Sheet No.
2

Biochemistry
hematolymphatic system

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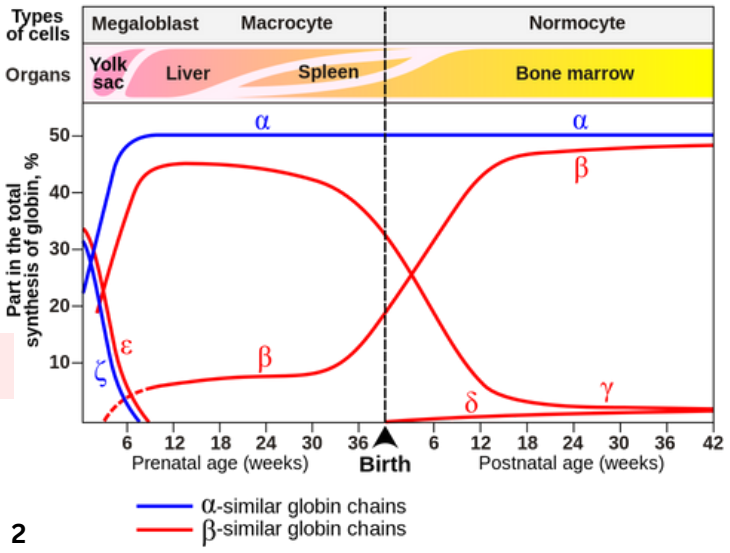
First: It is not only one hemoglobin!

We have **different** types/forms of hemoglobin -we always focus on **adult hemoglobin** (alpha 2 - Beta 2); hemoglobin molecules **do change during the different life stages from the time of fertilization**. (notice the following diagram)

The hemoglobin contains **4 polypeptides**; to form "**dimer of dimer**" each two are similar, the first two could be Alpha or Zeta, the second two could be Epsilon or Gamma or Beta or Delta.

Let's start the story of our hemoglobin from the beginnings!



1. The beginning after fertilization **(embryonic hemoglobin)**

In the yolk sac... for the first 4-6 weeks

At the beginning (after fertilization) and to form the **first 2 polypeptides**, the genetic codes choose **Zeta** polypeptides to be expressed in a **higher amount** than **Alpha polypeptides** - still there is an expression of Alpha polypeptides but in **a lower amount**.

- **Make sure that you understand this curve very will!**
- **Remember:**

ζ,ζ Zeta γ Gama β Beta
α Alpha ε Epsilon δ Delta

For the second 2 polypeptide, genes will choose **Epsilon**; the formed hemoglobins is **Zeta2 Epsilon2 (embryonic hemoglobin HbE Gower 1)** in **higher** amount **and Alpha2 Epsilon2 (HbE Gower 2)** in **lower** amount.

HbE Gower 1

ζ	ε
ζ	ε

Embryonic Hb (HbE G1) in a higher amount, so I drew it in a biger size .

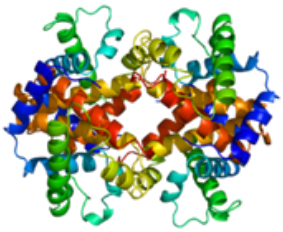
HbE Gower 2

α	ε
α	ε

Embryonic Hb (HbE G2) in a lower amount, so I drew it in a smaller size.

The percentages of expressed polypeptides **are not stable** (remember: they keep changing during the life); the expression of zeta and epsilon will **decrease**; they are almost parallel to each other.

Actually, Gower 1 and Gower 2 are not the only hemoglobins subtypes in the embryonic stage; there are also **HbE Portland 1 (ζ2γ2)**, **HbE Portland 2 (ζ2β2)**.



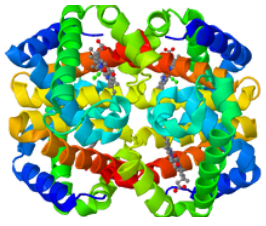
I searched and found that this structure is for HbE G2

To sum up.... Embryonic Hb (respectively) : G1, G2, P1 and P2.

2. Your second stage with **fetal Hemoglobin (HbF)**

By 6-8 weeks of gestation, the expression of **embryonic hemoglobin declines** dramatically and **fetal hemoglobin synthesis starts** from the **liver** and **spleen** will participate for a some extent.

Simultaneously with the decreasing of zeta and epsilon , we have increasing of alpha and gamma to form the fetal hemoglobin (HbF alpha2 gamma2); from this time, the alpha polypeptides remain on throughout life, while gamma starts to decrease just before the **natural** time of birth (the time of birth after 9 months of gestation), why am I clarifying that!? You will know under the genetic topic in this lecture.... And we have at the same time increasing in β expression, so we have also alpha2 beta2 subtype but in lower amount than fetal hemoglobin (we will call alpha2 beta2 adult hemoglobin HbA1 because it is the major hemoglobin in adults) .



At the time of birth (exactly after 9 months of gestation)

-We have 2 subtypes of hemoglobin (HbF **mainly** and HbA1 in **less amount**).
-HbF makes up 60% of the hemoglobin at birth.
-At birth, synthesis of both γ and β chains occurs in the bone marrow.

3. After the time of birth "switching from HbF to HbA"

In the bone marrow…

Gamma globin continues to decrease after the time of birth while beta increases. About the fourth week of age (after a 9 months of gestation and 4 weeks out the uterus), the beta globins will exceed the gamma globins, so HbA1 will be dominant over the HbF.
Also after birth, there is an expression for another gene which is delta globins but in minimal amount (to form $\alpha_2\delta_2$ HbA2).

