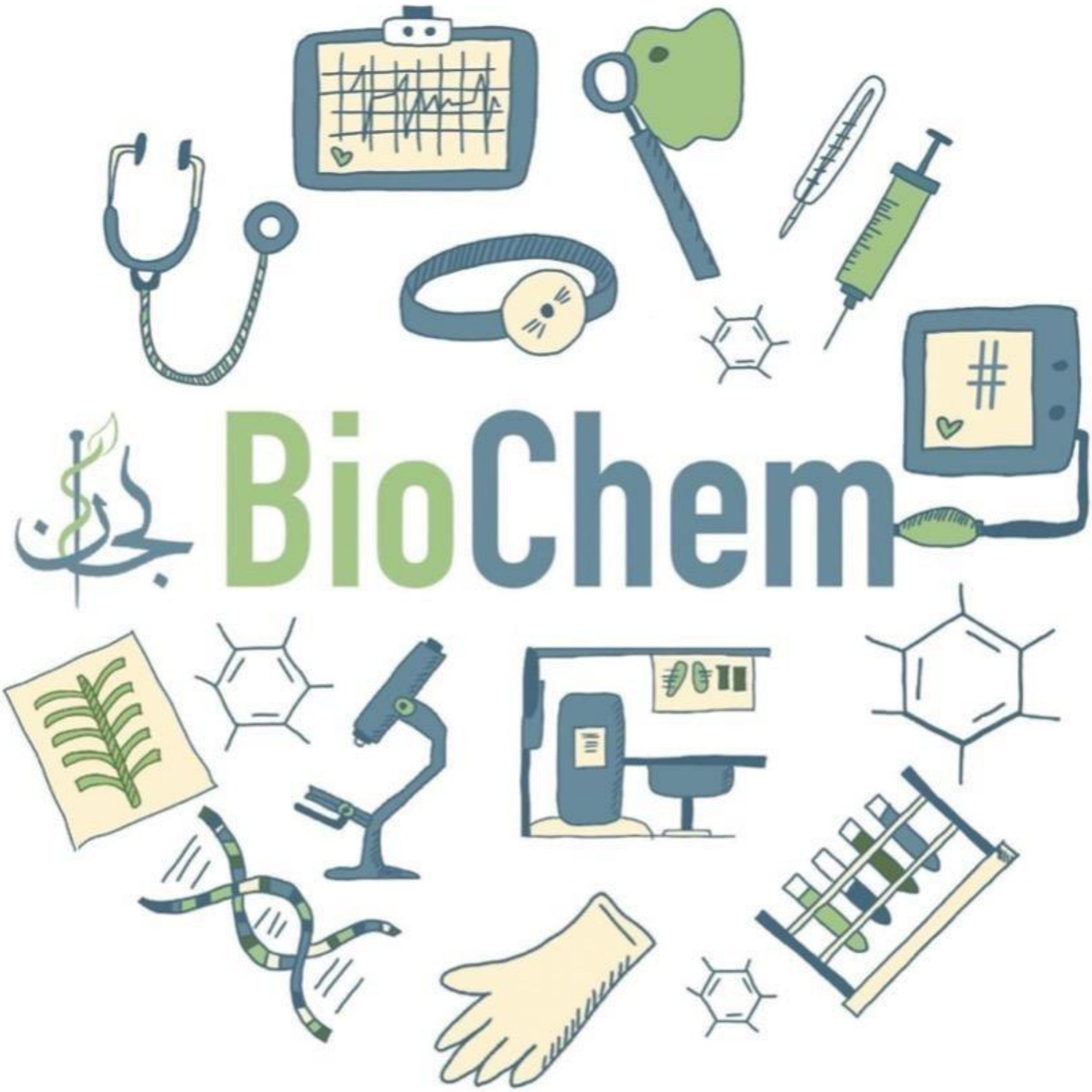

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Grammatical Correction: Ahmad Al Qatawneh

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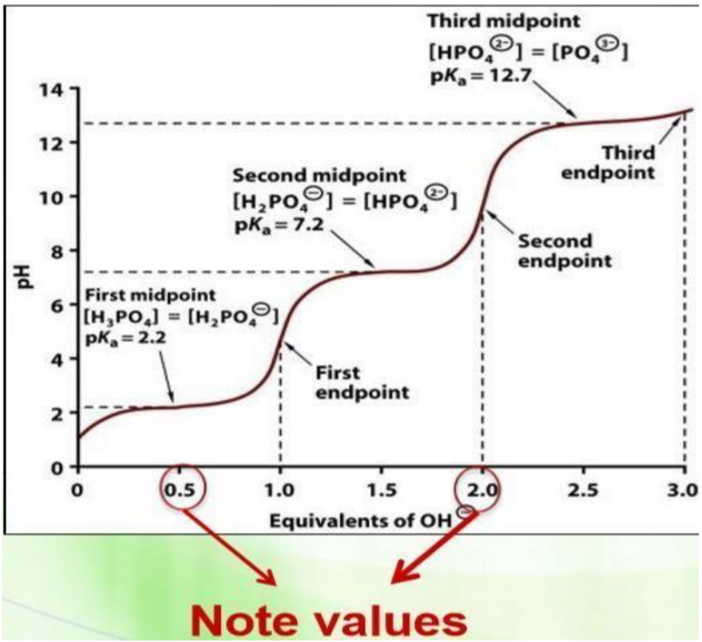
In the previous sheet, we discussed the titration of monoprotic acids, specifically the acetic acid. Now, we will talk about polyprotic acids specifically the H_3PO_4 (phosphoric acid) which will be titrated on several steps.



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

*First curve represents the titration of H_3PO_4 , giving its proton and becoming $H_2PO_4^-$, which will be titrated in the second curve and becoming HPO_4^{-2}, that will be titrated in the last curve to end with PO_4^{-3}

Notice that titration is gradual (H_3PO_4 lost each proton separately)

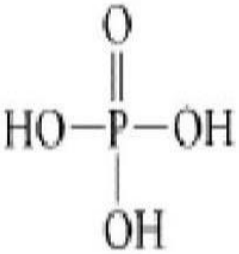
*As before, each curve has a concave down part & concave up part, with an inflection point.

