Wroclaw Medical University

Calculating the pH of a buffer

Buffer capacity

pH of blood

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Buffers

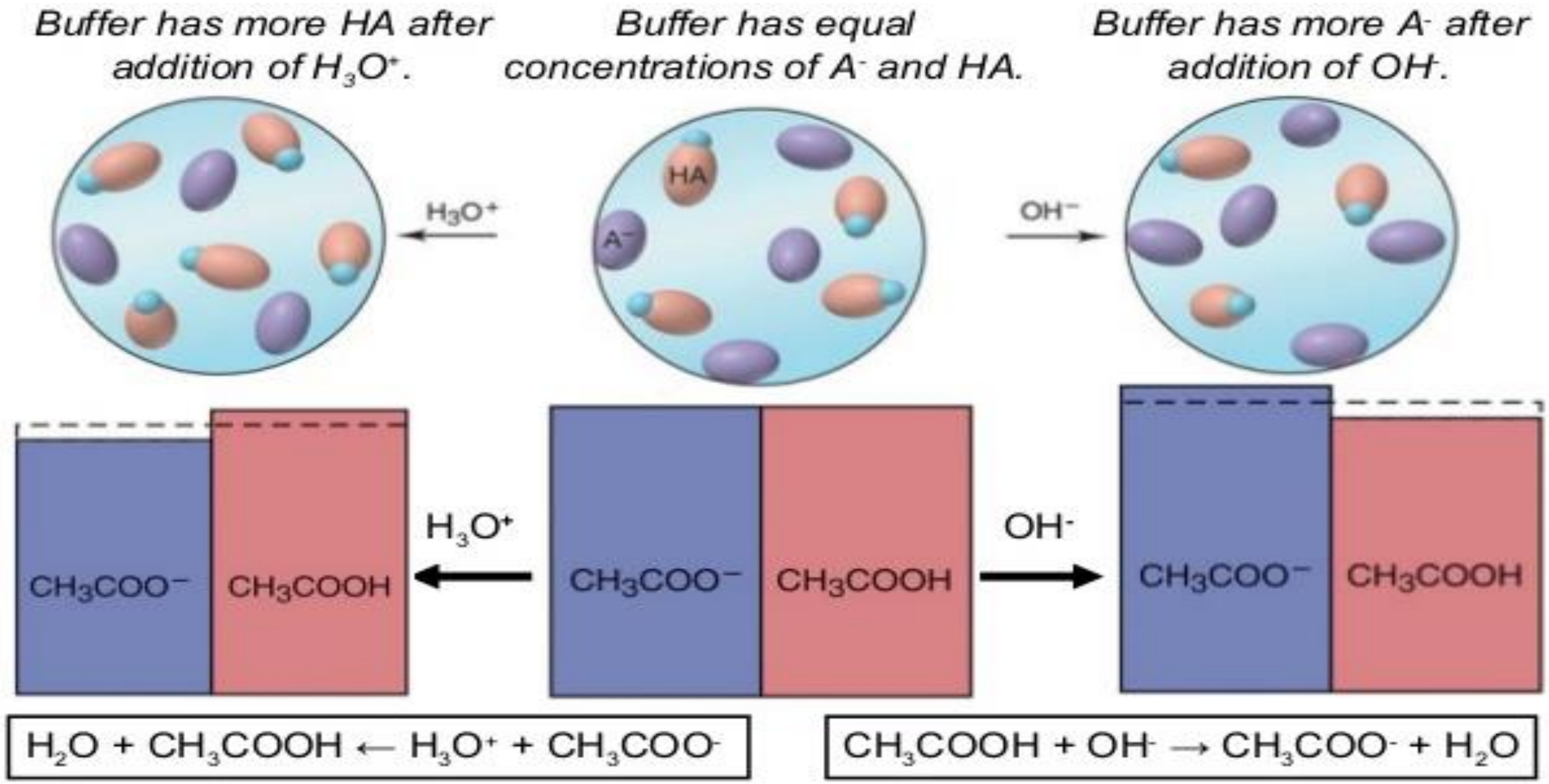
Buffers are chemicals that resist a change in the pH of a solution when either acids or bases are added to the solution.

The buffers in the body fluids contain salts of either weak acids or weak bases that combine with H+ when H+ increases in those fluids, or release H+ when H+ decreases in those fluids.

