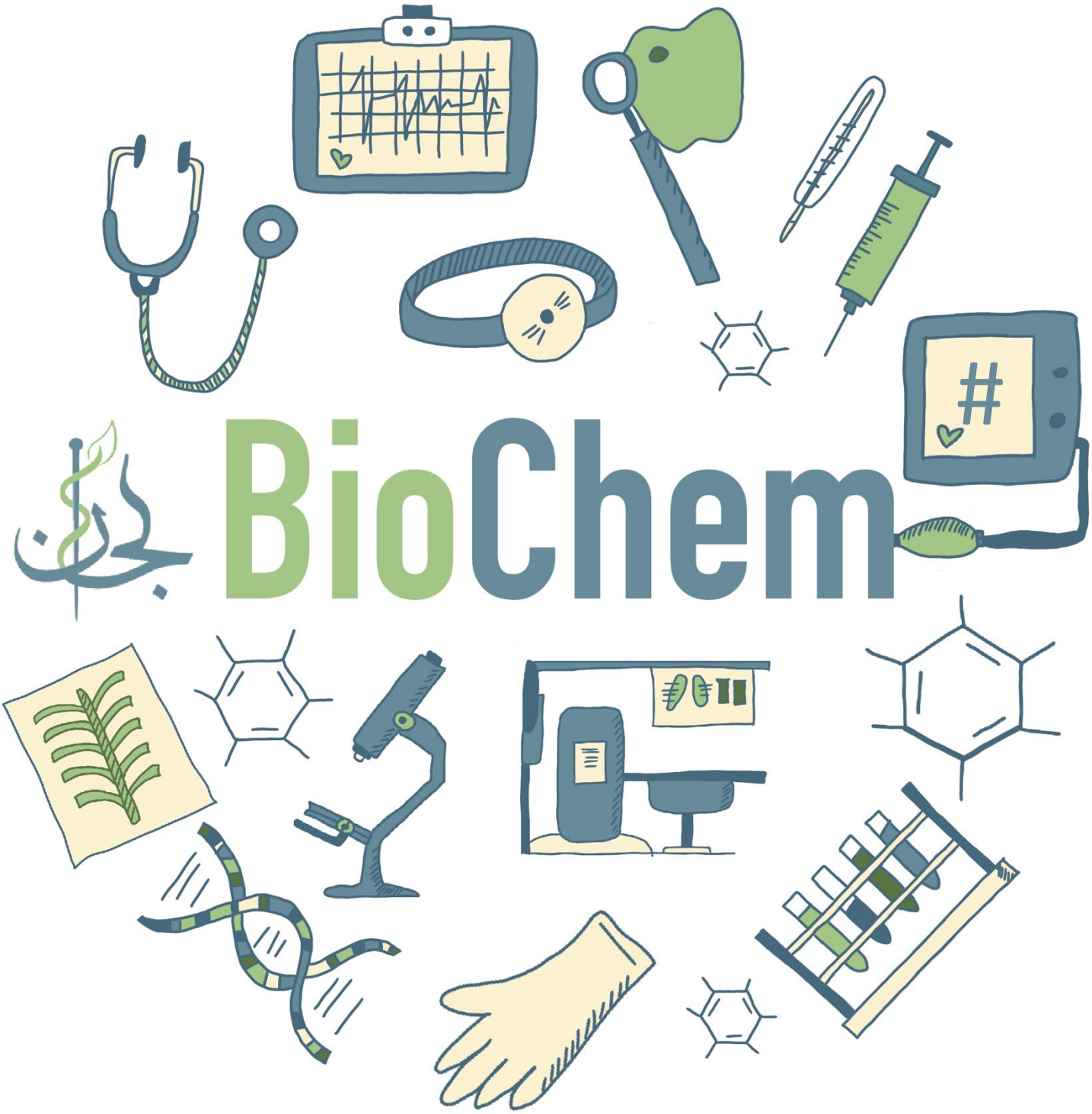

BioChem

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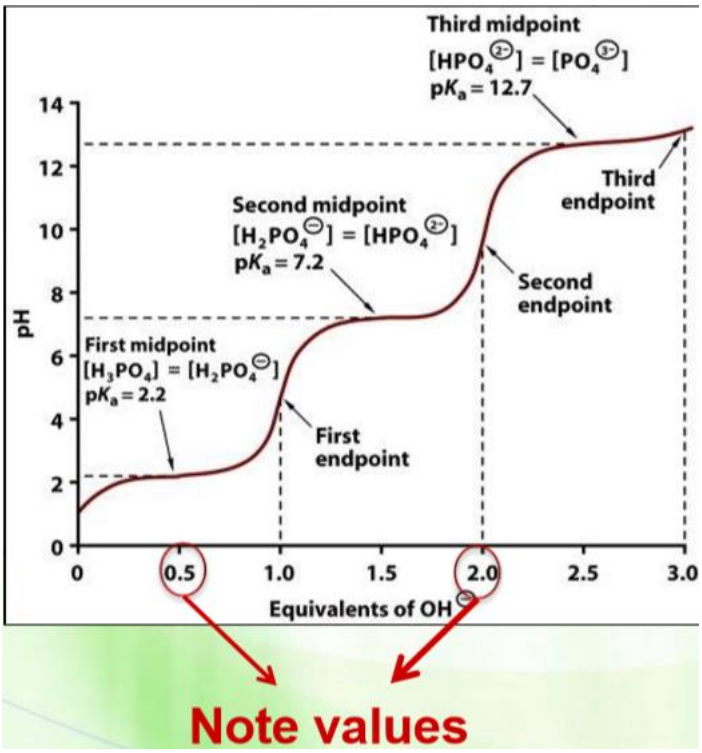
In the previous sheet, we discussed the titration of monoprotic acids, specifically the acetic acid. Now, we will talk about polyprotic acids specifically the H_3PO_4 (phosphoric acid) which will be titrated on several steps.

look at this figure to understand these points,



*Notice that we have 3 curves for 3 steps of titration, each has a product going to the next step as a reactant

*H_3PO_4 is titrated by a base at the beginning, it gives a proton and becomes $H_2PO_4^-$ which will give another proton and become HPO_4^{-2} which gives the last proton and becomes PO_4^{-3}

*Remember the properties of the curve like being fast at the beginning, slow in the middle and fast in the end, having an inflection point in the buffering region and equivalence point in the end.

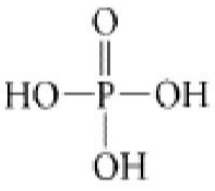
*We need equivalents of OH^- to titrate the acid at each step to reach the equivalence point.

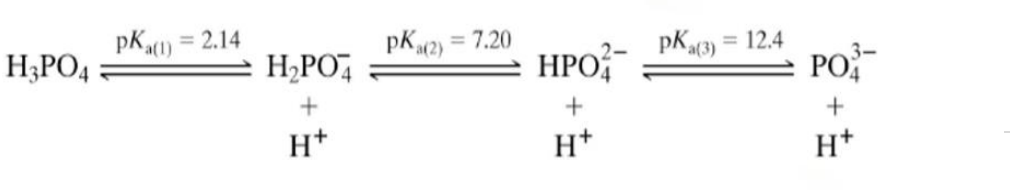
*From this figure, we can tell the pH for these molecules, for example, the pH for H_3PO_4 in the first step is 2.2 as well as the pKa, because the pH is equal to the pKa in the inflection point.