* For polyprotic acids, 1 OH eq is needed for each step to reach the equivalence point. H_3PO_4 contains 3 H+ thus 3 eq of OH are needed, first eq will ends at the first titration, second eq will ends at the second step and so on.

*From this figure, the pH for these molecules can be predicted, for example, the pH for H_3PO_4 in the first step is 1.6. Moreover, mid-point (inflection) for each step (curve) can be calculated by finding the Pka using H_3PO_4 ka for the first step, $H_2PO_4^-$ ka for the second step, HPO_4^{-2} for the third curve,

*Notice that the titration of the 1st step occurs at lower pH range compared to the 2nd step and the 2nd step occurs at a lower pH range compared to the 3rd step. According to this, the first acid is stronger than the second acid which is stronger than the third step. This is also reflected by their pka values, first acid has the lowest pka value → strongest acid.

*At the first endpoint, no more H_3PO_4 is left over, as all molecules are converted to $H_2PO_4^-$. At the second end point, no more $H_2PO_4^-$ is left as all molecules are converted to HPO_4^{-2} . Same thing happens with the last endpoint with only PO_4^{-3} molecules left over. **Look at the figure bellow:**



$$H_3PO_4 \xrightleftharpoons{pK_{a(1)} = 2.14} H_2PO_4^- + H^+ \xrightleftharpoons{pK_{a(2)} = 7.20} HPO_4^{2-} + H^+ \xrightleftharpoons{pK_{a(3)} = 12.4} PO_4^{3-} + H^+$$

- What is the pKa of a dihydrogen phosphate buffer when pH of 7.2 is obtained when 100 ml of 0.1 M NaH_2PO_4 is mixed with 100 ml of 0.1 M Na_2HPO_4?

Concentrations of reactants changed after mixing:

H_2PO_4 new conc. : 0.05 HPO_4 new conc. : 0.05

pH = pKa +log(A-/HA)

7.2 = pKa + log (0.05/0.05).   **pka = 7.2**

- a) A solution was prepared by dissolving 0.02 moles of acetic acid (HOAc; pKa = 4.8) in water to give 1 liter of solution. What is the pH?

(HOAc) conc. = 0.02/1 = 0.02
(HOAc) ka = $10^{-4.8}$ = 1.58 * 10^{-5}
Ka = 1.58 * 10^{-5} = X^2/0.02   **X = 5.63 * 10^{-4}**
pH = -log (5.63 * 10^{-4}) = 3.249

- b) To this solution was then added 0.008 moles of concentrated sodium hydroxide (NaOH). What is the new pH? (In this problem, you may ignore changes in volume due to the addition of NaOH).

0.008 NaOH will react with acid to
Produce conjugate base.
NaOH conc. = 0.008/1 = 0.008
Conc. of remaining HOAc = 0.02-0.008 = 0.012
Conc. of conjugate base = 0.008
pH = 4.8 + log(0.008/0.012) = 4.6

Buffers in the human body) biological buffers)

An average rate of metabolic activity produces roughly 22,000 mEq (not to memorize) of acid per day. If all of this acid were dissolved at one time in unbuffered body fluids, their pH would be <1. However, because of buffers, the pH of the blood is normally maintained between 7.36 and 7.44 and Intracellular pH at approximately 7.1.

- Carbonic acid – bicarbonate system (blood)
- Dihydrogen phosphate-monohydrogen phosphate systems (intracellular)
  ATP, glucose-6-phosphate, bisphosphoglycerate (RBC)
- Proteins (extra- and intracellular)
  - Hemoglobin in blood
  - Other proteins in blood and cells

Bicarbonate buffer (carbonic acid- bicarbonate system)

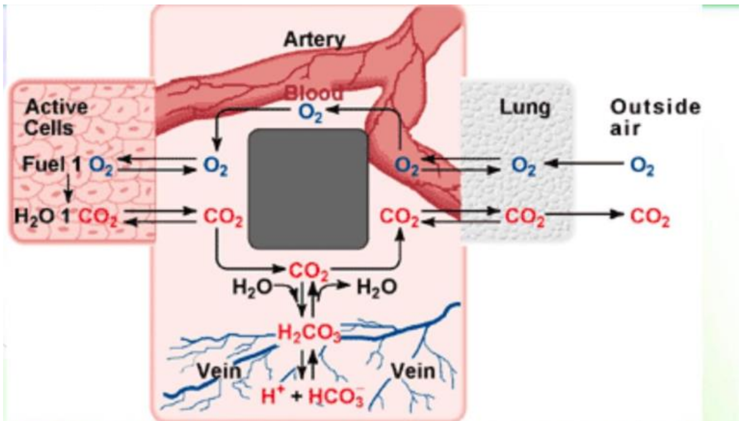
*Bicarbonate buffer system in the main buffer system, located in blood specifically. (extracellular)

* As we all know, oxygen is inhaled into the lungs. Once in the lungs, oxygen is moved into the blood stream (by gas exchange at alveolus) and carried through your body. At each cell in your body, oxygen gas (which will be used in combustion reactions) is exchanged with wastes including carbon dioxide. Blood stream will carry CO_2 through our body, reaching the lungs.

* CO_2 is always surrounded by H_2O all over its way; however, CO_2 will react with water only in RBC, where **carbonic anhydrase is present**.

* As we said before, CO_2 will react with H_2O (by the action of an enzyme known as **carbonic anhydrase**) forming **carbonic acid**(H_2CO_3), which will dissociates as any other acid, releasing H^+ & HCO_3^- .

Note: Dissociation of HCO_3^- is not favored by body environment.