Buffers tend to keep the H+ concentration, and thus the pH, within a narrow range of values because of these characteristics.

What is a buffer in chemistry and how does it work?

- A **buffer** is simply a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. **Buffers work** by reacting with any added acid or base to control the pH.



Example 1 An acetate buffer is made by mixing 250 mL of 0.5 molar acetic acid and 250 mL of 1 molar sodium acetate. What will be the pH value of the prepared buffer? The pKa of acetic acid is 4.7.

$$pH = pK_a + log\frac{C_{salt}}{C_{acid}} = pK_a + log\frac{n_{salt}}{n_{acid}}$$

$$pH = pK_a + \log\frac{C_{salt}}{C_{acid}} = 4.7 + log\frac{1}{0.5} = 5.00$$

or

$$pH = pK_a + \log\frac{n_{salt}}{n_{acid}} = 4.7 + log\frac{0.25\times1}{0.25\times0,5} = 5.00$$

Example 2

What is the pH value of a buffer prepared by adding 70 mL of 0.2 molar CH_3COOH to 60 mL of a 0.5 molar CH_3COONa solution? pKa = 4.7.

- What is the pH value of this buffer after adding 20 mL of 1 molar HCl
- What is the pH value of this buffer after adding 2 mL of 2 molar NaOH

$$pH = pK_a + log\frac{C_{salt}}{C_{acid}} = pK_a + log\frac{n_{salt}}{n_{acid}}$$

$$pH = 4.7 + log\frac{0.5}{0.2} = 4.7 + 0.4 = 5.1$$

- What is the pH value of this buffer after adding 20 mL of 1 molar **HCl**

$$CH_3COONa \quad + \quad HCl \quad \rightarrow \quad CH_3COOH \quad + \quad NaCl$$

CH_3COONa	HCl	CH_3COOH	
n=C×v=0.5×0.06=0.03		n=C×v=0.2×0.07=0.014	**at the beginning**
- 0,02	n=1×0.02=0,02	+ 0.02	**for the reaction**
0,01		0.034	**after the reaction**

$$pH = 4.7 + log\frac{0.01}{0.034} = 4.7 - 0.53 = 4.17$$

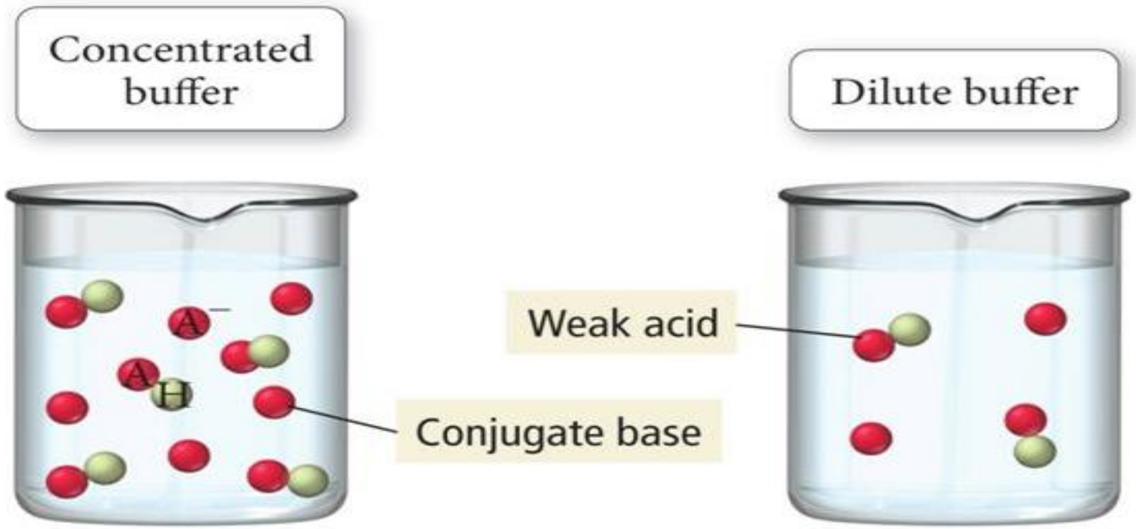
- What is the pH value of this buffer after adding 2 mL of 2 molar **NaOH**

$$CH_3COOH \quad + \quad NaOH \quad \rightarrow \quad CH_3COONa \quad + \quad H_2O$$

CH_3COOH	NaOH	CH_3COONa	
n=C×v= 0.2×0.07=0.014		n=C×v=0.5×0.06=0.03	**at the beginning**
- 0.004	n=2×0.002=0,004	+ 0.004	**for the reaction**
0.01		0.034	**after the reaction**

$$pH = 4.7 + log\frac{0.034}{0.01} = 4.7 + 0.53 = 5.23$$

Buffering Capacity



A concentrated buffer can neutralize more added acid or base than a dilute buffer.