Developmental transition of hemoglobins

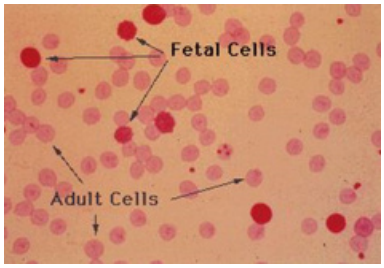
Be careful about globins relative percentages from the previous diagram…. Know which is the major, the minor and which in between like the No. in each square …

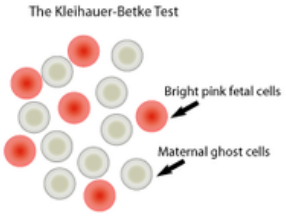
	ζ	α	ε	γ	β	δ
Yolk sac	✔ 2	✔ 3	✔ 1			
Liver		✔ 1		✔ 2	✔ 3	
Spleen		✔ 1		✔ 2	✔ 3	
Bone marrow		✔ 1		✔ 3	✔ 2	✔ 4

Correlate it with the original diagram

	ζ	α
ε	Gower 1 HbE	Gower 2 HbE
γ	Portland 1 HbE	HbF
β	Portland 2 HbE	HbA 1
δ	-	HbA 2

Embryonic hemoglobin | fetal hemoglobin | Adult hemoglobin | No hemoglobin with this formula

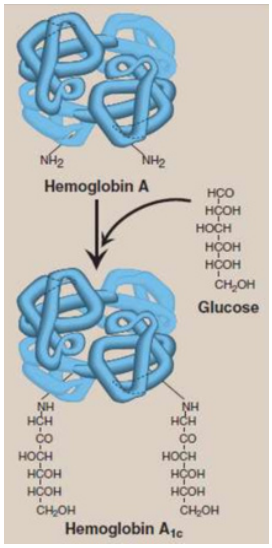




The doctor didn't discuss Kleihauer-Betke test, but it's worth be known by you, here is the link for clarification….

Clinical application of HbA1

- HbA can be glycosylated with a hexose and is designated as HbAc.
- The major form (HbA1) has glucose molecules attached to valines (amino terminus) of Beta chains and become (HbA1c).
- HbA1c is present at higher levels in patients with diabetes mellitus.
- The level or rate of glycosylation indicates the level of sugar in the blood and then the severity of diabetes, the commitment of drugs or to check their effects and effectiveness of the dose, the lifestyle and habits of patients …. to understand this point, you should distinguish the two different sugar tests:



1. **Blood fasting glucose level:**

Is concentration of glucose in your blood at a single point in time, i.e. the very moment of the test, to measure how the body deals with sugar within a short time.

2. **HbA1c فحص السكر التراكمي:**

- **Provides a longer-term trend**, similar to an average, of how high blood sugar levels have been over a period of time (2-3 months) because the glycosylation needs a long period.

- **HbA1c can be expressed as:**
  - a percentage (DCCT unit, used in the US).
  - a value in mmol/mol (IFCC unit), IFCC is new and used in Europe.

- **Limitations:**

It does not capture short-term variations in blood glucose, exposure to hypoglycemia and hyperglycemia, or the impact of blood glucose variations on individuals' quality of life.

good news !!

You should memorize the numbers!

BLOOD GLUCOSE		STATUS	HbA1c	
mmol/L	mg/dL		%	mmol/mol
5.4	97	Normal	5	31
7.0	126		6	42
8.6	155	Pre-Diabetes	7	53
10.2	184 (28 units)	Diabetes	8	64
11.8	212	Diabetes	9	75
13.4	241		10	86
14.9 (1.5 units)	268 (27 units)	Diabetes	11	97
16.5	297		12	108
The difference between the two consecutive values….	1.6 units	29 units	1 units	11 units

The normal blood range was 80-120 mg/dl, but now it is 80-110 (for commercial or scientific reason).
Prediabetic: patient is more susceptible for metabolic syndrome and diabetes, so he needs to manage himself by changing his lifestyle.

Second: Hemoglobin Genetics and Epigenetic Regulation.

This topic will be treated by 3 questions...

Q1: How are globins encoded in the genetic material? The answer is.....

1. By α gene cluster.

On chromosome 16, we have a cluster of genes which encode the two -primary*- globins (Alpha and Zeta); this cluster contains:

1. 2 Alpha encoding genes on each chromosome (alpha 1, Alpha2) so we have a total of 4 Alpha genes, in case of hemogloninopathies that affect one alpha gene, other 3 genes will fix the problem.

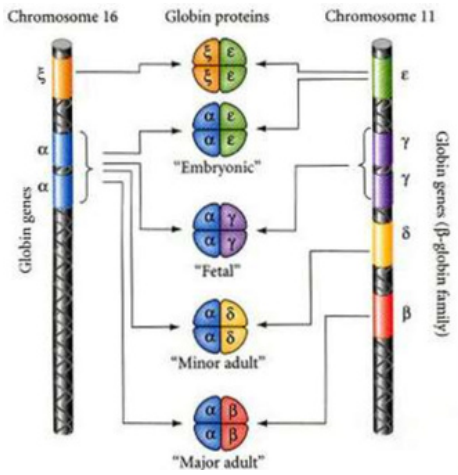
2. one Zeta encoding gene, each gene has a promoter.

2. By β gene cluster.

On chromosome 11, we have a cluster of genes which encodes the -secondary*- globins (Epsilon, Gamma, Beta and Delta); this cluster contains :

1. One encoding gene for Epsilon.
2. Two encoding genes for Gamma.
3. One encoding gene for Beta.
4. One encoding gene for Delta.

Each gene has its own promoter: RNA polymerase binding site, where transcription factors will bind to initiate the transcription, the promoter of Delta is weak! So its percentage is low.



Notice: The genes order on each chromosome parallels order of expression.

Q2: How is the globins' gene expression and switching from a subtype to another regulated and controlled epigenetically? The answer is.....

Transcription Factors.....

Genetic switching is controlled by **transcription factors** - dependent developmental clock, independent of the environment;

"Globins' synthesis is a urgent case that doesn't response to other emergencies even early childbirth."....

What does that mean?!

The globins are different during different life stages. The transcription factors are responsible for the gene expression and controlling of proteins. you must conclude that different globins have different transcription factors that control the expression of each one of them, and the different life stages have their different transcription factors; actually, the different life stages follow something that is called (development clock), the transcription factors are built in response for that development clock (one of transcription factors will be produced until 4-6 first weeks of gestation, it will initiate HbE expression, after that it will be turned off because the baby gets older and other transcription factor must be switched on to allow for the HbF to be expressed) even if the baby is premature neonate, this development clock will run as usual without being interrupted by the birth, if the baby completed his gestational period inside his mother's uterus, he will reach the level of HbA1 dominance after 4 weeks of birth, while if he was born a month earlier, he will reach that level at age of 8 weeks..... (the development clock is very restricted) the development clock starts the timing from the moment of fertilization of ovum by the sperm (the gestational age), this clock doesn't look for the time of birth or any other emergency.

Remember: Premature newborns follow their gestational age, after 9 months of fertilization, 60% HbF and 40% HbA1 ...

(development clock): decides that cardiogenesis should begin at the 3ed week of gestation and the menstual cycle in females should begin at the puberty age, same concept is applied for transcription factors.

