Buffer System

- **Acid** is a substance that can **release hydrogen ions** (protons H^+).
- **Base** is a substance that can **accept hydrogen ions.**
- **pH** is the concentration of hydrogen ions it determines the acidity of the solution
- The pH (Potential of Hydrogen) of a solution is the negative base 10 logarithm of its hydrogen ion concentration
- $$\mathbf{pH} = -\log_{10}[H^+]$$
- The "p" in pH or in pKa signifies -log

pH	$[H^+]$	
0	(10^{0})	1.0
1	(10^{-1})	0.1
2	(10^{-2})	0.01
3	(10^{-3})	0.001
4	(10^{-4})	0.0001
5	(10^{-5})	0.00001
6	(10^{-6})	0.000001
7	$(\mathbf{10^{-7}})$	**0.0000001**
8	(10^{-8})	0.00000001
9	(10^{-9})	0.000000001
10	(10^{-10})	0.0000000001
11	(10^{-11})	0.00000000001
12	(10^{-12})	0.000000000001
13	(10^{-13})	0.0000000000001
14	(10^{-14})	0.00000000000001

pH	$[H^+]$ (mol/l)	
1	10^{-1}	
2	10^{-2}	
3	10^{-3}	Increasing
4	10^{-4}	acidity
5	10^{-5}	
6	10^{-6}	
7	10^{-7}	Neutral
8	10^{-8}	
9	10^{-9}	
10	10^{-10}	Increasing
11	10^{-11}	alkalinity
12	10^{-12}	
13	10^{-13}	
14	10^{-14}	

- The following examples illustrate how to calculate the pH of acidic and basic solutions.
- ***Example 1:*** What is the pH of a solution whose hydrogen ion concentration is 3.2×10^{-4} mol/L?
- pH = -log $[H^+]$

  $= -\log (3.2 \times 10^{-4})$
- $= -\log (3.2) - \log(10^{-4})$
- $= -0.5 + 4$
- pH = 3.5

Dissociation Constants (Ka)

- When an acid loses a proton, its conjugate base is formed.
- The tendency of any acid (HA) to lose a proton and form its conjugate base (A-) is called **dissociation constants (Ka) and thus measure the strength of an acid.**

$$\underset{\text{Acid}}{HA} \rightleftharpoons \underset{\text{Conjugate Base}}{A^-} + H^+$$

$$K_a = \frac{[A^-]\,[H^+]}{[HA]}$$

- The stronger the acid, the greater its tendency to lose its proton.
- **<u>Strong acids</u>**: are acids that dissociate completely in solution like HCl. $HCl \longrightarrow Cl^- + H^+$
- **<u>Weak acids</u>**: are acids that dissociate only to a limited extent like H_2CO_3. $H_2CO_3 \longrightarrow HCO_3^- + H^+$
- The weak **acid** (proton donor) dissociates into a hydrogen ion H+ and an anionic component (A-), called the **conjugate base** (or salt).