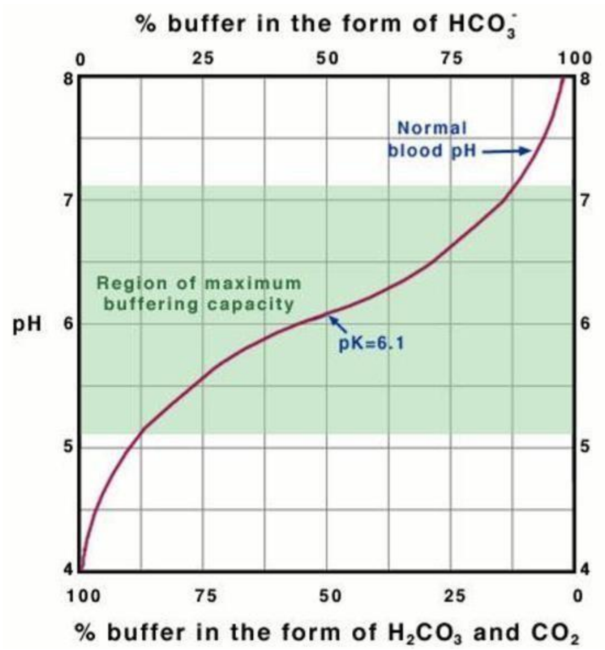
Weak acid

Conjugated base

*This biological buffer (H_2CO_3 + HCO_3^-)$_3$is the first working buffer system to maintaining the pH of the blood within its normal ranges (7.4). According to this, this system must work quickly just to rescue the situation; however, the system work is not permanent and another system should interact to finish the job, such as :

- The respiratory system(2nd line of defense), by affecting the exhalation of CO2
- Renal system (3rd line of defense) which affect the absorption & excretion of HCO_3^- outside the body.

Titration curve of bicarbonate buffer



Just like any other buffer system, pH change starts very quickly, then pH increases slowly.

The pKa for this system is 6.1, thus buffer system will act in the range 7.1-5.1. This means that blood pH (7.4) is not included in the buffering capacity. **Then How does the bicarbonate buffer resist changes in the blood pH that is outside its capacity?!**

1) It is an open system (not a closed system like in laboratory)

   An open system is a system that continuously interacts with its environment

2) The components of the buffer system are effectively under physiological control: the CO_2 by the lungs, and the bicarbonate by the kidneys (as we said it is an open system)

3) bicarbonate is present in a relatively high concentration in the ECF (**24mmol/L**), According to Henderson-Hasselbalch Equation, for pH value to reach a value higher than pka, A- must be larger than HA. (pKa = 6.1 pH= 7.4, thus log (HCO_3^-/ H_2CO_3) must equal 1.3, hence [HCO_3^-] : [H_2CO3] is 20 : 1 under physiological conditions)

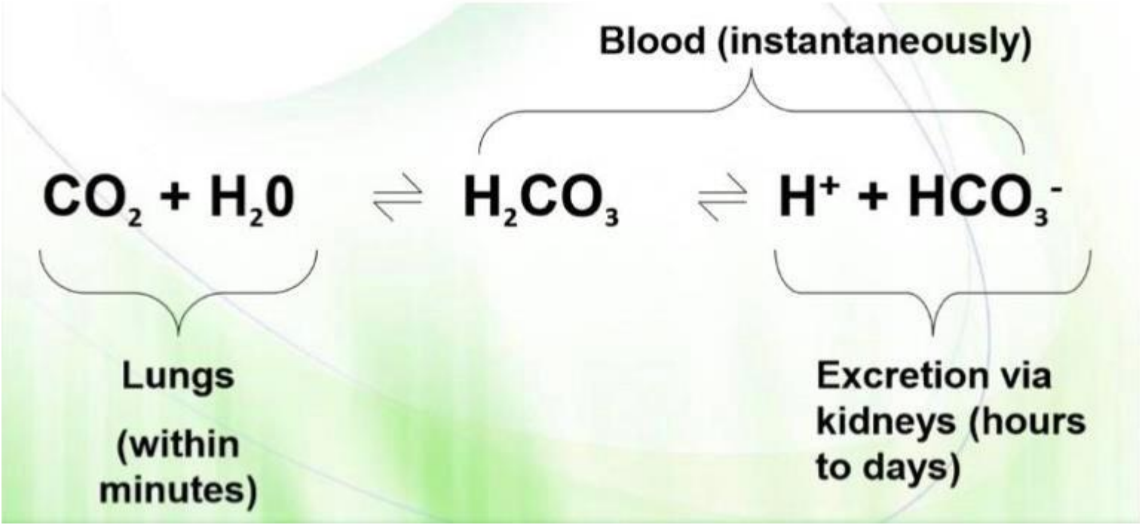
Bicarbonate buffer and interaction with other systems

With the help of two others systems (the respiratory and renal), the Bicarbonate buffer system is effective against high concentrations of acids and bases:

When High concentrations of acids are added to blood, many molecules of **H^+** will be released into the blood, **HCO_3^-** will react with excess **H^+** forming **H_2CO_3** which will be broken to **H20** and **CO2.** Starting from here, the function of bicarbonate buffer system ends and the respiratory system (the collaborator) starts functioning by releasing **CO2** ,hence keeping the **CO2** levels low , preventing our body from converting **CO2** back to **acid**. By this excess acid is removed. The renal system is also involved in regulating the pH, it does so by reabsorbing HCO_3^- restoring reacted molecules.

When high concentrations of bases are added to blood, many molecules of **OH^-** will be released into the blood, **H_2CO_3** will react with excess **OH^-** producing salt (**HCO_3^-**) and water. However, concentration of **H_2CO_3** decreases, thus respiratory system will accumulates CO2 in blood (respiration rate decreases) which will be converted back to acid, and the renal system will excrete more $HCO3^-$.

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).



Acidosis and alkalosis (pathological conditions)

Both pathological conditions can be either metabolic or respiratory.