*Notice that the range of the 1st step is narrower than the 2nd narrower than the 3rd, which reminds us of the inverse relation between pH or pKa and the strength of the acid (pH decreases → acid strengthens) ($H_3PO_4 > H_2PO_4^- > PO_4^{-3}$)

*At the end of the first step, we do not have H_3PO_4 anymore, only $H_2PO_4^-$ is there, by the end of the second step we only have HPO_4^{-2}, finally, at the end of the last (third) step we only have PO_4^{-3}.

*Take a look at the formula below



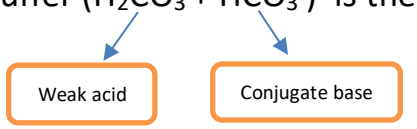


Buffers in the human body

Bicarbonate buffer (carbonic acid- bicarbonate system)

Inhaling air → O_2 enters the lungs → goes to vessels surround the bronchioles and binds hemoglobin → reaches capillary where gas exchange occurs → is absorbed by cells during metabolism (combustion reactions) to generate energy → CO_2 is released and binds hemoglobin → reaches the lungs → binds water forming carbonic acid H_2CO_3 by the enzyme carbonic anhydrase in the red blood cells → H_2CO_3 gives its proton as an acid to become HCO_3^- which does not give its proton due to environmental circumstances.