- Buffering capacity is the amount of acid or base that can be added to a buffer without causing a large change in pH.

- The buffering capacity increases with increasing absolute concentration of the buffer components.

How to calculate buffer capacity?

Example 3

$[CH_3COOH]=0,16$ mol/L
$[CH_3COONa]=0,04$ mol/L
} pH=4,13

$$\beta = \frac{\Delta C}{\Delta pH}$$

β – buffer capacity

ΔC – molar concentration added strong acid (base)

ΔpH – change of pH

For 100 mL of buffer was added 1 mL of 0.9 M HCl, calculate the buffer capacity:

Step 1: Calculate pH of the buffer after adding 1 mL 0.9M HCl →

0.9 mol	------	1000 mL
0.0009 mol	------	1 mL

$$pH = pK_a + \log\frac{n_{salt}}{n_{acid}} = 4.73 + log\frac{0.004-0.0009}{0.016+0.0009} = 3.99$$

Step 2: Calculate ΔpH of the buffer: Δ<u>pH = 4.13-3.99 = 0.14</u>

Step 3: Calculate β

$$\beta = \frac{\Delta C}{\Delta pH} = \frac{0,0089\ mol/L}{0.14} = 0.064$$

→

0.0009 mol	------	101 mL
0.0089 mol	------	1000 mL

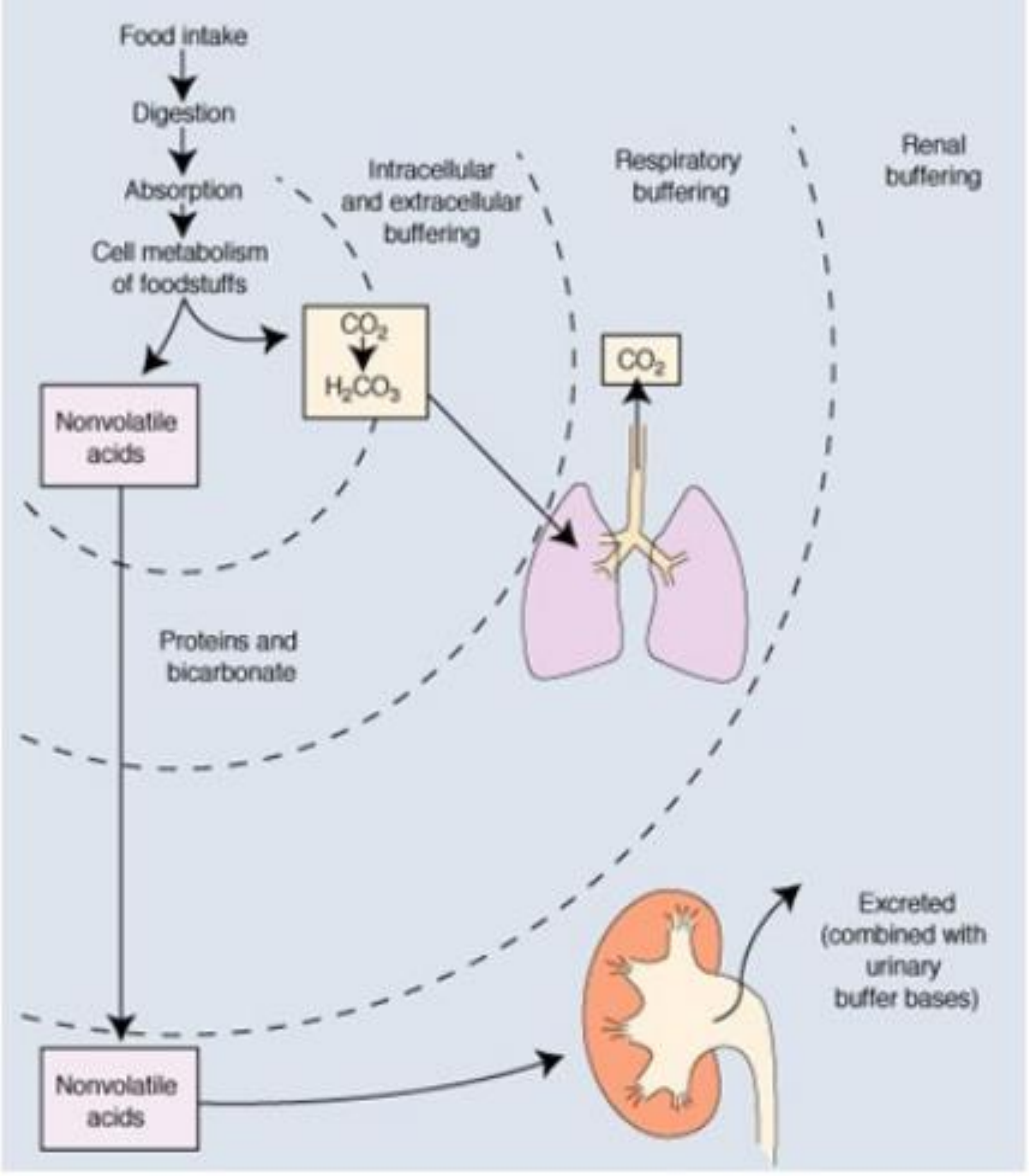
Mechanisms of pH regulation in the body

Organ regulation:

- pulmonary regulation (respiratory system)
- kidney regulation
- liver regulation
- bone regulation

Buffer regulation:

- **Protein buffer (including hemoglobin buffer):** Hemoglobin and Protein of plasma
- **Phosphate buffer:** $H_2PO_4^-/HPO_4^{2-}$
- **Bicarbonate buffer:** $H_2CO_3^-/H_2CO_3$



Buffers in Biological Systems

Biological functions: The equation of buffer system in blood is,

$$H_2CO_3 + H_2O \leftrightarrow H_3O^+ + HCO_3^-$$

If any acid (H+) is added, the reaction will be,

$$H^+ \text{ (acid)} + HCO_3^- \leftrightarrow H_2CO_3$$

$$H_2CO_3 \rightarrow H_2O + CO_2$$