Acidosis (condition when the blood pH is less than 7.35):

Metabolic:

1) Starvation: human body relies on food as the main source of glucose. (glucose level increases after a meal (this increase lasts for 2 hours) Blood glucose is absorbed by cells under the action of insulin and extra glucose is stored as glycogen in the body. Starvation reduces the main source of glucose and because glucose is an essential molecule, cells start degrading stored glycogen which will supply body with glucose for 10-18 hours (depending on the size and amount of glycogen stored in the body). After that, your body will start breaking lipids which releases large amounts of acetyl coA compared to sugar. Some acetyl coA will be used in Krebs cycle to produce energy; however, excess acetyl coA will activates the production of ketone bodies (ketoacids), increasing H^+ conc.

2) Uncontrolled diabetes: for diabetic patients who do not take insulin or follow a specific diet, blood sugar is not absorbed by cells, thus cells will degrade lipids for energy and as explained before, production of ketone bodies is activated.

Respiratory: pulmonary (asthma; emphysema)

Difficulties in excreting (breaking out) CO2 very well, thus CO2 will accumulates in blood and reacts with H2O carbonic acid which dissociates into bicarbonate and H^+, resulting in more H^+ and more acidic blood.

Alkalosis (a condition when the blood pH is more than 7.45):

Metabolic

This is a result of administration of salts. In case of some patients, there would be excess salts in their veins that increases the negative ions (bases) leading to affect the balance in the constituents of the blood, thereby resulting in less acidic blood (that is, more PH).

Respiratory

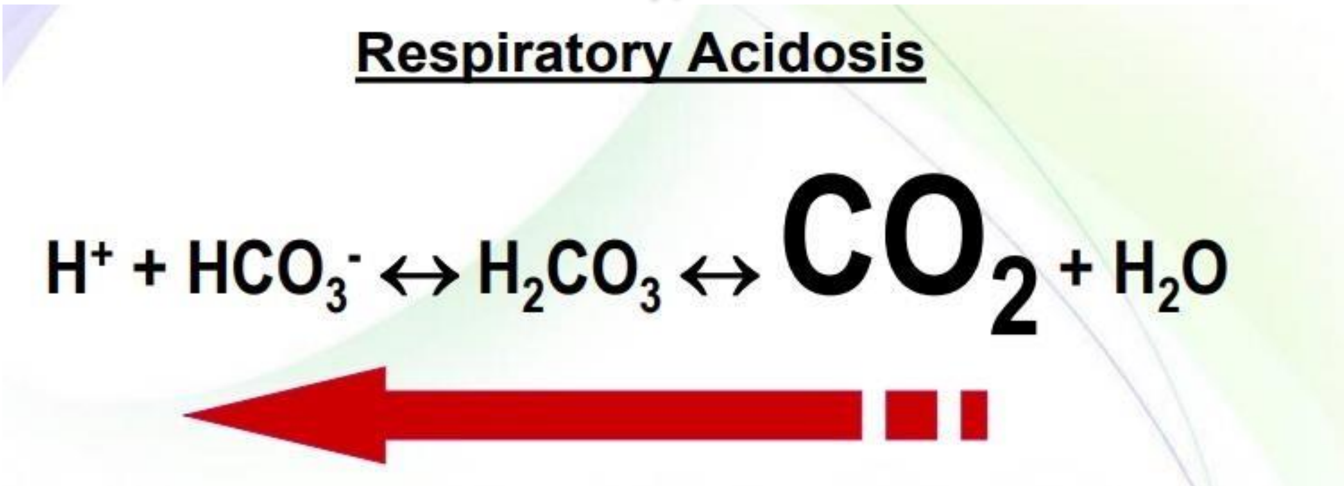
This is the result of **hyperventilation**(تسارع في التنفس)associated with **anxiety**, or going up to **high altitudes** like climbing high mountains and you are not used to it, there will be less oxygen in a such environment with our body being not used to it, so he will respond to that by increasing the respiration rate to compensate (يعوض) the lack of oxygen in our bodies. Same thing happens with people that are having anxiety (mental illness condition).

Less oxygen inhaled → **Less CO2 produced** → **Less acid produced (H2CO3)** → **Higher PH (alkalosis)**

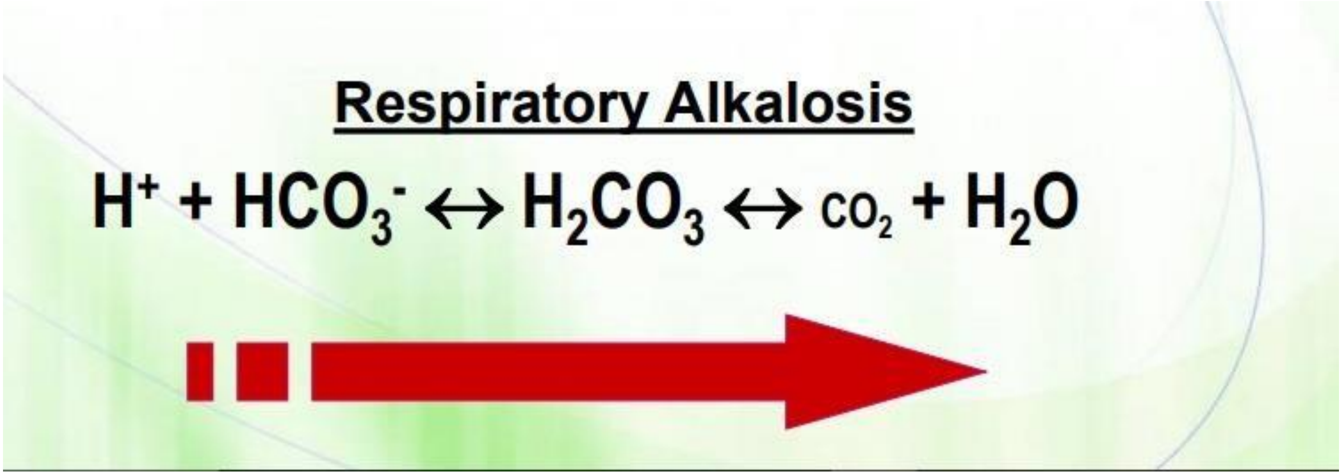
NOTE: You have to understand this well, so the following relations gets easier…. CO_2 is the main precursor for making H_2CO_3, So when the concentration of CO_2 gets higher, expect to see high concentration of the acid H_2CO_3 and less PH blood (Acidosis), and vice versa. And also when there is a high concentration of protons (H+), it will react with HCO_3^- producing high concentration of H_2CO_3 and less PH blood (Acidosis).