*This biological buffer (H_2CO_3 + HCO_3^-) is the main one maintaining the pH of the blood around 7.4



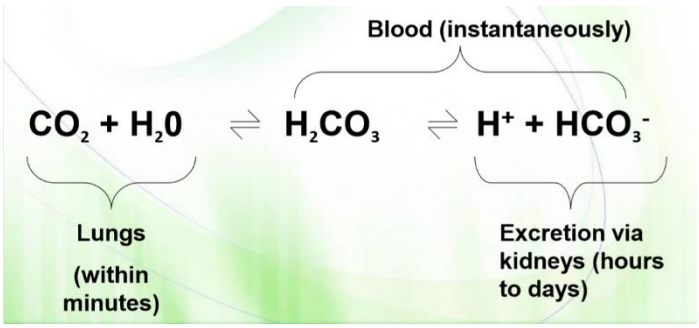
*How does this buffer work? Well, when the blood becomes acidic and H^+ increases abnormally, the respiratory rate increases, thus it gets rid of CO_2 and decreases formation of H_2CO_3 as well as the H^+

However, when the blood becomes basic and H^+ decreases abnormally, the respiratory rate decreases, thus increases the formation of H_2CO_3 as well as the H^+

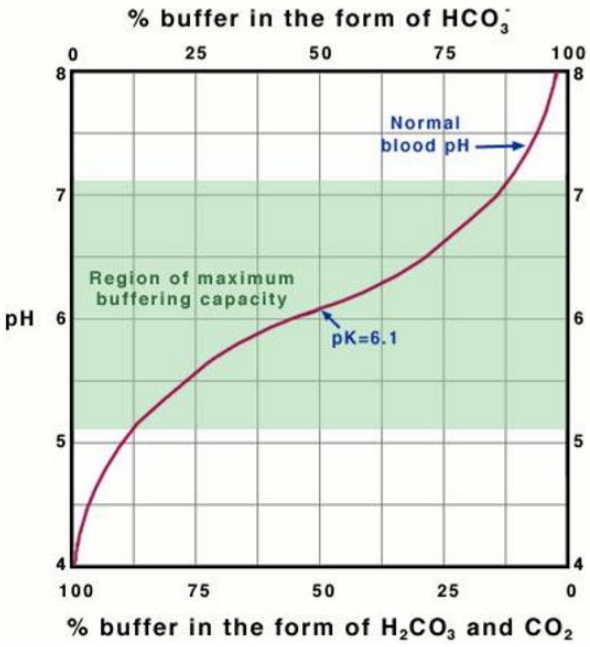
(Keep in your mind that wherever we have CO_2 we have H^+, that makes the relations above a bit easier)

*The renal system is also involved in regulating the pH through reabsorbing HCO_3^-, so it increases or decreases the rate of absorption according to the change in H^+ concentration (CO_2 conc. as well).

So there are 2 mechanisms involved in regulating blood pH (respiratory and renal systems) but they are different in speed, as the respiratory is fast (minutes) and renal is slow (hours to days).



Notice that the pKa of the bicarbonate buffer is 6.1 so the capacity ranges between (5.1-7.1) and we know that the blood pH is 7.4 (outside the buffering capacity). How does this occur? How does the bicarbonate buffer resist changes in the blood pH that is outside its capacity?!



At first, you have to know that the biological buffers slightly differ than the chemical buffers, and the body is an open system (continually interacts and exchanges with the environment), so it can control the blood pH through changing the rate of breathing unconsciously.

لو محلول منظم كيميائي بره الجسم بكون نظام مغلق كتير محدد ودقيق، ما بتفاعل مع إشي حواليه، لكن هون بالجسم، في جهاز تنفسي بحل مشكلة تغير تركيز مادة معينة زي الهيدروجين أو البايكربونات عن طريق زيادة الشهيق أو تقليله، وفي جهاز بولي بحل مشكلة تغير تركيز ثاني أكسيد الكربون عن طريق زيادة إعادة امتصاص البايكربونات من البول أو تقليلها، ف ما في مشكلة لو كان بي إتش الدم بره البفرنج ريجن تبعت البفر(هاد الكلام رح يتوضح قدام)

Secondly, Bicarbonate (HCO_3^-) is present in a relatively high concentration in the ECF(24mmol/L) (can be controlled easily), we need a large amount of bicarbonate because it does most of the buffer's job. (By Henderson-Hasselbalch Equation, we know the pKa and pH, so we could know the ratio (HCO_3^-:H_2CO_3) or (HCO_3^-:CO_2) because the carbonic acid conc. is equal to the CO_2 conc. as it dissociates releasing it.
((High conc. of the buffer → more efficient))