pKa

- pKa = -log Ka $Ka = 10^{(-pKa)}$

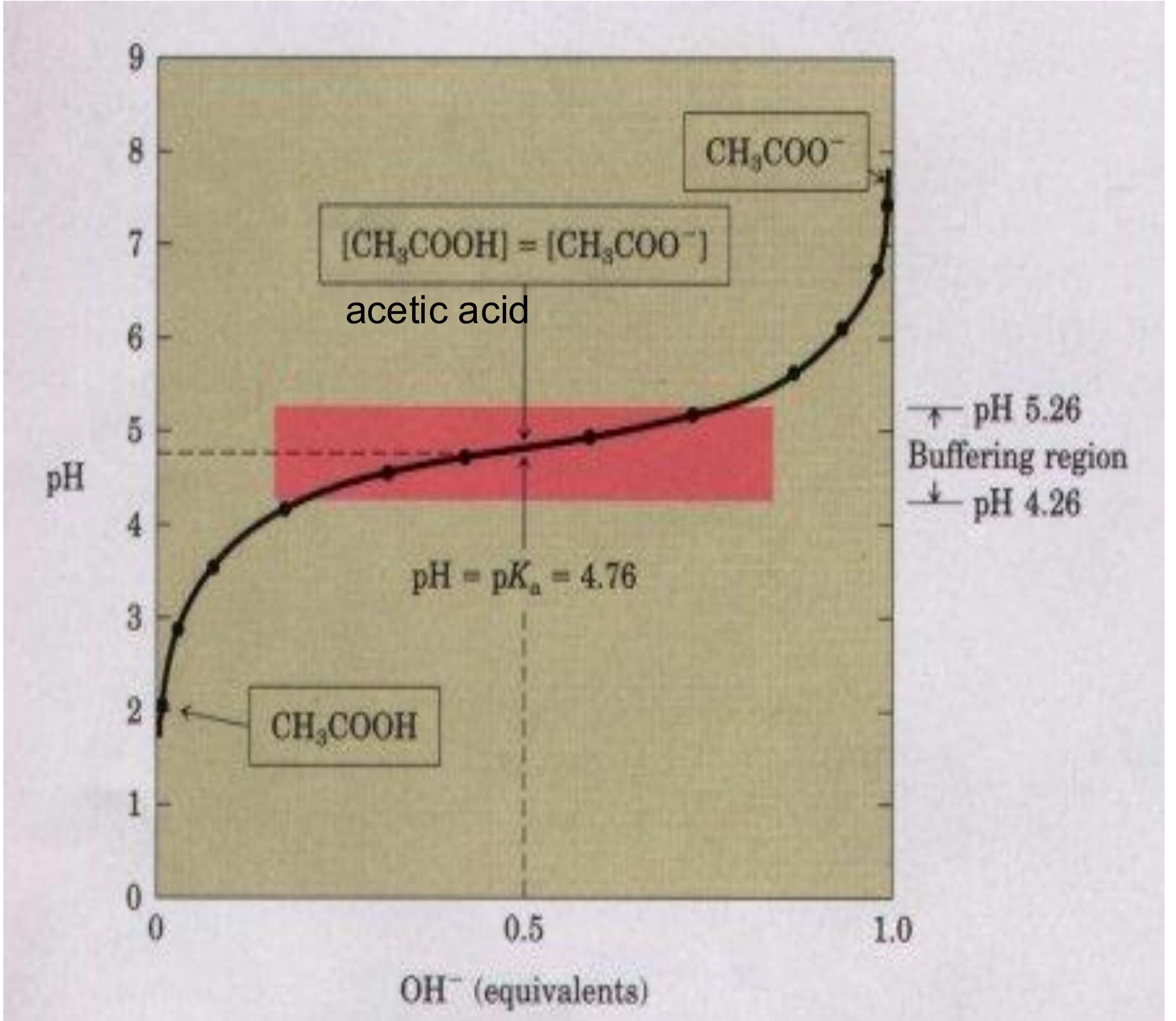
- **pKa of an acid is the pH at which 50% dissociation occurs**
- pKa value is easier to work with and remember than Ka value as of H and pH.

- Strong acids has strong tendency to dissociate and thus has high Ka value and low p*K*a value and thus the lower the pH the compound will produce in solution.

- Example a <u>strong acid</u> with Ka of 10^{7} has a pKa of -7, while a <u>weak acid</u> with Ka of 10^{-12} has a pKa of 12

How to determine the pKa of an acid

The maximum buffering capacity exists when the pH of the solution equals the pK' of the buffer, [conjugate base] = [acid], the buffer can then respond equally to both added acid and added base



Buffers

- A buffer is a solution that <u>resists</u> pH changes when acids or bases are added to the solution.
- Buffer solutions consist of a weak acid (undissociated acid) and its conjugate base (the form of the acid having lost its proton).

$$\underset{\text{Acid}}{HA} \rightleftharpoons \underset{\text{Conjugate Base}}{A^-} + H^+$$

- A buffer works because added acids (H^+) are neutralized by the conjugate base (A^-) which is converted to the acid (HA).
- Added bases are neutralized by the acid (HA), which is converted to the conjugate base (A^-).
- <u>Two factors determine the effectiveness of a buffer</u>:

1- its pKa relative to the pH of the solution

2- its concentration.

Henderson-Hasselbalch Equation

- Henderson-Hasselbalch adjusted equation shown below describe the relationship between the acid and its conjugate base with pH and pKa

$$\boxed{pH = pKa + \log \frac{A^{-}}{HA}}$$

$$\mathbf{pH = pK_a + \log_{10} \frac{[Conjugate\ Base]}{[Acid]}}$$

- The most effective buffers is when pH=pKa means it has equal concentrations of acid [HA] and its conjugate base [A-] **(**50% of both forms AH & A- present in solution).
- At pH = p*K*a ± 1 the buffer capacity falls to 33% of the maximum value. Therefore the buffer is effective one point up or down the pH pKa value.