كل جلوبين اله transcription factor خاص فيه ... وكل life stage الها جلوبين او اكثر خاص فيها.... اذن كل life stage الها مجموعة transcription factors خاصين فيها
وبس تخلص هاي المرحلة بصير في switch of لهاي ال transcription factors وبتشتغل بدالها transcription factors لجلوبينات المرحلة اللي بعدها..... والمعلومة المهمة
انه مراحلنا العمرية وتطوراتنا الجسمية والنفسية والاجتماعية مرتبطة بساعة بيولوجية دقيقة جدًا تبدأ من مرحلة الإخصاب، وهاي الساعة البيولوجية هي وبس هي اللي بتتحكم بانتاج
ال transcription factors اللي بتتحكم بنوع الهيموجلوبين.... يعني لو انولد البيبي أبكر ب شهر، ساعته البيولوجية ما بتتأثر لأنه العد تبعها ببلش من الإخصاب وعليه انتاج نوعيات
مختلفة من الهيموغلوبين راح يضل بتوقيته الطبيعي وراح يحتاج البيبي مدة أطول خارج رحم والدته لحتى يطور HbA1 & HbA2 لإنه قضى وقت أقل داخل رحم أمه؛ باختصار،
بالطبيعي الحمل ٤٠ اسبوع و تطوير HbA بحتاج كمان ٤ اسابيع بعد الولادة يعني المجموع ٤٤ اسبوع... فلو الطفل انولد بالاسبوع ٣٦ هو بحتاج ٨ اسابيع بعد الولادة عشان يضل
المجموع ٤٤ اسبوع ليبدأ الجسم بانتاج ال transcription factors المسؤولة عن ال HbA (وهاي هي الساعة البيولوجية المسؤولة عن تطورنا).....

Q3: How do the transcription factors actually regulate the switching from a subtype to another ? The answer is.....

By epigenetic regulation (e.g. acetylation, methylation), modifying histones and chromatin looping.

There is something smart here

Transcription factors will exert there effects mainly by 2 mechanisms :

1. The general transcription factors will bind to the promoter region for the desired gene, and this will initiate the gene transcription.
2. There are special transcription factors each one is responsible for the expression of a certain globin, these transcription factors will exert there effect by binding to 2 enhancers:

- The enhancer of Alpha globin cluster is called **HS40 region** (It controls Alpha cluster).
- The enhancer of beta globin cluster is called **locus control region (LCR)**; it's a master enhancer that controls beta cluster.

You should revise some concepts:

promoter: a sequence of DNA to which RNA polymerase binds to build up m-RNA molecules.

Silencers: regulatory DNA elements that reduce transcription from their target promoters.

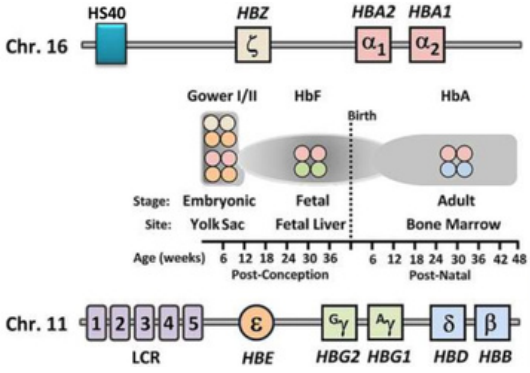
Enhancers: DNA-regulatory elements that activate transcription of a gene or genes to higher levels than would be the case in their absence.

Activator: is a protein (transcription factor) that increases transcription of a gene or set of genes.

DNA looping: is a mechanism in which the DNA bends to form a loop so two different DNA regions will meet each other, and this will affect metabolism, DNA replication, DNA transcription, etc.

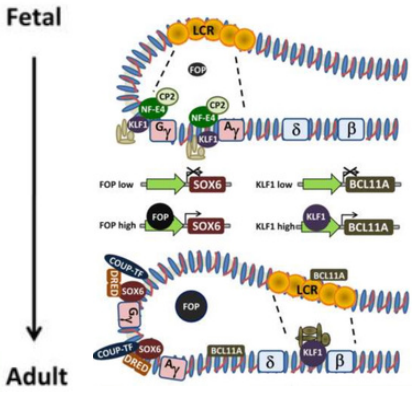
These enhancers with their corresponding transcription factors exert their effect by a special mechanism.... **DNA looping!!!**

When the transcription factors bind to the enhancers, the DNA structure will loop, so the transcription factors on the enhancer will react with the transcription factors on the promoter, and that will initiate the expression of epsilon globin for example.
During development the transcription factors of epsilon on LCR enhancer will decrease, and the transcription factors of Gamma will increase, these transcription factors will bind to the same enhancer, DNA will loop, but the transcription factors on that enhancer will meet the transcription factors on other promoter, which is in this case (Gamma promoter).
Remember: the producing of transcription factor is somehow timed....

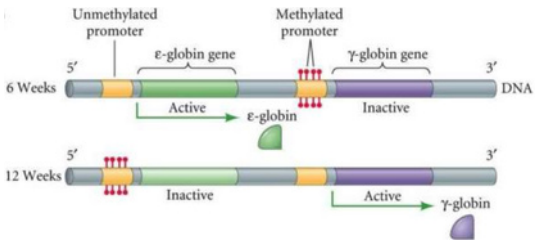


This is how switching takes place....

DNA Looping!



Epigenetic modifications



Regarding epigenetic regulation, the scientists try 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 the scientists try to induce the expression of gamma globin instead of beta. Here is a recently published research, that comes with a finding, there is a factor that induces the expression of Gamma globin, so we can use this factor to compensate the loss of beta globin.