Thirdly, the components of the buffer system are effectively under physiological control: the CO_2 by the lungs, and the bicarbonate by the kidneys

*Know that increasing the H^+ in the blood results in its interaction with the basic component of the buffer (HCO_3^-) forming H_2CO_3 which dissociates into water and CO_2 which is exhaled quickly to keep it away from cells, by doing this we prevent forming the acid and getting back to the problem again. On the other hand, increasing OH^- and relatively decreasing H^+ in the blood results in the interaction between OH^- and H_2CO_3 (the acidic component of the buffer), forming water and salt, by doing this, we got rid of the high OH^- but unfortunately we consumed the acid (H_2CO_3) too!!! That is why the respiration rate decreases, so we could have enough CO_2 in the red blood cells reacting with water and producing the acid, that's how we compensate the lost H_2CO_3.

Let us get into medicine now…

Acidosis and alkalosis (pathological conditions)

Acidosis is the condition when the blood pH is less than 7.35 while alkalosis when it is more than 7.45

*Both of them are classified into metabolic and respiratory

*Metabolic acidosis is caused by:

Starvation: when you eat a meal, the glucose conc. in the blood increases → insulin is secreted to bind cells and get the sugar into them → excess sugar is stored in the liver as glycogen → decreasing glucose conc. in the blood → after a while, all the sugar is consumed and you will feel hungry → your body starts breaking down glycogen (almost first 10-18 hours) and lipids (fat) → breaking fats releases large amounts of acetyl coA which will activate the synthesis of ketone bodies or keto acids (Because it is an acid, it will cause acidosis (reduces the PH)).

(When the sugar is broken down, there will be some acetyl coA going to krebs cycle producing energy, but when burning fats, there will be a very large amount of acetyl coA, some of them will get into krebs, and the rest will form keto acids)

-The reason behind starvation, is that we start relying on meat, fats and proteins as a source of energy (NO USE of sugars) .

Uncontrolled diabetes: for diabetic patients who do not take insulin or follow a specific diet, when they have a meal, their sugar conc. in the blood increase (there's no reduction in their blood sugar it will still high), but because there is no insulin, cells will not recognize that there is a sugar in the blood (cells do not sense insulin because of

the insulin resistance), they will feel very hungry and this stimulates other molecules to be degraded like fats, the same story in the starvation happens. (Fat degradation → acetyl coA → ketone acids → acidosis)

في سكر بالدم، بس ما في إنسولين يحكي للخلية امتصي السكر، فالخلية مش فاهمة إنه في سكر فراحت نبّهت الدماغ إنه يكسّر الجلايكوجين والدهون (ما انتبهت انه في طريق سهل فراحت ع الصعب)

*respiratory (pulmonary) acidosis is caused by: asthma; emphysema

People with this problem cannot exhale CO_2 very well, CO_2 stays more in their lungs → reacts with water forming carbonic acid which dissociates into bicarbonate and H^+ → more H^+ → more acidic → acidosis

*metabolic alkalosis is caused by: the administration of salts, when we give a patient an excess amount of salts, we disrupt the balance of salts in the blood, making the blood less acidic → causing alkalosis

*respiratory alkalosis is caused by: Hyperventilation(exhaling more than inhaling):

Climbing high altitudes: when you go up, the O_2 going to your lungs becomes less than CO_2 getting out. Increasing CO_2 that gets out of your lungs makes the blood less acidic so alkalosis happens.

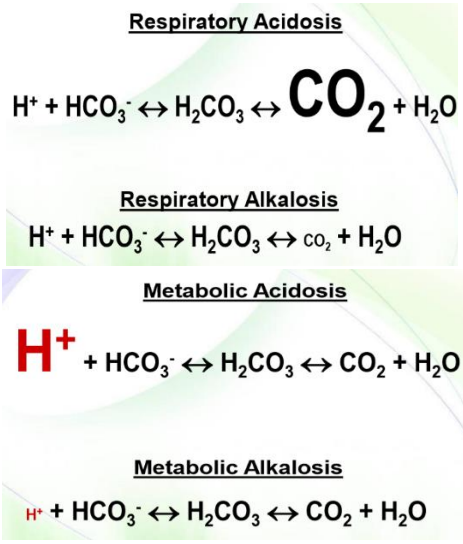
قال تعالى: "ومن يُرِد أن يُضِلَّهُ يَجعَل صَدرَهُ ضَيّقًا حَرَجًا كَأَنّما يَصَّعَّدُ في السَّماءِ"