Solving Problems Using the Henderson-Hasselbalch Equation

1. Calculate the pK_a of lactic acid, given that when the concentration of lactic acid is 0.010 M and the concentration of lactate is 0.087 M, the pH is 4.80.

$$pH = pK_a + \log \frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$pK_a = pH - \log \frac{[\text{lactate}]}{[\text{lactic acid}]}$$

$$= 4.80 - \log \frac{0.087}{0.010} = 4.80 - \log 8.7$$

$$= 4.80 - 0.94 = 3.9 \quad (\textit{answer})$$

2. Calculate the pH of a mixture of 0.10 M acetic acid and 0.20 M sodium acetate. The pK_a of acetic acid is 4.76.

$$pH = pK_a + \log \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$= 4.76 + \log \frac{0.20}{0.10} = 4.76 + 0.30$$

$$= 5.1 \quad (\textit{answer})$$

Organs controlling pH

1. Lungs function to regulate blood pH through bicarbonate system. The respiratory tract can adjust the blood pH upward in minutes by exhaling CO_2 from the body.

2. Kidney maintain a normal pH through:

A. Reabsorption of filtered bicarbonate.

B. Excretion of acids.

- The renal system can also adjust blood pH but this process takes hours to days to have an effect.

Acids in our Body

1. Volatile acid: represented in our body by carbonic acid which is originated from CO_2. So the main source of volatile acid is CO_2 which can evaporate and get rid of it through lungs.

2. Nonvolatile acids: include all acids produced in the body except the one that is produced from CO2 example lactic acid (fermentation), phosphoric acid, sulfuric acid (Protein breakdown), acetoacetic acid and *beta*-hydroxybutyric acid (ketone bodies).

- Nonvolatile acids elimination is through the kidney.

Transport of CO_2

- **CO_2 is carried in the blood by 3 ways:**

1- About 10% of the CO_2 in blood is simply dissolved in plasma.

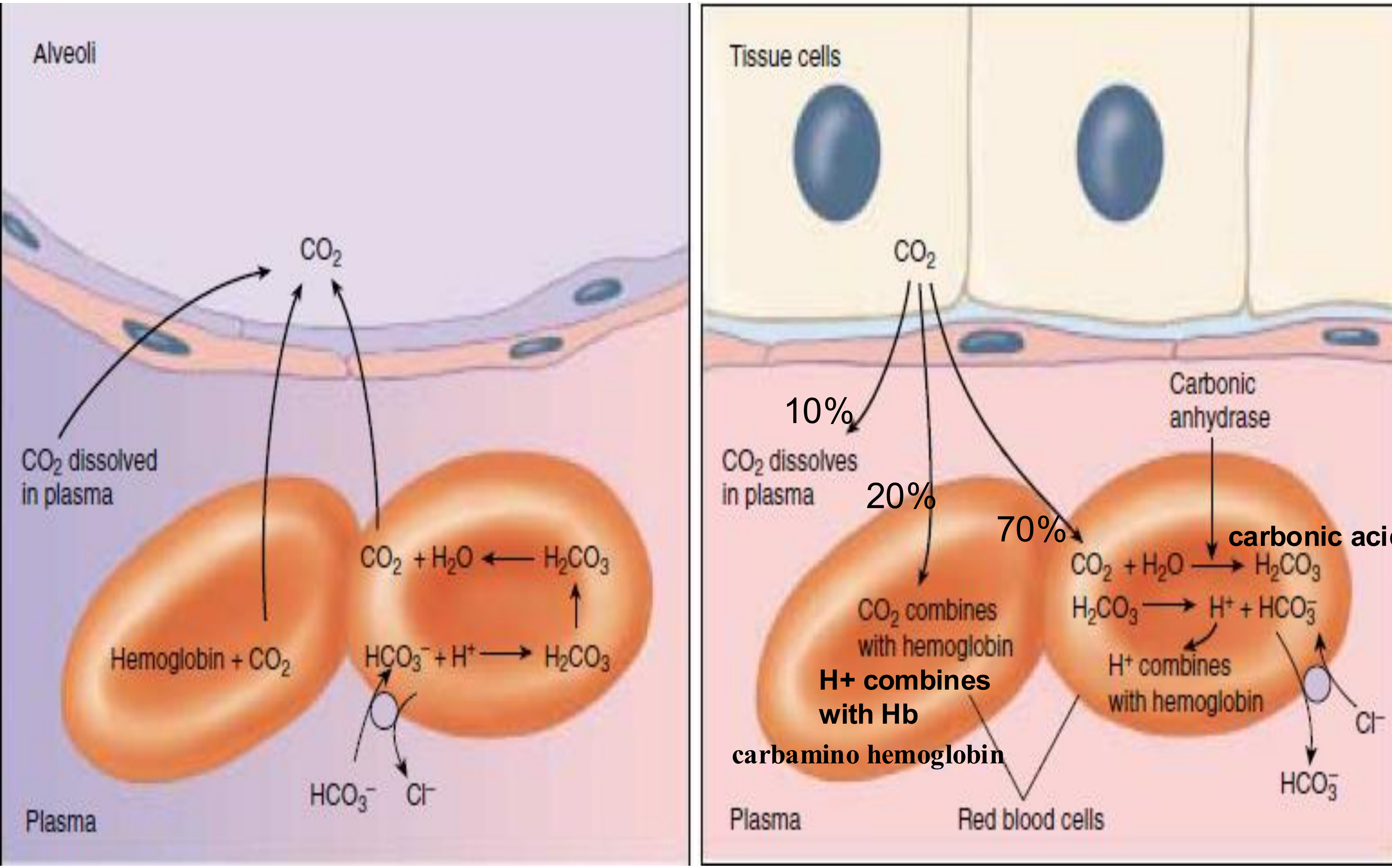
2- About 20% of CO_2 react nonenzymaticaly with amino groups (NH_2 terminal amino group) of hemoglobin to form **carbamino hemoglobin (carbamate)**