If any base (OH-) is added, the reaction will be,

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

As such, the buffer of blood neutralizes the addition of acids and bases and keeps the pH constant.

Why is pH so important?

In body: [H^+] ~ nmol/L, while [K^+], [Cl^-], [HCO_3^-] ~ mmol/L

<u>However, [H^+] is crucial:</u>

- **pH affects function of proteins (hydrogen bonds → 3-D structure = function**
- **All the know low molecular weight and water soluble biosynthetic intermediates possess, groups that are almost completely ionised at neutral pH**
  - ✓ **pH-dependent ionisation (i.e. charge) serves to an efficient intracellular trapping of ionised compounds within the cell and its otganelles**

Respiratory system

The respiratory system responds rapidly to a change in pH and helps bring the pH of body fluids back toward normal. Increasing CO_2 levels and decreasing body fluid pH stimulate neurons in the respiratory center of the brain and cause elevated rate and depth of ventilation. As a result, CO_2 is eliminated from the body through the lungs at a greater rate, and the concentration of CO_2 in the body fluids decreases. As CO_2 levels decline, the concentration of H^+ also declines. The pH therefore rises back toward its normal range

If CO_2 levels become too low or the pH of the body fluids is elevated, the rate and depth of respiration decline. As a con-sequence, the rate at which CO_2 is eliminated from the body is reduced. Carbon dioxide then accumulates in the body fluids because it is continually produced as a by-product of metabolism. As CO_2 accumulates in the body fluids, so does H+, resulting in a decreased pH.