إعجاز الآية العلمي عن إنه صعود المرتفعات زي السماء بضيّق الصدر بتقليل كمية الأكسجين اللي بتدخل للرئتين

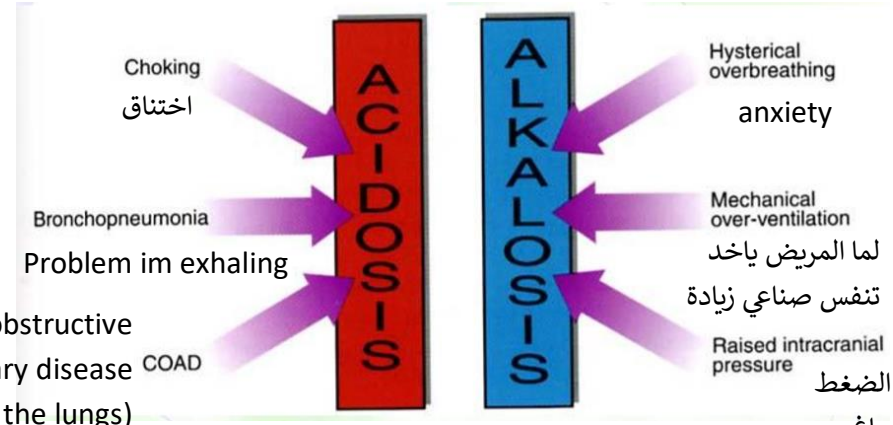
anxiety: people who suffer from phobias or pathologic anxiety have hyperventilation and take short breaths (They get rid of high conc. of CO_2 making their blood less acidic → alkalosis)

In the respiratory acidosis or alkalosis, the problem is the CO_2. In the acidosis, we have high conc. of CO_2 → high conc. of acid. In the alkalosis, we have low conc. of CO_2 (it is mostly exhaled) → low conc. Of acid.

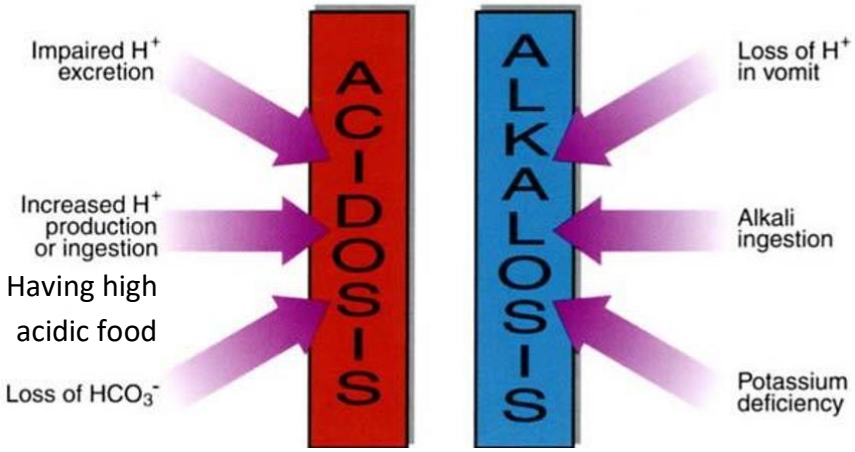
In the metabolic acidosis or alkalosis, the problem is the H^+. In the acidosis, we have high conc. of H^+→ high conc. of acid. In the alkalosis, we have low conc. of H^+ → low conc. Of acid.



Respiratory causes



Metabolic causes



The doctor asked us to search and figure out the relationship between Hypokalemia (K^+ deficiency) and metabolic alkalosis.

When Hypokalemia occurs, the kidneys try to compensate the loss of potassium by reabsorbing K^+ in the collecting ducts and excreting H^+ to maintain the electrical balance.

(low H^+ → less acidic → alkalosis)

Now, how does our body compensate the change in the pH?

The compensation is the change in HCO_3^- or CO_2 results from the primary event.

If the problem is **metabolic (change in H^+ conc.) we will have **respiratory** compensation whether it is hyperventilation or hypoventilation (change in CO_2 conc.)

If the problem is **respiratory (change in CO_2 conc.) we will have **metabolic** (renal) compensation (change in HCO_3^-)

*This compensation might be complete if we get the pH back to 7.4 or partial if it does not reach 7.4 exactly.