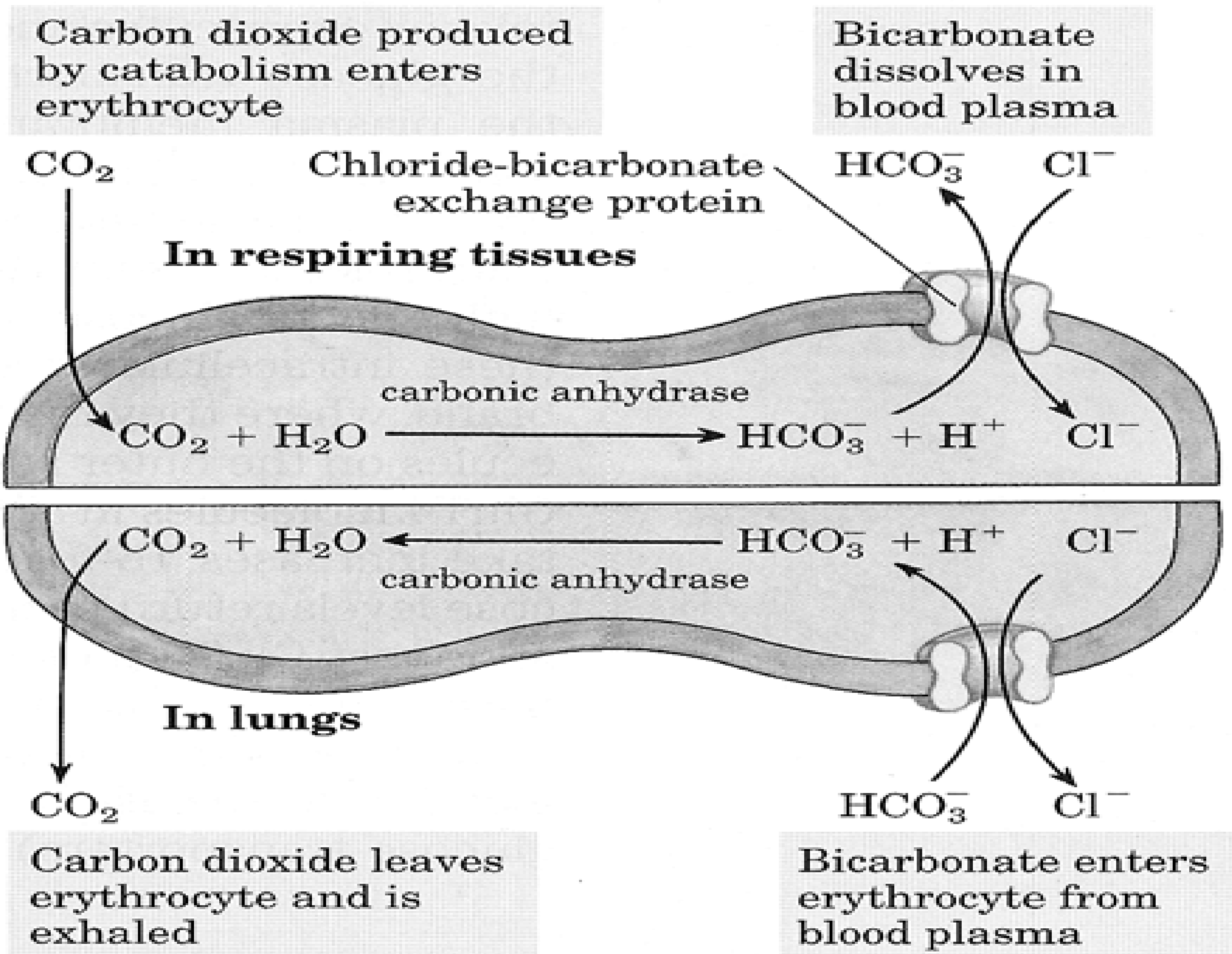
- $$\mathbf{HbNH_2 + CO_2 \leftrightarrow HbNHCOO^- + H^+}$$
- The excess H^+ produced binds with Hb and stabilize the deoxy form and promoting the release of O_2 to cells.