Activation of γ-globin expression by hypoxia-inducible factor 1α

Ruopeng Feng, Thivagarai Mavuranathan, Peng Huang, Phillip A. Doerfler, Yichao Li, Yu Yao, Jingjing Zhang, Lance E. Palmer, Kalin Mayberry, Georgios E. Christakopoulos, Peng Xu, Chunliang Li, Yong Cheng, Gerd A. Blobel, M. Celeste Simon & Mitchell J. Weiss

Abstract

Around birth, globin expression in human red blood cells (RBCs) shifts from γ-globin to β-globin, which results in fetal haemoglobin (HbF, $\alpha_2\gamma_2$) being gradually replaced by adult haemoglobin (HbA, $\alpha_2\beta_2$)[1]. This process has motivated the development of innovative approaches to treat sickle cell disease and β-thalassaemia by increasing HbF levels in postnatal RBCs[2]. Here we provide therapeutically relevant insights into globin gene switching obtained through a CRISPR–Cas9 screen for ubiquitin–proteasome components that regulate HbF expression. In RBC precursors, depletion of the von Hippel–Lindau (VHL) E3 ubiquitin ligase stabilized its ubiquitination target, hypoxia-inducible factor 1α (HIF1α)[3,4], to induce γ-globin gene transcription. Mechanistically, HIF1α–HIF1β heterodimers bound cognate DNA elements in *BGLT3*, a long noncoding RNA gene located 2.7 kb downstream of the tandem γ-globin genes *HBG1* and *HBG2*. This was followed by the recruitment of transcriptional activators, chromatin opening and increased long-range interactions between the γ-globin genes and their upstream enhancer. Similar induction of HbF occurred with hypoxia or with inhibition of prolyl hydroxylase domain enzymes that target HIF1α for ubiquitination by the VHL E3 ubiquitin ligase. Our findings link globin gene regulation with canonical hypoxia adaptation, provide a mechanism for HbF induction during stress erythropoiesis and suggest a new therapeutic approach for β-haemoglobinopathies.

Third: The regulation of hemoglobin function.

- Ligands or small molecules that induce conformational changes in allosteric proteins and so change the affinity of them, are referred to as :
  modulators, modifiers, effectors or regulators.

- The switching between R-state and T-state depends on these modulators.

- 2 types of modulators:
  1. Homotropic modulators are identical to the ligand that the protein is committed to bind with (like oxygen).
  2. Heterotropic modulator are different from the normal ligand (like CO).

- **Modulators may be inhibitors or activators.**

- Previously, we talked about **Oxygen** as **homotropic** effector.
  Now, we will talk about heterotropic effectors:

The major **heterotropic allosteric effectors** of hemoglobin:

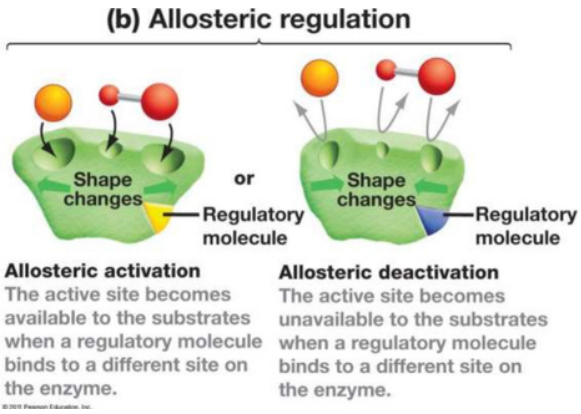
- Hydrogen ion
- Carbon dioxide
- 2,3-Bisphosphoglycerate
- Chloride ions
- A competitive inhibitor,Carbon Monoxide

Let's start discussing each one of them !

You should revise some concepts:

Allosteric enzymes: enzymes that can switch their structure between two states, one is very active, and the other is less active but still functioning .

Allosteric proteins: proteins that can switch their structure between two states, one with high affinity (R-state), and the other with less affinity (T-state).



1. The effect of pH (Hydrogen ion)

REMEMBER: pH is an indication of hydrogen ion concentration.

1. **The effect** of increasing hydrogen ion concentration (low pH) is..... Decreasing the affinity (shifting the state towards T-state Releasing the oxygen from Hb.

2. When you are exercising, your tissues produce protons, decreased pH will decrease the affinity of Hb. **The mechanism or phenomena** by which the pH exerts its effect is called **(Bohr effect)**.

⬆ [H ions] .... ⬇ pH .... ⬇ Affinity .... ⬆ Released oxygen

Before, diving in depth of Bohr effect, I should introduce the pKa concept for you...

- When you ask someone to give you the pKa value of a weak acid or weak base, the answer will be easily a pH value; so **pKa is a special pH value**.
- What special about this pH value that makes it pKa, is..... When this pH value is achieved, then **50% of this weak acid will be converted into deprotonated state (conjugated base)** (50% charged 50% uncharged) and the other 50% will stay at the weak acid state; same concept for the weak base and its conjugated acid).
- Each weak acid or weak base has its own pKa value that can't be change for it although the pH of the medium could be change; **pKa of the compound is a constant, pH of the medium is a variable.**
- Because some amino acids have a charged and polar R chain, they have some degree of acidity (usd to describe acidic and basic nature), and then they have their own pKa.
- If pH of the medium = pKa of the R chain of an acidic amino acid, then 50% of this amino acid will be deprotonated and converted into the conjugated base, while the remain 50% will stay at the acidic form.
- When the the surrounding pH is higher than the compound pKa, then **the equilibrium will be shifted towards the deprotonated form**, so more then 50% of the compound will loss its proton; so, in case we use a buffer that makes the pH of the medium stable then the lower the pKa, the easier to lose the proton. (Low pKa indicates that the weak acid is relatively strong, you remember from Altawjihi that strong acids give their protons easier).
- When the surrounding pH is lower than the compound pKa, the equilibrium will be shifted towards the protonated state, more than 50% of the strong acid will keep its proton ; inside a buffer, the higher the pKa the more difficult to lose the proton.
- To sum up, if the pKa of the compound is lower than the pH of the medium, that indicates that the compound is more acidic than the surrounding environment, so the compound will lose its protons to balance the difference of acidity with the medium, same thing will happen if the pH of the surrounding is lower than the pKa (the medium is more acidic than the compound), so the medium will give the protons for the compound to balance the difference between the medium and the compound (will favor the protonated state).
- you know that the protonated form represents the acidic form 😁

Bohr effect:

When the muscles exercise, they produce a lot of protons as a result of metabolism , then the pH in tissues will decrease (there is a lot of protons there), the pH will be lower than the pKa of some amino acids in the Hb …. So these amino acids will be shifted towards the protonated form, this protonated form favores the T-state, so the affinity of Hb towards oxygen will decrease and that will release the oxygen to the tissues and muscles (actually, the muscles need oxygen right now because they exercise).

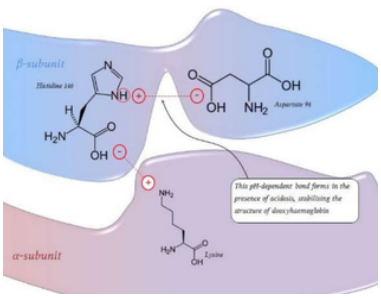
The binding of H+ to hemoglobin converts the Hb from R-state to the T-state and so lowers the affinity which promotes the release of O2 from hemoglobin and vice versa.