To sum up,

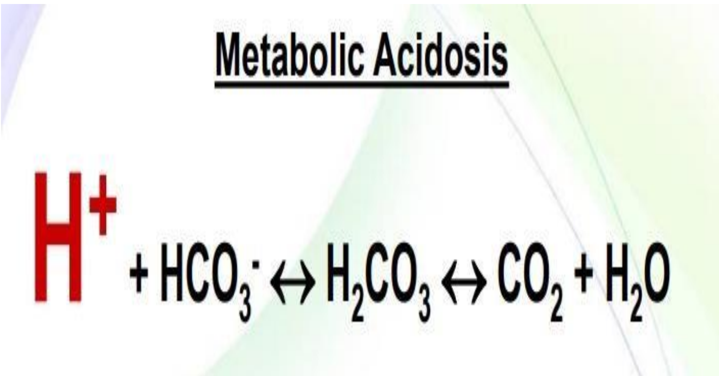
High CO_2 OR high H^+ ⟶ Acidosis	**low CO_2 OR low H^+ ⟶ Alkalosis**

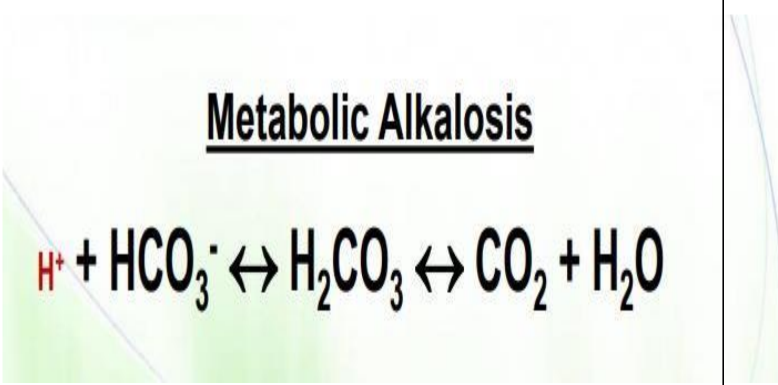


In respiratory conditions, whether it is **Acidosis** or **Alkalosis** the problem is the **CO_2**, as we said…. High CO_2 means high acid (H_2CO_3) in the blood, so **Acidosis.**



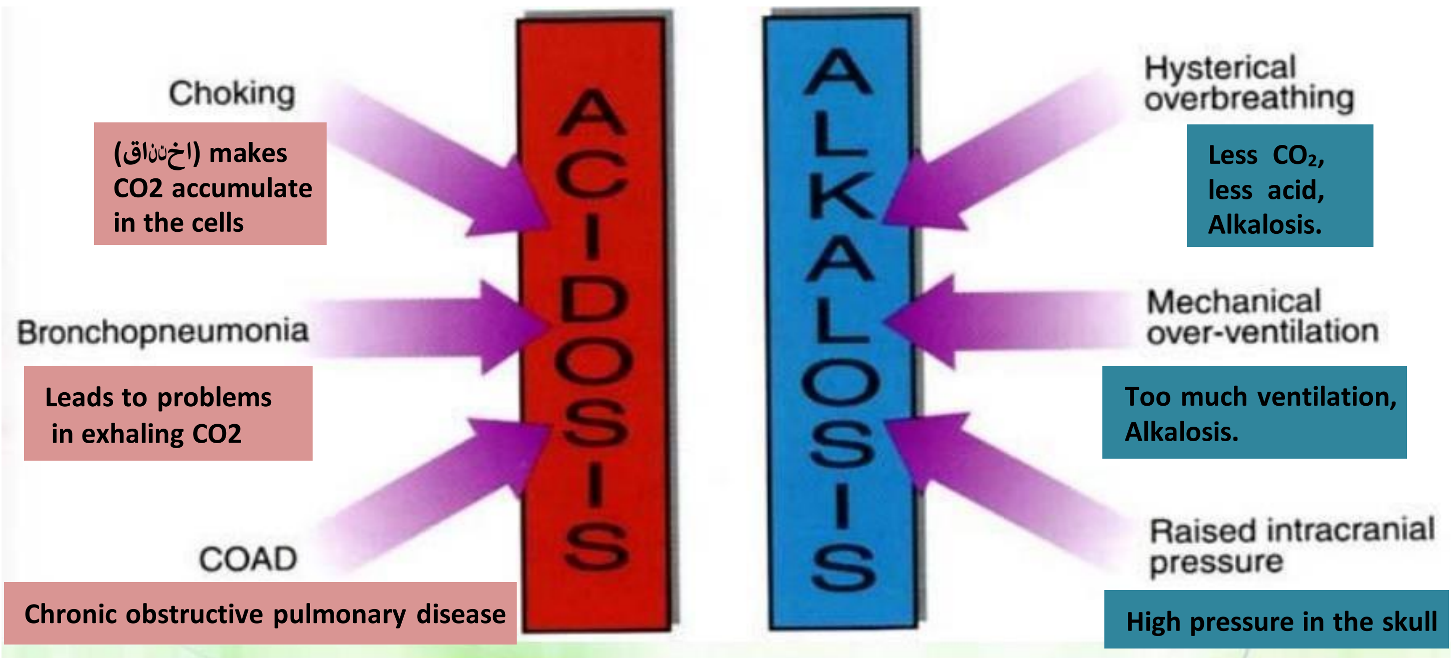
In **Alkalosis**, it is the other way around, the concentration of CO_2 is low (mostly exhaled out the body) and subsequently, less acid is produced leading to a PH higher than 7.45.