3- The remaining 70% of the CO_2 diffuses into the red blood cells, where the enzyme carbonic anhydrase catalyzes the combination of CO_2 with water (hydration reaction) to form carbonic acid (H_2CO_3).

- Carbonic acid dissociates into bicarbonate (HCO_3–) and hydrogen (H+) ions. The H+ binds to hemoglobin and force $Hb(O_2)_4$ to dissociate its O_2 which diffuses out of RBC. While the bicarbonate moves out of the erythrocyte into the plasma via a transporter that exchanges one chloride ion for a bicarbonate (this is called the "chloride shift").
- The blood carries bicarbonate to the lungs. The lower pCO_2 of the air inside the alveoli causes the carbonic anhydrase reaction to proceed in the reverse direction that leads to formation of CO_2. The CO_2 diffuses out of the red blood cells and into the alveoli, so that it can leave the body in the next exhalation.

Transport of CO_2 by the blood




Carbon dioxide produced by catabolism enters erythrocyte
Bicarbonate dissolves in blood plasma
CO_2
Chloride-bicarbonate exchange protein
HCO_3^-
Cl^-
In respiring tissues
carbonic anhydrase
$CO_2 + H_2O \longrightarrow HCO_3^- + H^+$
Cl^-
$CO_2 + H_2O \longleftarrow HCO_3^- + H^+$
Cl^-
carbonic anhydrase
In lungs
CO_2
HCO_3^-
Cl^-
Carbon dioxide leaves erythrocyte and is exhaled
Bicarbonate enters erythrocyte from blood plasma

The Bicarbonate Buffer System

- Bicarbonate and other buffers normally maintain the pH of extracellular fluid in human's body between 7.35 and 7.45.
- The major source of metabolic acid in the body is the gas CO_2, produced principally from fuel oxidation in the TCA cycle (tricarboxylic cycle).

$$\mathbf{CO_2 + H_2O \underset{}{\overset{\text{carbonic anhydrase}}{\rightleftharpoons}} H_2CO_3 \underset{\text{carbonic anhydrase}}{\rightleftharpoons} HCO_3^- + H^+}$$

- The pH of a bicarbonate buffer system depends on the concentration of CO2 (H_2CO_3) as proton donor and HCO_3^- as proton acceptor.
- This bicarbonate buffer system works with kidney as an open system to regulate body pH: the lung excel CO2 and the kidney reabsorb or excrete bicarbonate.