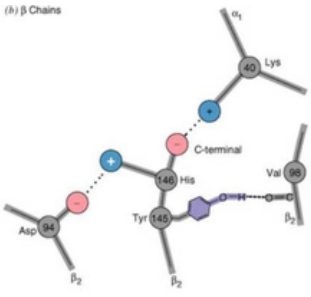
3. **The biochemistry** behind Bohr effect… how the protonation of amino acids decreases the affinity?

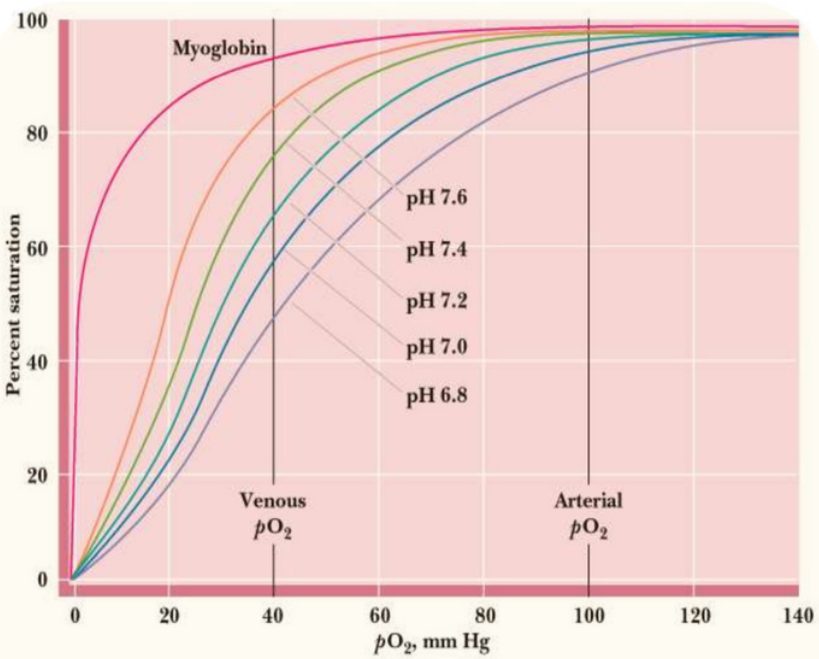
The protonation of some amino acids will enhance some interactions inside the structure of Hb, these interactions and bonds will stabilize the T-state and shifting the Hb structure from R-state to T-state, thus lowering the affinity and releasing the attached oxygen to the tissues.

Some examples of interactions that will take place when the structure is protonated…

- The beta chains have histidine (146) at the end, this histidine has a titrable R-group (it will be positively charged when it is protonated).
- Because histidine is the C-terminus, it has also a caboxylic group that is negatively charged (it has different pKa than the R-group, the c-terminus is mostly deprotonated (negative charge) because of its very low pKa compared with the physiological surrounding).
- The negatively charged carboxylic group interacts **electrostatically** to the R group of lysine on the alpha subunit (which has been positively charged - it is protonated because the pH now is lower than the lysine R-group pKa).
- The R-group of histidine is also protonated bacause of the low pH compared with its pKa, so it now has a positive charge that will build a **salt bridge** with the negatively charged carboxylic part of the R-group of aspartate (94) amino acid on the same beta globin chain (aspartate has 2 carboxylic groups: one is the c terminus and the other in the R-group).
- These interactions will stabilize the T-state and shift the strucuter from R-state to T-state.





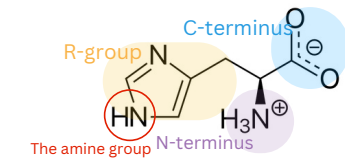


How can we conclude from the curve that the protonation will lower the affinity?

You should look to the diagram twice!!!
The first one: look to the saturation 50% point , choose a curve for a specific pH (like 7), the oxygen pressure that is needed to saturate 50% of Hb is about 37 mm Hg.
The second one: at the same 50% saturation point, choose another curve with a different pH value (like 6.8), the oxygen pressure is about 42 mm Hg.
At the same saturation point, the oxygen pressure that is needed to saturate 50% of Hb will increase by decreasing the pH, so lowering the pH will decrease the affinity because you need much more oxygen to reach 50% saturation point and the curve will shift to the right.

Something special about histidine

- The histidine amino acid (outside the polypeptide chain) has a pKa value 6.4 for the R-group.
- The histidine residue (inside the polypeptide chain for any protein other than Hb) has a pKa value 6 for the R-group.



The structure of histidine (the R-group is in the deprotonated state, remember the amine group, is a basic group, the deprotonated state of basics is uncharged)

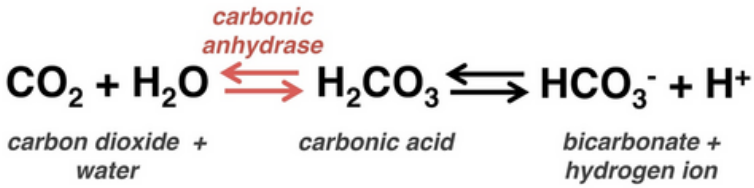
- The histidine inside Hb has 2 different pKa values:

1. inside the T-state it has a value 7.7, which is relatively high, so the majority of Hb in low pH will be protonated to stabilize the T-state.
2. inside the R-state it has pKa value 7.3, it is much lower than T-state, and the R-state will be unstabilized in low pH because much more molecules will be protonated (protonation doesn't favor R-state) (R loses its protons easier than T).

- The stabilizing interactions of R-state (which depend on the deprotonated state) will be broken when the pH goes down in the tissues, and the deprotonated amino acids inside R-state will be protonated, and so the stabilizing interactions of T-state will be built (meaning that this will favor the deoxygenated T form of hemoglobin), and the shifting to the right (shifting from R to T) will eventually happen. If you want to back for the R-state, you should break down the interactions of T-state.

Protons' sources

- CO2 and H+ are produced at high levels in metabolically active tissues by carbonic anhydrase.
- This is accompanied by generation of H+, facilitating the release of O2.



- In the lungs, the reverse effect occurs and high levels of O2 cause the release of CO2 from hemoglobin.

2. The effect of Carbon dioxide (CO2)

Q1: How can we produce carbon dioxide?

By the different **metabolic reactions**....

1. The conversion of one pyruvate molecule to one Acetyl CoA produces 1 CO2.
2. Inside Kreb's cycle we produce 2 CO2.

Q2: What is the fate of this carbon dioxide?