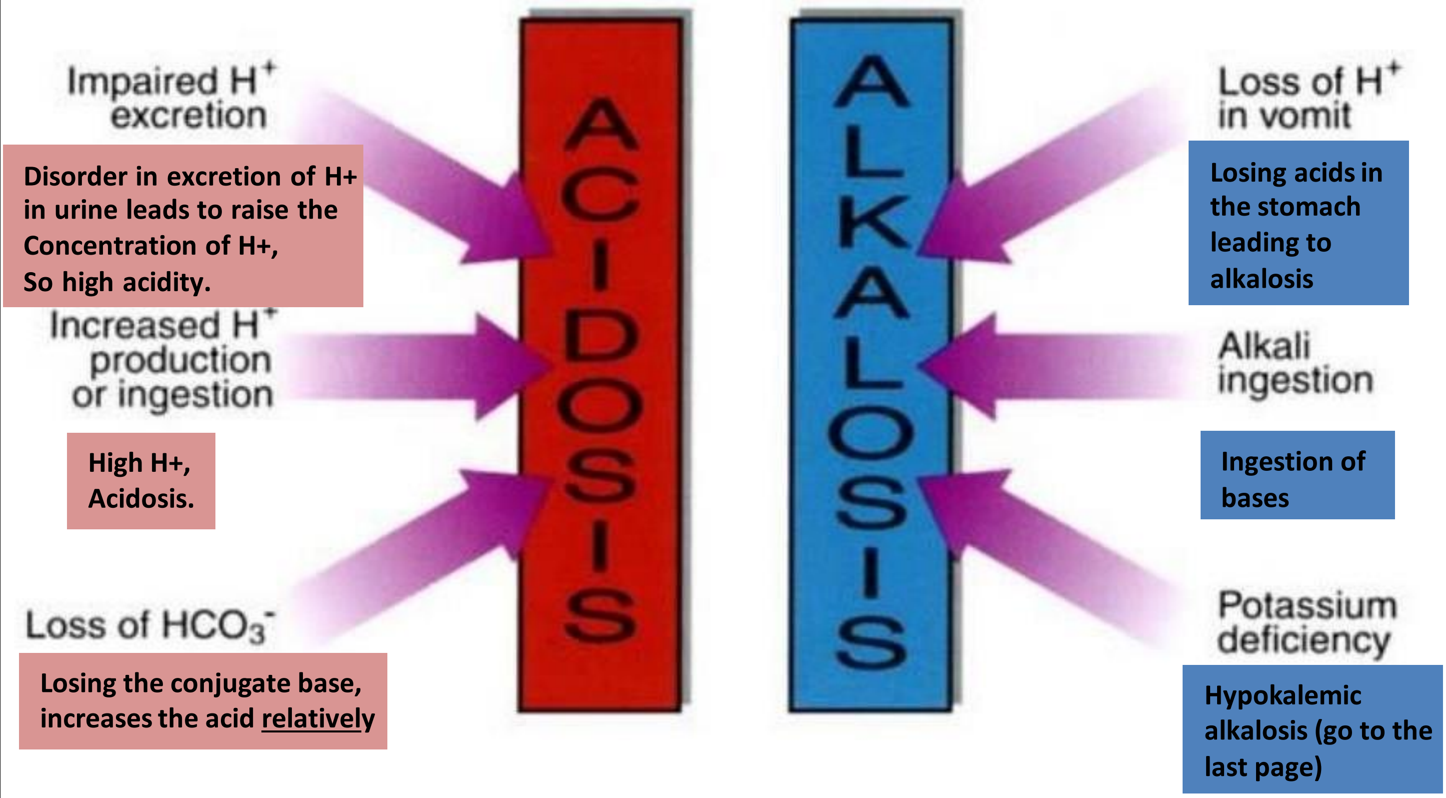


In **metabolic conditions**, **metabolic Acidosis** is due to the **high concentration of protons** leading to the production of high amounts of H_2CO_3 (**Acidosis**).while in **metabolic Alkalosis** it is quite the opposite, low concentration of protons leading to less production of the acid (**alkalosis**).

We previously talked about some examples about **respiratory causes** about **Acidosis** and **Alkalosis**, but here are another examples;



There are also another **metabolic causes**, look at the picture below;



Now, How can our buffer systems deal with situations whenever we have an increase or decrease in PH value?

Compensation

There is always a cooperation between **renal system** and **respiratory system** in maintaining the PH around its normal range, The renal system controls the concentration of HCO_3^- either by excretion or reabsorption, While the respiratory system controls the PCO_2 (the pressure of **CO_2**) inside our cells either by exhalation or inhalation.

- If there is a **metabolic problem** whether it is **Acidosis** or **Alkalosis**, the compensation will be done by the respiratory system by increasing or decreasing PCO_2 (hyperventilation or hypoventilation): **Respiratory compensation.**

- If the problem is **respiratory**, the compensation will be done by the renal system by excretion or reabsorption of HCO_3^-: **Metabolic compensation.**

The compensation can be:

1- **complete** if brought back within normal limits.
2- **Partial compensation** if range is still outside norms (the PH doesn't reach 7.4 back).

In summary, we established that if there is a problem in one system, the other system compensates and fixes that problem. For example; if the PCO_2 goes up (respiratory Acidosis), the renal system reabsorbs more HCO_3^- effectively getting the PH back to normal. The idea here is to **maintain the ratio between H_2CO_3 and HCO_3^-** according to Henderson-Hasselbalch equation.

Acid-Base Disorder	Primary Change	Compensatory Change
Respiratory acidosis	pCO_2 up	HCO_3^- up
Respiratory alkalosis	pCO_2 down	HCO_3^- down
Metabolic acidosis	HCO_3^- down	PCO_2 down
Metabolic alkalosis	HCO_3^- up	PCO_2 up

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

There are two ways for understanding the examples above, either you **maintain the ratio**, If the PCO_2 goes up…..the HCO_3^- also goes up, if PCO_2 goes down…..the HCO_3^- also goes down and so on. The other way is **to look to the effect of the change that happens and do the opposite thing**, if PCO_2 goes up the blood becomes more acidic, resulting in a need to decrease the acidity by increasing the concentration of the conjugate base "HCO_3^-".