The Bicarbonate Buffer System

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

When acid is added:

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

When a base is added:

$$H_2CO_3 + OH^- \rightleftharpoons HCO_3^- + H_2O$$

- **The Buffer Equation(s)**: The Henderson-Hasselbalch equation, is important for understanding buffer action in the blood and tissues
- **For bicarbonate system in our blood**: the normal average level of plasma bicarbonate of plasma is 24 mmol/litre. The normal CO_2 dissolved concentration in blood is 1.2 mmol/L (therefore the ratio of HCO_3^- to H_2CO_3 at pH 7.4 is 20 to 1 because most of the body's metabolic wastes, such as lactic acid and ketones, are acids). The pKa for carbonic acid is 6.1:
- pH = 6.1 + log 24/1.2
- = 6.1 + log20
- = 6.1 + 1.3
- = 7.4 which is the normal pH of arterial blood.

$$pH = pKa + \log \frac{[HCO_3^-]}{Pco_2}$$

- **What happen when CO_2 is increased**? Suppose that the CO_2 concentration doubled from 1.2 to 2.4. The doubling of CO_2 is achieved by increasing the PCO_2 in the atmosphere. Thus calculating pH from the above equation = **7.1**
- **What happen when increasing the concentration of HCO_3^-.** Increasing the conc of HCO_3^- from 24 to 48 will cause a change in pH from 7.4 to **7.7**
- Thus, removing CO_2 through lungs from the blood helps increase the pH and removing HCO_3^- from the blood helps lower the pH.

- The respiratory center in brain which controls the rate of breathing, is sensitive to changes in pH.
- As the pH falls, individuals breathe more rapidly and expire more CO2.
- As the pH rises, they breathe more slowly.
- Thus, the rate of breathing contributes to regulation of pH through its effects on the dissolved CO2 content of the blood.

Hemoglobin as Protein Buffer

- Hemoglobin in blood is made up of 574 amino acid, 36 of them are histidine
- The pKa of the various histidine residue (imidazole groups) on the different plasma proteins range from about 5.5 to about 8.5 depending on its microenvironment (because its imidazole ring can interact with surrounding amino acids) thus providing a broad spectrum of buffer pairs.
- As stated before, the carbonic acid dissociates into bicarbonate anion and H+.
- The H+ released bind the side chain of the amino acid histidine (His-146 (β) in the two β chains of hemoglobin.
- Increasing H+ (in tissues) causes the protonation of histidine (His-146 (β) that promote the formation of salt bridge with Aspartic acid 94 in the same chain which stabilize the T-state.
- Only a small number of hydrogen ions generated in the blood remains free not attached to Hb. This explains why the acidity of venous blood (pH = 7.35) is only slightly greater than that of arterial blood (pH = 7.45).

Phosphate buffer the Intracellular pH

- Phosphoric acid (H_3PO_4) dissociate to conjugate base dihydrogen phosphate ion ($H_2PO^{-}{}_4$) and H+
- Dihydrogen phosphate ion dissociate to conjugate base hydrogen phosphate ($HPO^{2-}{}_4$) and H+ with a pKa of 7.2 which is very close to physiologcal pH
- **$H_2PO_4^- \leftrightarrow HPO_4^{-2} + H^+$**
- Thus, phosphate anions play a major role as an intracellular buffer in the red blood cell and in other types of cells, where their concentration is much higher than in blood and interstitial fluid.
- Organic phosphate anions, such as glucose 6-phosphate and ATP, also act as buffers.

- **Respiratory acidosis**
- Blood pH reflects changes in pH in tissues and values above or below the normal range 7.35—7.45 indicates a potential pathological condition. Blood pH below 7 or above 7.8 are life threatening and medical intervention is necessary. If the blood pH falls below 7.35 the condition is referred to as an acidosis and above 7.45 as alkalosis.
- Conditions of acidosis and alkalosis are divided according to the source into metabolic or respiratory.
- Respiratory acidosis is caused by hypoventilation so there is retention of CO_2 and a drop in pH thus the concentration of dissolved CO_2 in the blood increases, making the blood too acidic and is caused by condition restricting the exhaling of CO_2 from the lungs such as
- Diseases of the airways (such as asthma and chronic obstructive lung disease)
- Diseases of the chest (such as sarcoidosis)
- Diseases affecting the nerves and muscles that "signal" the lungs to inflate or deflate
- Depression of the respiratory centres in the medulla by different drugs.
- Severe obesity, which restricts how much the lungs can expand

- **Respiratory alkalosis**
- Results from hyperventilation that causes too much dissolved CO_2 to be removed from the blood, which decreases the carbonic acid concentration, which raises the blood pH. Often, the body of a hyperventilating person will react by fainting, which slows the breathing.
- Respiratory alkalosis may be caused from hysteria (any psychological dysfunction of unknown cause), central nervous system diseases, overdose of some drugs (e.g salicylate) and fever.