Complete compensation

For Resp. acidosis we have a pH which is close to the normal but less, as the cause is respiratory (increasing CO_2) the response is metabolic (increasing HCO_3^-) so we make an acid-base balance to restore the normal pH.

For Resp. alkalosis, we have a problem which is low amount of CO_2 , the response will be reducing HCO_3^- to reduce the basicity of the blood and restore our optimal pH.

$$H^+(aq) + HCO_3^-(aq) \rightleftharpoons H_2CO_3(aq) \rightleftharpoons H_2O(l) + CO_2(g)$$

	pH	pCO_2	HCO_3^-
Resp. acidosis	Normal But<7.40	↑	↑
Resp. alkalosis	Normal but>7.40	↓	↓
Met. Acidosis	Normal but<7.40	↓	↓
Met. alkalosis	Normal but>7.40	↑	↑

For metabolic acidosis, we have a problem which is decreasing HCO_3^- , the response will be respiratory, CO_2 will go down to restore the normal pH.

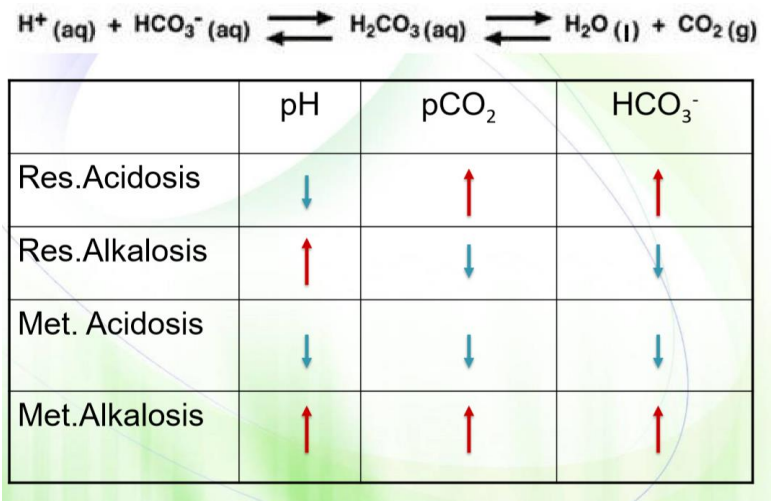
For metabolic alkalosis, the problem is having more base (HCO_3^-), the response is having more acid (CO_2) to get back to 7.4

Think about it as a positive feedback to get homeostasis.

Partial compensation

The same thing will happen, except that the action of the body will not be enough to get the pH back to its normal value.

Look at the Res.Acidosis for example, the pH is **still** less than 7.4



	pH	pCO_2	HCO_3^-
Res.Acidosis	↓	↑	↑
Res.Alkalosis	↑	↓	↓
Met. Acidosis	↓	↓	↓
Met.Alkalosis	↑	↑	↑

Other buffers in the body:

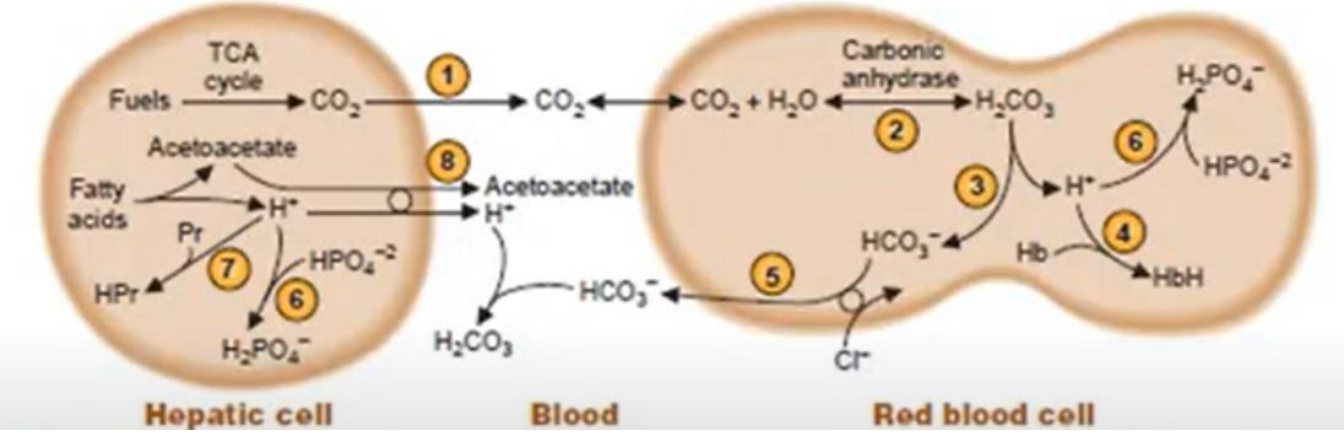
***Dihydrogen phosphate-monohydrogen phosphate system (intracellular)**

