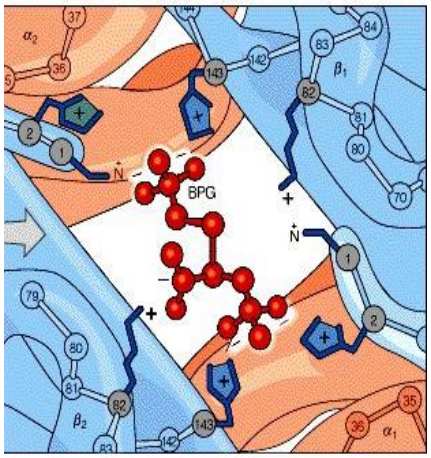
OTHER CONSIDERATIONS

FETAL HEMOGLOBIN

- **Fetal Hb (HbF) has higher affinity towards oxygen than adult hemoglobin (HBA).**
- **HbA = α2β2** (adult hemoglobin)
- **HbF = α2γ2** (fetal hemoglobin)
- **His143 residue in the β subunit is replaced by a serine residue in the γ subunit of HbF.**
- **Since serine cannot form a salt bridge with 2,3-BPG, it binds weaker to HbF than to HbA.**







Remember we said that 2,3-BPG forms salt bridges with the terminal amino groups of both β chains and with a lysine and His143 our focus here is on His143, this is the case in the adult's hemoglobin since it has β chains ending with His143, so 2,3-BPG will bind to this hemoglobin and make more electrostatic interactions which decrease its affinity to oxygen.

However, in the fetal hemoglobin it has γ chains ending serine residue where 2,3-BPG cannot bind. So, the fetal hemoglobin will have more affinity to oxygen and will mostly be in the R state rather than the T state.

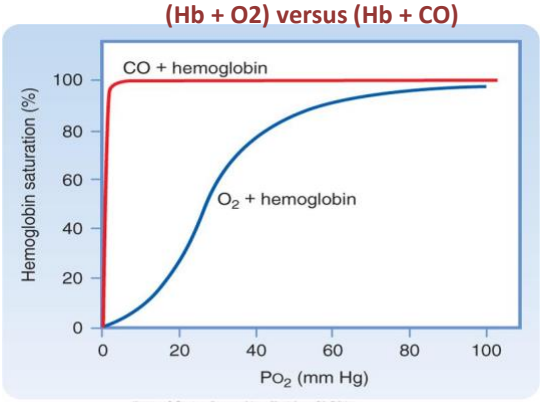
EFFECT OF CO

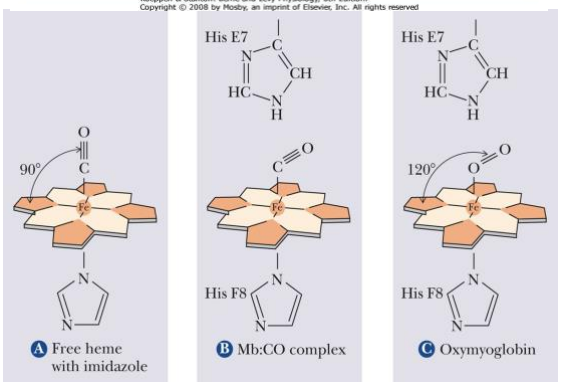
• **In addition to competing with oxygen in binding to hemoglobin, the affinity of Hb-CO towards oxygen increases resulting in less oxygen unloading in peripheral tissues.** Saturation with oxygen when there is carbon monoxide won't reach 100% because of the competition on the binding on hemoglobin.

Carbon monoxide acts as a positive allosteric effector, CO will increase the affinity of hemoglobin to oxygen, and the p50 of oxygen will decrease.

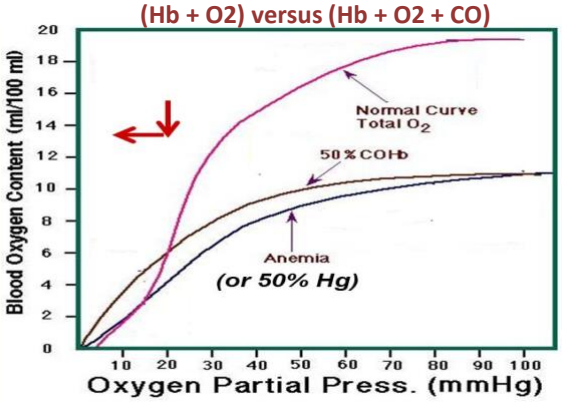
When CO binds to hemoglobin it will change its form from the T to the R state, which makes oxygen binding easier and tighter, and when this hemoglobin (which have CO and O2 bound strongly to the hemoglobin) reach the peripheral tissues, the oxygen won't be released, and it will stay bound to the hemoglobin because it will be in the R state all the time.