1. It will diffuse from the cells to the blood.
2. inside the RBCs it will be convarted to carbonic acid by carbonic anhydrase enzyme.
3. Carbonic acid will disassociate to bicarbonate io HCO3- and **proton H+**.
4. Carbon dioxide is transported in the blood from the tissue to the lungs in three ways:1 (i) dissolved in solution; (ii) buffered with water as carbonic acid; (iii) bound to proteins, particularly haemoglobin.

Q3: How does this carbon dioxide exert its effect?

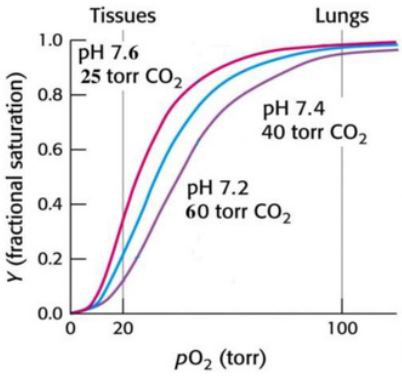
By 2 mechanisms:

1. Production of H+.
2. Production of carbamate.

1. Production of H+.

- As we said previously when the carbon dioxide enters the RBC, it will be converted into carbonic acid which disassociate to for bicarbonate ion and proton.

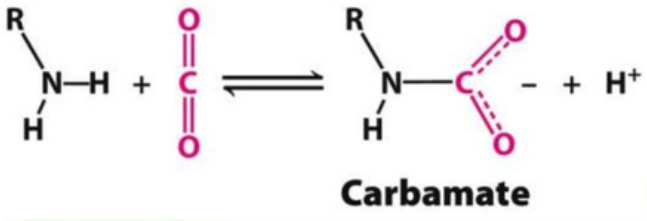
- By increasing the pH of the medium, the affinity of Hb toward oxygen will decrease (Bohr effect).
- This mechanism will decrease the affinity indirectly by (Bohr effect).



Notice: when we increase the pressure of CO2, the pH will decrease, and the curve will be shifted to the right.

2. Production of carbamate ion.

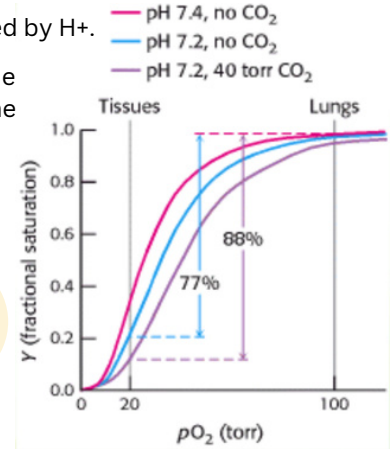
- Hemoglobin transports some CO2 directly.
- When the CO2 concentration is high, it combines with the free alpha-amino terminal (N-terminal) groups (which is positive) to form carbamate which is negatively-charged group.
- The increased number of negatively-charged residues increases the number of electrostatic interactions that stabilize the T-state of hemoglobin.



Q4: which one of these two mechanisms has the major effect?

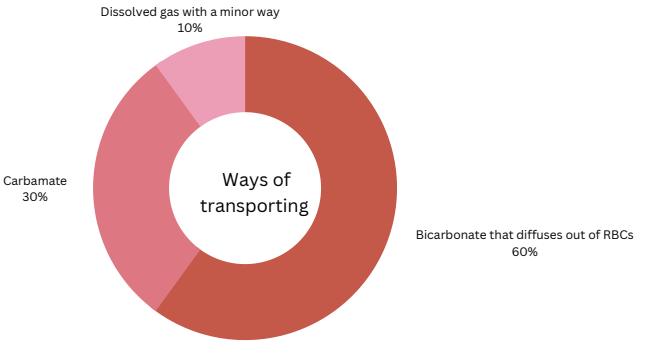
To examine the effect of each mechanism, we use three curves, as shown in the diagram:

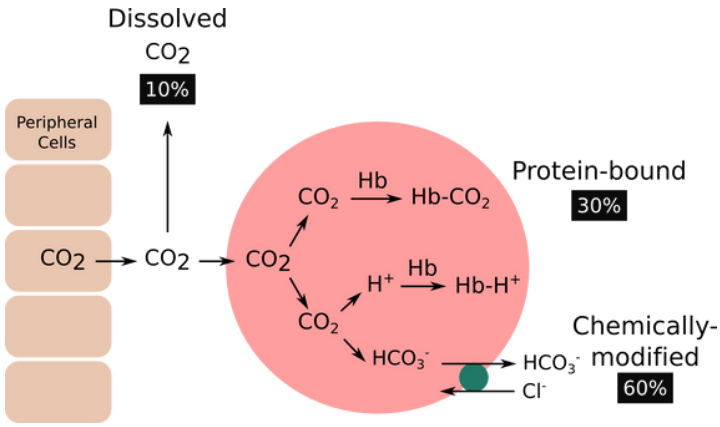
- About 75% of the shift is caused by H+.
- About 25% of the effect is due to the formation of the carbamino compounds.
- How do we know about the carbamate mechanism ?



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

Transport of CO2 into lungs





- The movement of CO2 in/out of cells during metabolism does not change the pH dramatically, a phenomenon called isohydric shift, which is **partially** a result of **hemoglobin being an effective buffer**.

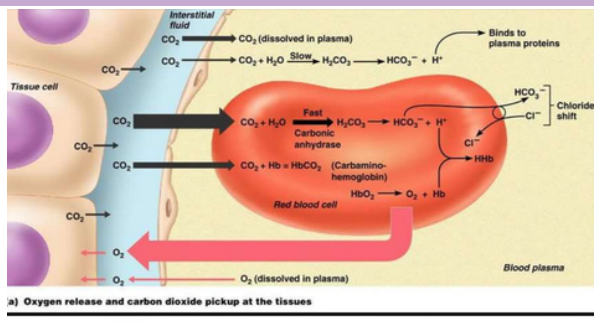
- There are three physiological buffers in the body:

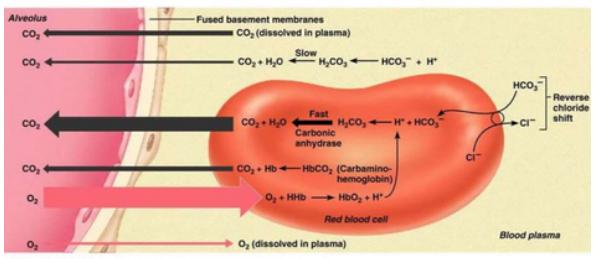
(1) Proteins buffer (by exchanging the protons between the environment and the amino acids, so they can stabilize the pH), like Hb.
(2) Phosphate buffer (inside the cells).
(3) Bicarbonate buffer (in the plasma).