PCO_2 up: decreased respiratory rate(decreased exhalation) and vice versa for **PCO_2 down**.
HCO_3^- up: absorption of HCO_3^- **HCO_3^- down**: secretion of HCO_3^-.

As we mentioned earlier the compensation could be a **complete** or **partial compensation**.

1- complete condensation:
The two systems collaborate to get the PH back very close to 7.4, in case of Acidosis a little bit lower, in case of Alkalosis a little bit higher.

	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	↑	↑

2- partial compensation:
The reaction might not be able to return the PH back to the normal 7.4 or close to it, so it could be far from 7.4.

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

We talked about **Carbonic acid-bicarbonate system** in the blood, now we will talk about the other two buffer systems ;

- **Dihydrogen phosphate ($H_2PO_4^-$) - monohydrogen phosphate (HPO_4^{-2}) system (intracellular)**

This system is about molecules that have "HPO_4^{-2}" or "H2PO4⁻" like, ATP, glucose-6-phosphate, bisphsphoglycerate (RBC). This system exists **intracellularly** and act as a buffer by converting the phosphate group from one form to another by accepting or losing a proton. (with $H_2PO_4^-$ acting as an acid and HPO_4^{-2} as the conjugate base).

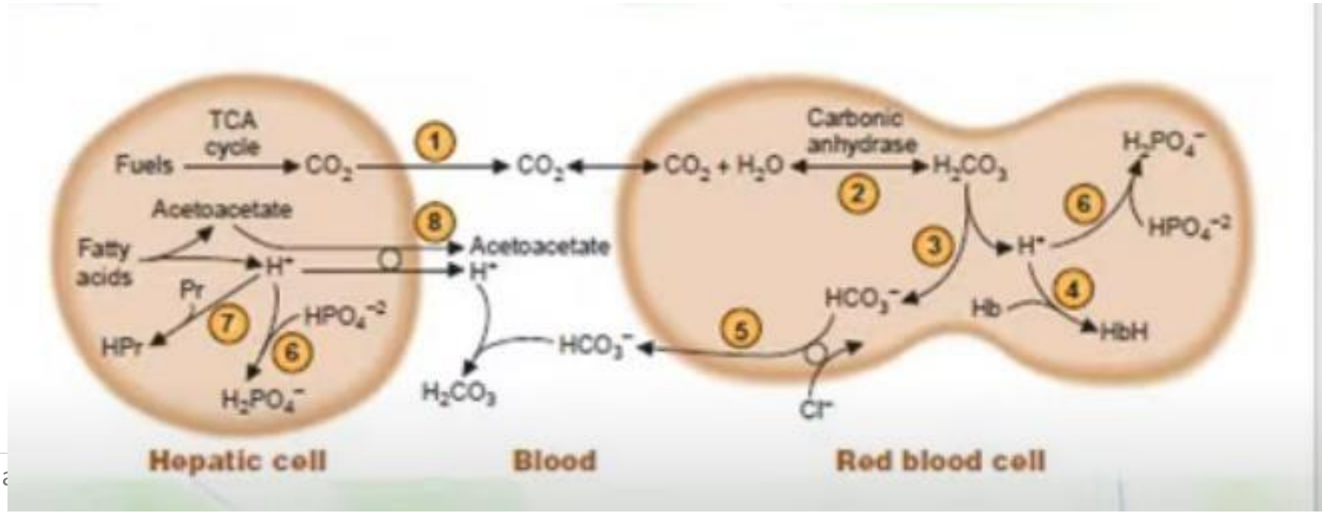
- **Protein buffer system (intracellular and extracellular)**

Recall that proteins are composed from amino acids with 20 different amino acids considered as building blocks for those proteins. However, there is only one amino acid that can act as a functional buffer under physiological conditions called **histidine** ,this amino acid has a side chain called **imidazole group**, this group has a pKa that is very close to the PH of the blood which is 7.1, so this group can be protonated or deprotonated according to the surrounding environment. Although there are another amino acids that can act as bases or acids, but they have pka values (around 9,11 or even 3) that are very far from the PH of the blood, so they can't act as functional buffers.