Kidney regulation

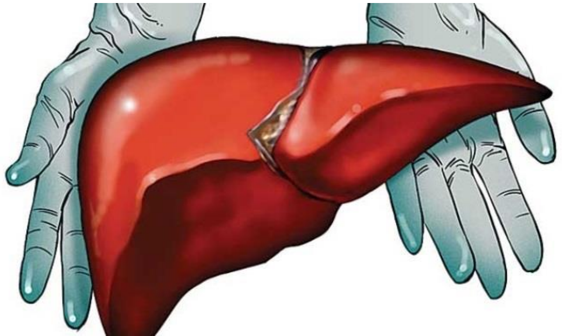
The nephrons of the kidneys secrete H+ into the urine and therefore can directly regulate the pH of the body fluids. The kidney is a powerful regulator of pH, but it responds more slowly than does the respiratory system. Cells in the walls of the distal convoluted tubule are primarily responsible for the secretion of H^+. As the pH of the body fluids drops below normal, the rate at which the distal convoluted tubules secrete H+ increases.

At the same time, reabsorption of HCO_3^- increases. The increased rate of H^+ secretion and the increased rate of HCO_3^- reabsorption both cause the blood pH to rise toward its normal value. On the other hand, as the body fluid pH elevates above normal, the rate of H+ secretion by the distal con-voluted tubules declines, and the amount of HCO_3^- lost in the urine increases. Consequently, the blood pH drops toward its normal value.

Liver and Bone regulation

<u>Metabolic regulation of liver:</u>

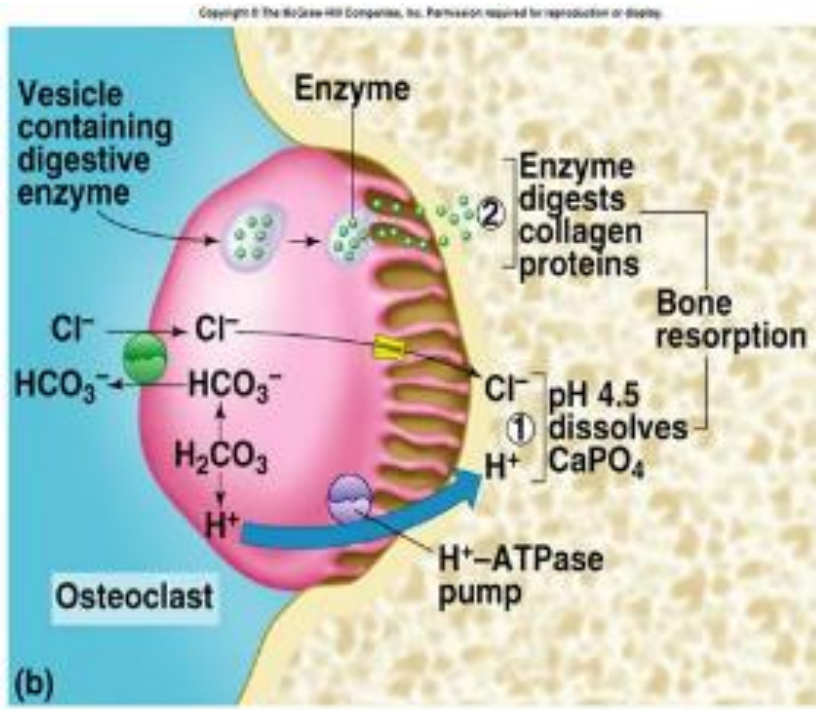
- Metabolism of organic acids anions such as lactate, ketones and mino acids
- Metabolism of ammonium
  - Conversion of NH_4^+ to urea in the liver consumes HCO_3^-
  - Production of glutamate → urine buffering
- Production of plasma proteins e.g. albumin contributing to the anion gap



<u>Bone</u> inorganic matrix consists of hydroxyapatite crystals $(Ca_{10}(PO_4)_6(OH)_2$

Bone can take up H^+ in exchange for Ca^{2+}, Na^+ and K^+ (ionic exchange)