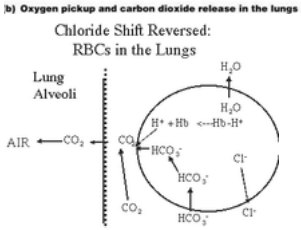
- Because of the huge amount of Hb, it is considered a major buffer, so the CO2 movement can't change the pH, and that's called (**isohydric shift**); there is a shift of CO2 but the pH doesn't change.

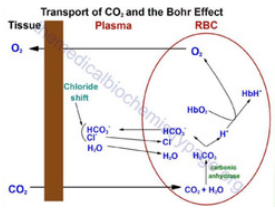
3. The effect of Chloride ion

- You remember that a lot of carbon dioxide is produced from the metabolism, and the pathway is that when CO2 diffuses from the tissues to the RBCs, they will be converted into negatively charged bicarbonate ion (HCO3-) in a large amount.
- This large amount of bicarbonate will leave the RBCs, and will cause a huge loss of negative charge inside the RBCs.
- To compensate this negative charge loss, chloride ions will enter the RBCs.
- This phenomena is called, **Chloride ion shift.**





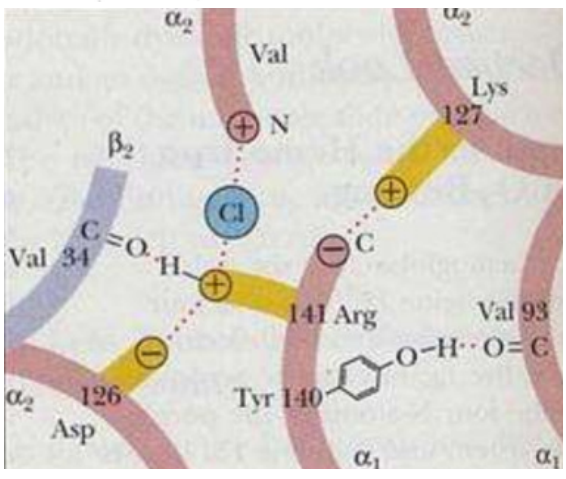




- In the **arterial blood (alveoli)**, Cl- diffuses out of the RBCs, and **bicarbonate enters RBCs** again to compine with H+ to form carbonic acid that disassociate to water and CO2, CO2 will diffuse to the lung to be exhaled out.

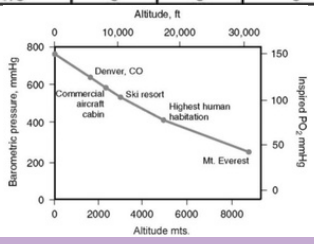
Can the chloride decrease the affinity of Hb?

- **Yes**, it decreases the affinity by affecting the electrostatic interactions, when the venous blood reachs the tissue, the chloride ions shift will happen.
- Chloride ions will build some electrostatic interactions that stabilize the T-state; so the affinity will decrease and oxygen will be discharged to the tissues which need oxygen right now; Increasing the concentration of chloride ions (Cl-) shifts the oxygen dissociation curve to the right (lower affinity).
- Chloride ions interact with N- terminus (val1) of α2 chain and (Arg141) of α1 chain.



Oxygen at high altitude

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

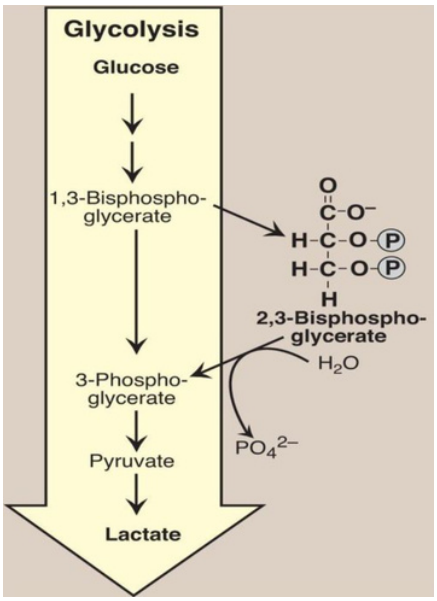


- Respiration is difficult at high altitude, because of low atmospheric pressure of oxygen,
- the body needs 2-3 days to adapt (histology said one week), because:

1. the body needs a time to build up more RBCs.
2. The major role is for 2,3-bisphosphoglycerate:

4. The effect of 2,3-bisphosphoglycerate

Q1: How to produce 2,3 BPG?



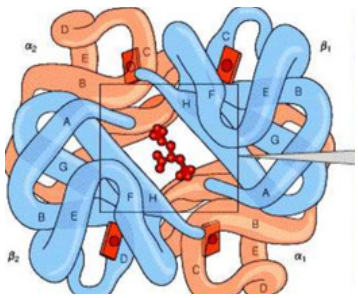
- By the glycolysis metabolic pathway is the only metabolic pathway in RBCs.
- when the glycolysis is turned on, there is a production of 1,3-bisphosphoglycerate from its precursor (glyceraldehyde-3-p).
- 1,3-BPG will be converted into 3-phosphoglycerate, and this will produce one ATP molecule.
- 1,3-BPG could be isomerized to form 2,3-BPG.
- The structure of 2,3-BPG contains 2 negatively charged phosphate groups and one negatively charged carboxylic group, it is highly negative.

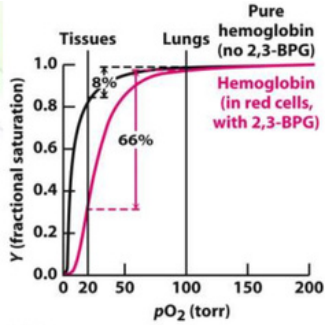
Q2: How does 2,3 BPG exert its effect?