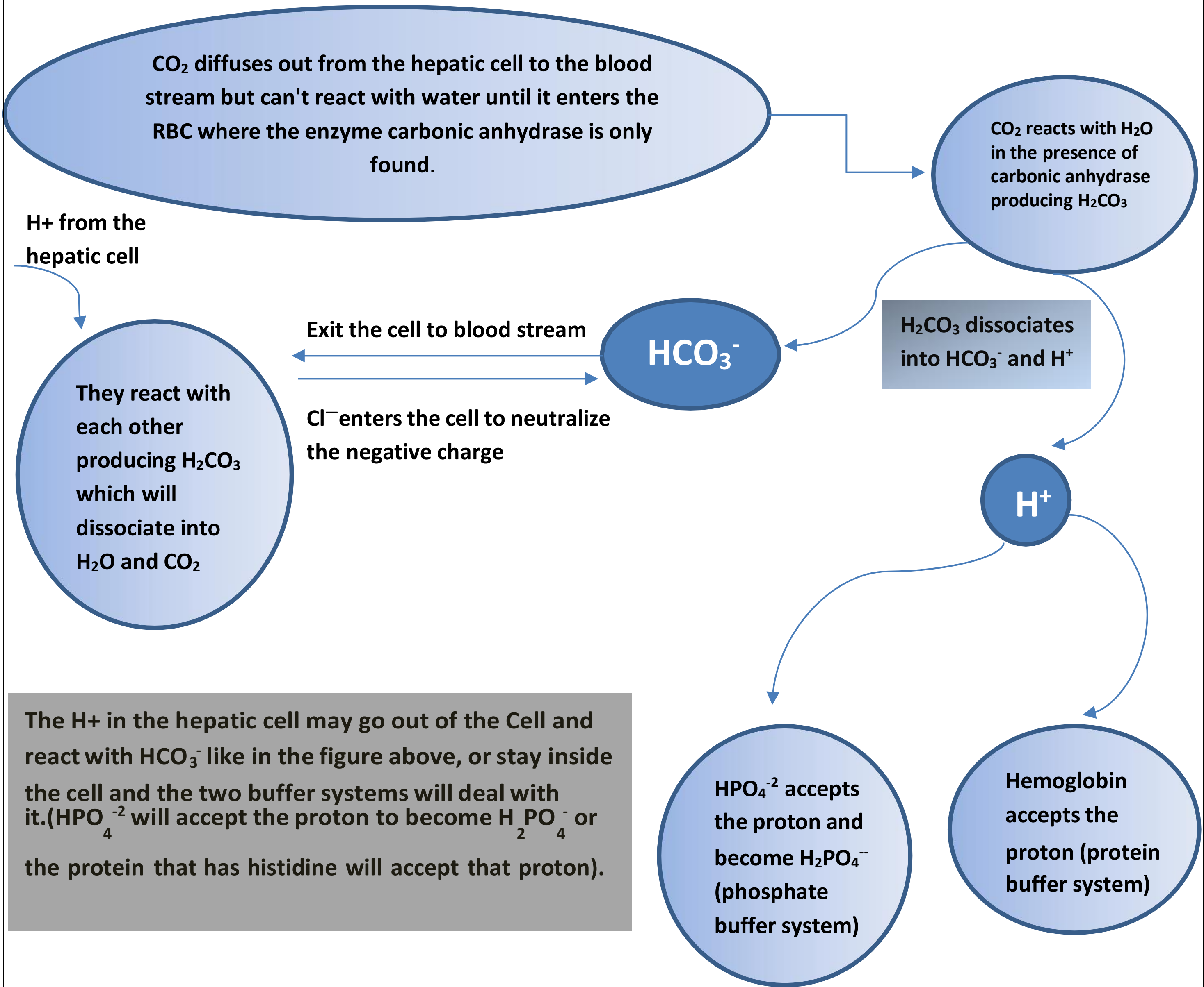
The more histidines we have in the protein structure, The better it acts as a buffer.

Recall that a slight change in pH is significantly reflected on Proton concentration leading to different effects within our body. For instance Death, if the pH reached a value of 7, the patient is going to die, which also the case if the pH value reached 7.6-7.7 , Or it could lead to certain problems in our central nervous system.

✓ **Hemoglobin** in **RBCs** contains 38 histidines, each one of them can accept or donate a proton depending on the situation, so it can act as a buffer system. Other proteins like albumin or proteins inside or outside the cell that have histidines can act as buffers as well. In conclusion, to summarize **all the buffer systems** and their interacting function:



On the left side there is a **hepatic cell (liver cell)**, which is an example of a very active cell, on the right side is a red blood cell and the blood stream between them. Like any cell the hepatic cell inhale the oxygen and use it in combustion reactions to produce energy and CO_2.



- **a metabolic condition, known as Hypokalemic Alkalosis due to Potassium deficiency, in which Potassium (K) depletion causes potassium to move from cells into the extracellular fluid and this transcellular K+ flux causes extracellular Hydrogen ions H+ to move into cells to maintain the electroneutrality resulting in a decrease in [H+] in the plasma of the blood Hence an increase in pH (Alkalosis).**

THE END

Self-assessment Questions

1- 1- All 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 accompanied with doing exercise.
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 $[HCO_3^-]/[H_2CO_3] = 20$.
c- Its buffering range is less than the desirable pH and that's compensated by CO_2 mobility.
d- When adding a strong acid, it will react with HCO_3^-
e- When adding a strong base, it will react with CO_3^{-2}

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

a- 100 mmol
b- 150 mmol
c- 200 mmol
d- 300 mmol

4- A Female patient is presented to the clinic suffering from Insulin dependent diabetes mellitus (diabetes type 1) with decreased PCO_2 , pH=7.35 and $[HCO_3^-]$ =17 mmol/L, which one of the following is most appropriate based on these values: -

a- Respiratory Compensation, Metabolic Acidosis.
b- Respiratory Compensation, Metabolic Alkalosis.
c- Metabolic Compensation, Metabolic Acidosis.
d- Uncompensated Respiratory Acidosis
e- Uncompensated Respiratory Alkalosis

5- The Greatest Buffering capacity at physiological pH would most likely be provided by a protein rich in which of the following Amino acids: -

a- Lysine
b- Histidine
c- Aspartic acid
d- Leucine

6- The pH of the body fluids is stabilized by buffer systems. Which of the following compounds is the **most effective** buffer system at physiological pH?

a- Bicarbonate buffer
b- Phosphate buffer
c- Protein buffer
d- All the above

7- A 35-years old male Patient is presented at the clinic with Obstructive lung disease complaining from shortness of breath, you would most likely expect: -

a- Low pH, low CO2, and low bicarbonate
b- High pH, high CO2, and high bicarbonate
c- Metabolic acidosis
d- Respiratory acidosis

8- All the following features are present in the blood chemistry in **compensated metabolic alkalosis** except?

A) Increased pH
B) Increased bicarbonate
C) Normal chloride
D) Normal pCO2

9- A medical student is feeling anxiety for a big exam and is breathing rapidly, what do you expect out of the following you would expect: -

a) Metabolic Acidosis
b) Metabolic Alkalosis
c) Respiratory Acidosis
d) Respiratory Alkalosis

10- What initial effects does hyperventilation have on the human blood pH and H2CO3 Concentration?

A) pH increases and [H2CO3] increases
B) pH increases and [H2CO3] decreases
C) pH decreases and [H2CO3] increases
D) pH decreases and [H2CO3] decreases

Answer-Key: -

1- D 2- E 3- B 4- A 5- B 6- A 7- D 8- D 9- D 10- B