*It consists of ($H_2PO_4^-$/HPO_4^{-2}), it is the most important buffer inside the cells because of having phosphate group.
*Examples (molecules having phosphate): ATP, glucose-6-phosphate, bisphsphoglycerate (RBC)

***proteins:**

*Proteins as you all know consist of 20 types of amino acids
*1 amino acid called histidine can act as a buffer under the physiological conditions of a blood pH 7.4, because the side chain of this amino acid (imidazole) has a pKa (7.1) that is very close to the pH of the blood. This group (imidazole) keeps the pH by acting as a base accepting protons (protonation) then donating protons (deprotonation) according to the environment.
*There are other amino acids that could be acidic or basic but their pKas are very far away from the blood pH, they could have pHs like 9,11,5,… and these numbers are not appropriate for the blood (Remember that any slight decrease of 7.35 or increase above 7.45 of the blood pH causes death or serious problems in the CNS)
*As histidine increases → more efficient buffer
*Hemoglobin in blood contains 38 histidines, each one could be protonated or deprotonated depending on the situation (So they can act as buffer system) .
*Other proteins like albumin in the blood or other proteins in the cell have different numbers of histidine and they could act as buffers.
*Notice that buffer proteins do not exist only extracellularly, instead, there are some acting intracellularly.

Look at this figure:



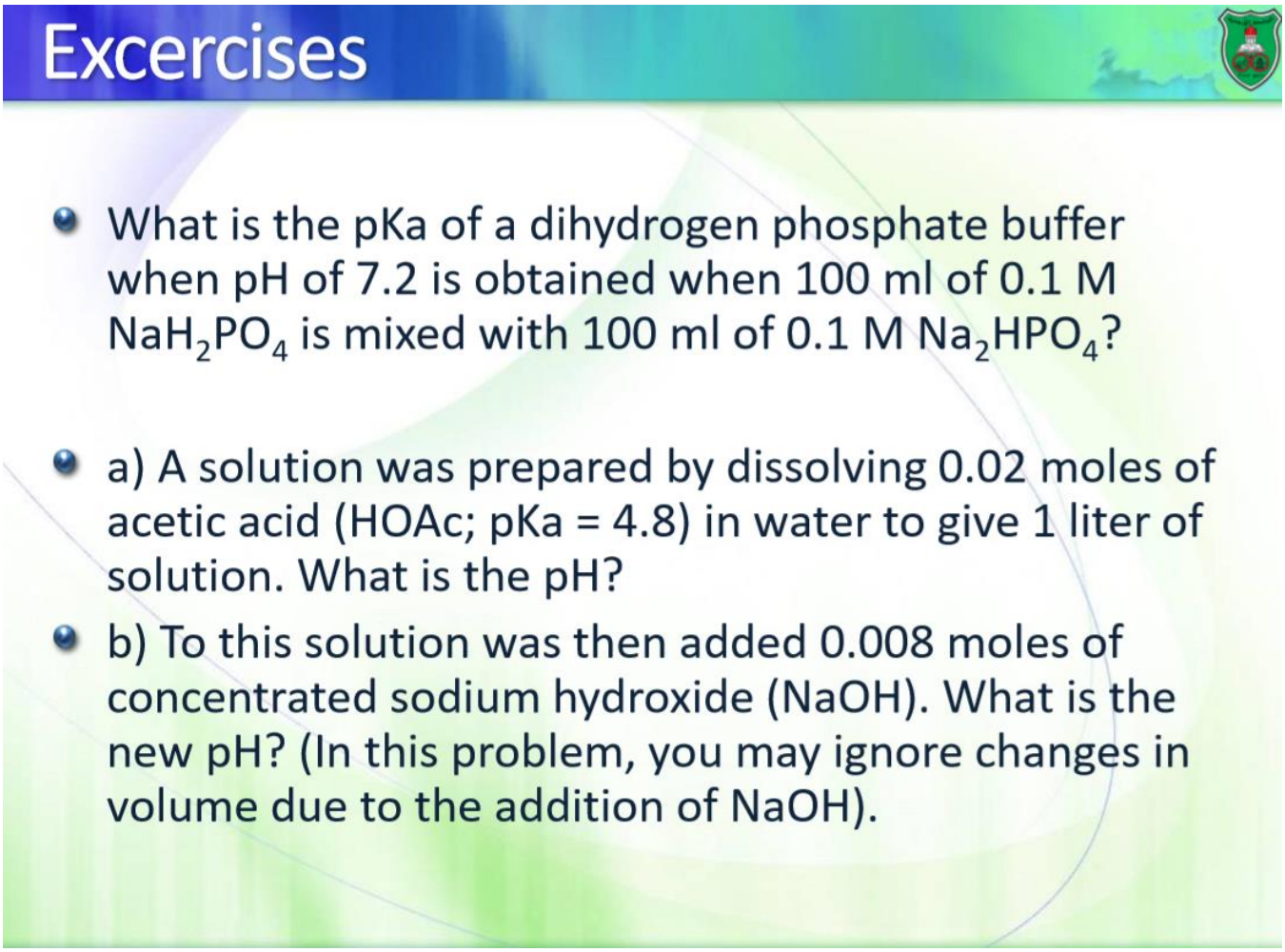
When we inhale air, this hepatic (liver) cell absorbs O_2 and uses it in combustion reactions, CO_2 is released to the blood stream and enters RBC to react with water by the action of (carbonic anhydrase enzyme) which is not existed in the blood, that's why this reaction does not happen in the blood. When carbonic acid gives its proton, the H^+ increases in the cell making it more acidic, **Here**, we actually have **2** buffers helping the cell to restore the normal pH which are the **hemoglobin buffer** (the histidine takes this proton to solve the problem) **And the phosphate buffer system** (HPO_4^{-2} becomes $H_2PO_4^-$). When the carbonic acid gives its proton, HCO_3^- is also released in the cell, but the original place it works in is the blood, that's why it leaves the cell to the blood.
Does this disrupt the electrical balance between ECF and ICF? Of course it does, that's why negative chlorine ions enter the cell after HCO_3^- ions have left it to restore the balance.