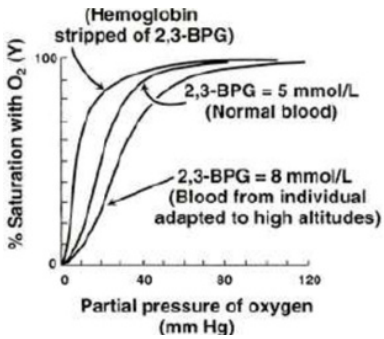
- BPG (a major heterotropic effector) binds to hemoglobin (specifically T-state) and reduces its affinity towards oxygen by stabilizing T-state (through some reversible interactions) and preventing it to be converted into R-state again because this binding increases the energy needed to transform hemoglobin from the T state to R state.
- BPG binds in the central cavity of deoxyhemoglobin only in a ratio of (1 : 1) BPG/hemoglobin tetramer.
- So, bound 2,3-BPG reduces binding of oxygen to hemoglobin and facilitates oxygen release.
- The biochemistry behind that; 2,3-BPG forms salt bridges with the terminal amino groups of both β chains and with a lysine and His143; a lot of negative charges in the 2,3-BPG will make interactions with positive amino acids.

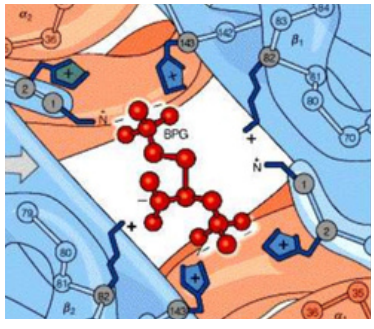
Q3: How does 2,3 BPG exert its effect regarding the binding of oxygen?

- In the presence of 2,3-BPG, the p50 of oxyhemoglobin is 26 torr (normal conc.of 2,3-BPG).
- 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 (the second factor in addition to increasing RBCs synthesis).









Q4: Does 2,3-BPG affect the binding of oxygen forever?

- The answer is, No. Because the binding of 2,3-BPG is reversible (not covalent).
- But !!!! It will decrease the binding of oxygen in alveoli for a certain time, mmmmm!

You may ask yourself now, if the body produces 2,3-BPG in a higher rate to adapt the low atmospheric pressure of oxygen and to supply the tissues with more oxygen; then why 2,3-BPG decreases the saturation level in the alveoli which will eventually decrease the level of oxygen-bound Hb?

The answer is: at the same time that hemoglobin has a decreased saturated level in the lunge, it will has a very high disassociation ability in the tissues, so it can release a very high amount of oxygen even if it doesn't have a lot of them.

I want to make the situation more obvious for you by this image:
If you have an emergency and you need money, you think to borrow some money from a friend,
If you asked a miserly rich friend, you will never believe that he might give you enough money; in contrast to your trustful generous friend, who have a little money, but he still can give you much more than your miserly friend.
you are the tissues, the generous friend is the Hb with 2,3-BPG, and the miserly friend is the Hb without 2,3-BPG.

The scientific translation for this story:
At sea level the lungs pick up oxygen with 100% saturation of Hb, and when the oxygen pressure drops to 40 mm Hg in the tissues the Hb will be 55% saturated in these tissues..
So the Hb has released 45% of oxygen.

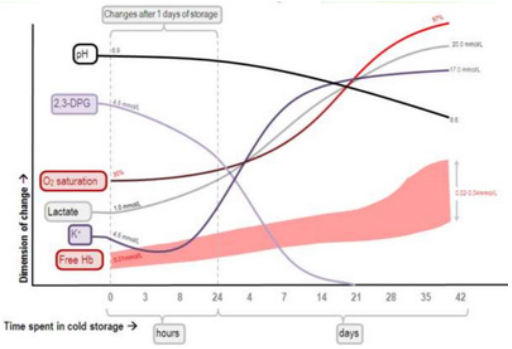
At high altitude (in case of no adaptation), Hb is only 80% saturated. Thus at 40 mm Hg in the tissues when Hb is only 55% saturated it will only have released 25% of its oxygen.

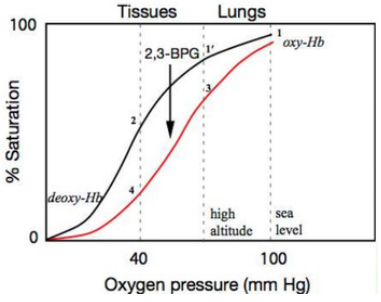
At high altitude (with increased 2,3-BPG production- in red), At the lunges, the Hb will be less charged with oxygen (only 70% saturation) but at 40mm Hg in the tissues, it will be much less saturated than on the black curve — 30%. Thus, it will have made available 40% of its oxygen.

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 (2,3-BPG will be destroyed in the transfused blood).
- Transfused RBCs are able to restore their depleted supplies of 2,3-BPG in 6– 24 hours.
- Severely ill patients may be compromised, so both 2,3-PBG and ATP are rejuvenated right before the transfusion to help them restore the homeostasis.
- Patients with stable situation can adapt without adding ATP or 2,3-BPG

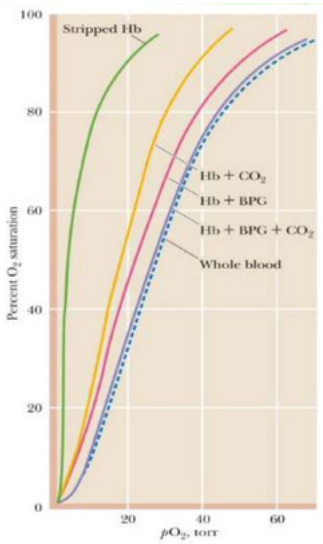




2,3-BPG and CO2 are important players

Look to the nice colorful diagram :

- The first (green curve) represents the Hb without CO2 and 2,3-BPG, it looks like myoglobin with a very high affinity (that isn't the normal case).
- The second yellow curve represents the Hb with CO2, the curve is shifted to the right.
- Ghe third red curve reported the Hb with 2,3-BPG, is shifted to right slightly more than the previous situation.
- The purple fourth cure is too much close to the normal physiological situation and it overlaps with the normal curve, it represents (Hb + CO2 + 2,3-BPG).
- Generally, we can't say that one effector is more important than the others.



5. The effect of temperature

When you are exercisin, your temperature is normal, but if you measure the muscles temperatur, or will be a little bit higher (around 38.5), because of the heat that result from the metabolic reactions.
The heat decreases the affinity by two mechanisms:

1. Increased temperature increases the metabolic rate of RBCs, increasing the production of **2,3-BPG**, which also facilitates oxygen unloading from HbO2.
2. The temperature shifts Hb structure towards T-state (increase in temperature decreases oxygen affinity and therefore increases the P50), more oxygen will be discharged to meet the demand of muscles.

The heat will break down some non-covalent interactions theoreticall, but actually we don't know which typeof interactions, and the observational results shows just a decreased affinity