(Hb + O2) versus (Hb + CO)





(Hb + O2) versus (Hb + O2 + CO)



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 to 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.**

SUMMARY \(·ᴗ·)/

■ **The electrostatic bonds that stabilize the hemoglobin in the T state and release oxygen, shifts the blot to the right:**

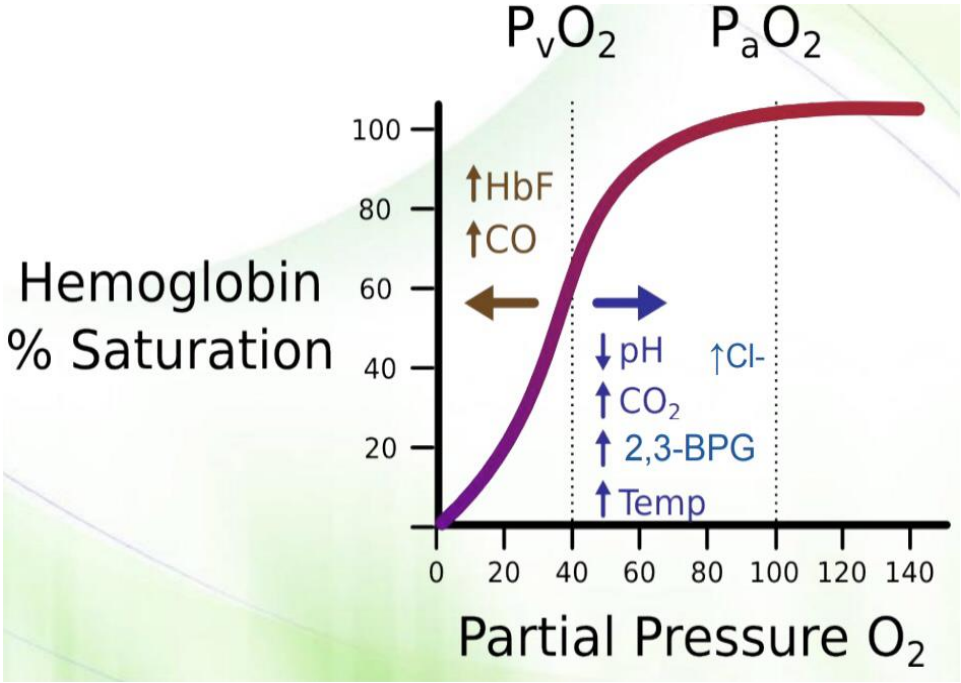
- **His146 binds to lysine of the α chain and forms a salt bridge to Asp94 within the same chain.**
- **CO2 binds to the free α-amino terminal groups to form carbamate.**
- **Chloride ions interact with both the N-terminus of α2 chain and Arg141 of α1 chain.**
- **2,3-BPG forms salt bridges with the terminal amino groups of both β chains and with a lysine and His143, and it increases at high altitudes.**

■ **Factors that shift the oxygen dissociation curve to the right (which indicates that hemoglobin has a decreased affinity for oxygen, oxygen actively unloads):**

1. **Decrease in the pH,**
2. **Increase in the Cl-,**
3. **Increase in the CO2,**
4. **Increase in the 2,3-BPG and living at high altitudes,**
5. **Increase in the temperature.**

■ **Factors that shift the oxygen dissociation curve to the left:**

1. **Increase in the CO,**
2. **Increase in the HbF (fetal hemoglobin).**



PAST PAPERS

1.Which of the following is wrong about HbF (Fetal hemoglobin)?

a. It can bind 8 oxygen atoms.

b. It has similar affinity to myoglobin.

c. It is only found in adults.

d. It has higher affinity than adult hemoglobin.

2. Which of the following increases p50 of the curve of O2 binding to Hb?

a. Decreased temperature.

b. Increased pH.

c. Living in high altitude.

d. Mutation at His146 of β-chain.

3. Fetal hemoglobin (HbF) has low amounts of 2,3-bisphosphoglycerate (2,3-BPG) because:

Fetal pyruvate kinase has high activity.
(Typical ahramic question)

اللهم سلّم غزّة وأهلها من كل سوء وشر، اللهم إنّا نستودعك غزّة وأهلها، وأرضها، وسمائها، رجالها ونساءها وأطفالها يا ربّ العالمين، اللهم احرسهم بعينك التي لا تنام واجعل دائرة السوء على عدوك وعدوهم، اللهم استرعوراتهم وآمن روعاتهم، اللهم انصرهم وثبت أقدامهم وكن لهم ناصرًا ومعينا، اللهم انصر دينك وكتابك وسنَّة نبيّك وعبادك الصالحين، اللهم أنجِ المستضعفين من المؤمنين في كل مكان، اللهم كن لهم عونًا ونصيرا، ومؤيدًا وظهيرا، وحَافظاً وأمينا، اللهم كن معهم، ثبتهم وألحقنا بهم يا ربّنا.

Answers: C, C

V3: in page 5 the words lungs and peripheral tissue were switched and make sure there are like this also in the picture (Lungs have the higher pH)