Remember: When H^+ is released from the hepatic cell due to reactions in it, the acidity of the blood increases, the HCO_3^- react with H^+ forming H_2CO_3 which is dissociated into water and CO_2 which is exhaled by the respiratory system to reduce the acidity. But if these H^+ ions remain in the hepatic cell, 2 buffer systems will deal with this problem to reduce the acidity inside the cell **1) phosphate buffer system 2) Histidine of different proteins inside the cell ((We have already talked about these systems in details))**

To sum up, these buffer systems act as first line of defense to maintain the pH in the body, they get help as well from the respiratory and renal systems.

For this slide, try to solve these problems and check the answers from the link:

https://www.facebook.com/groups/doctor2019/permalink/1170224016691641/



الله يوفقكم ☺

1- All of the following will cause mild or severe acidosis except:

a- the presence of ketone bodies in untreated diabetic patient
b- The production of acids like lactic acid during metabolism
c- Excessive breathing
d- Repeated vomiting from the stomach containing HCL.

2. One of the following statements is not true about Carbonic acid/Bicarbonate buffer:

a- The most common extracellular buffer.
b- Under physiological conditions the ratio of [HCO3-]/ [H2CO3] = 20.
c- Its buffering range is less than the desirable pH and that's compensated by CO2 mobility.
d- When adding a strong acid, it will react with HCO3-
e- When adding a strong base, it will react with CO3-2

3. 100 mmol of a triprotic acid were titrated with KOH. PKa values = 3, 6, 9. - How many mmoles of KOH must be added to have pH=6?

a- 100
b- 150
c- 200
d- 250
e- 300

4. $H_2PO_4^-$: HPO_2^- = 25:75 24, pka=6.3 *10^-8,
find the ph

5. Which one of the amino acids could serve as the best buffer at pH 7?

A) Glutamic acid
B) Arginine
C) Valine
D) Histidine

6. What initial effects does hyperventilation have on the human's blood pH and H_2CO_3 concentration?

A) pH increases and [H_2CO_3] increases
B) pH increases and [H_2CO_3] decreases
C) pH decreases and [H_2CO_3] increases
D) pH decreases and [H_2CO_3] decreases

7. Which of the following features are present in blood chemistry in uncompensated metabolic alkalosis except?

A) Increased pH
B) Increased bicarbonate
C) Normal chloride
D) Normal pCO_2

ANSWERS

Q1	Q2	Q3	Q4	Q5	Q6	Q7
D	E	B	7.7	D	B	D