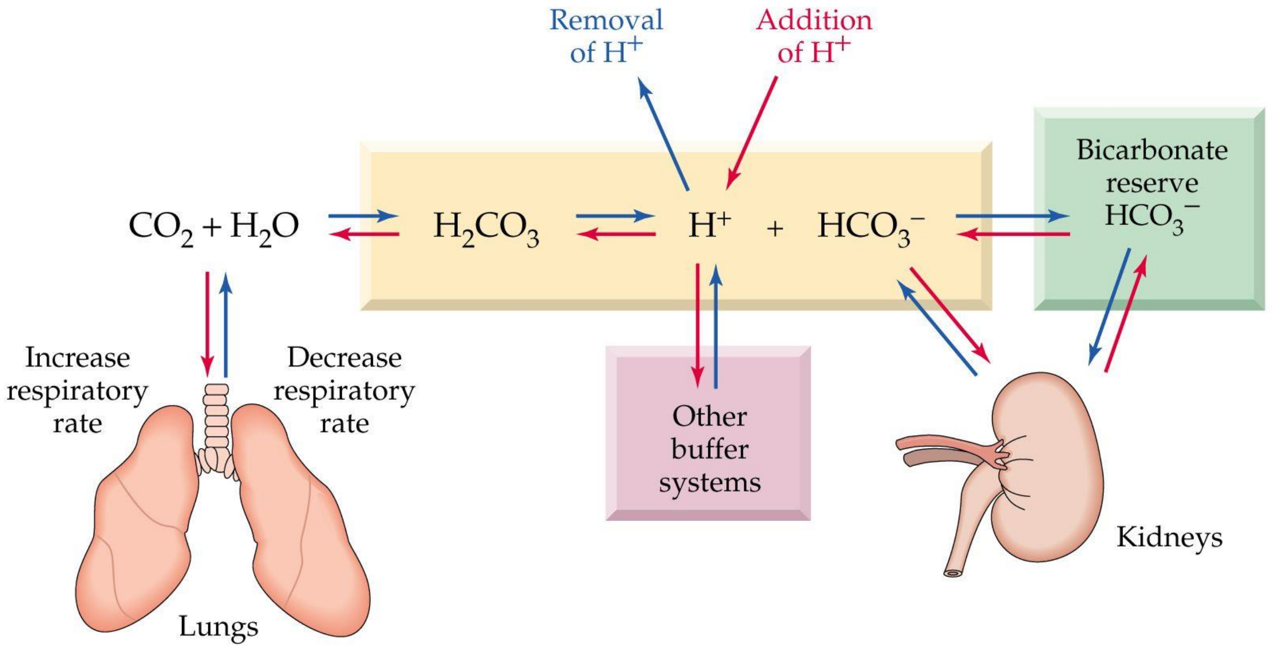
Release of HCO_3^-, CO_3^- or HPO_4^{2-}



Bicarbonate buffer

The most important buffer system

- **Buffer capacity is strong, its content account for 53% of total buffer system**
- **A open system:**
  - **Regulated by respiratory regulation**
  - **Regulated by renal regulation**



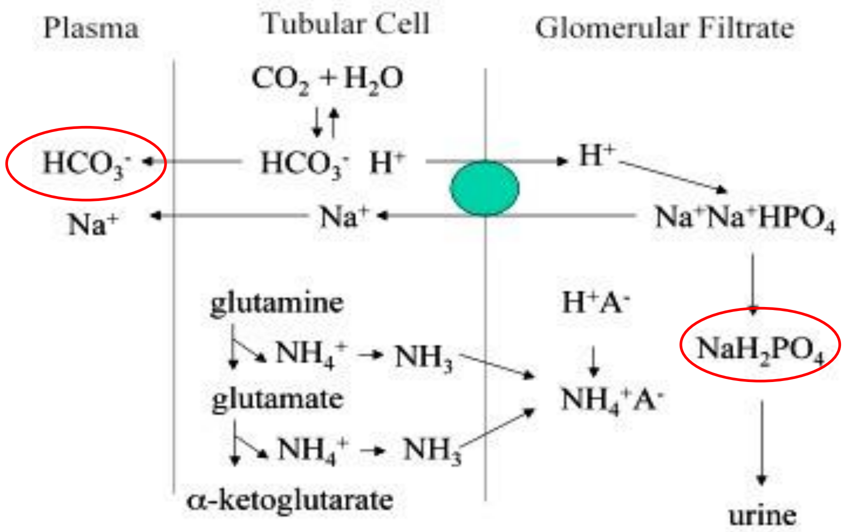
Phosphate buffer

Kidneys help regulate blood pH by excreting H^+ and reabsorbing HCO_3^-

- **Most of the H^+ secretion occurs across the walls of the proximal convoluted tubule in exchange for Na^+ (antiport mechanism → moves Na^+ and H^+ in opposite directions)**
- **Normal urine normally is slightly acidic becease the kidneys reabsorb almost all HCO_3^- and excrete H^+ (returns blood pH back to normal range)**

$$H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$$

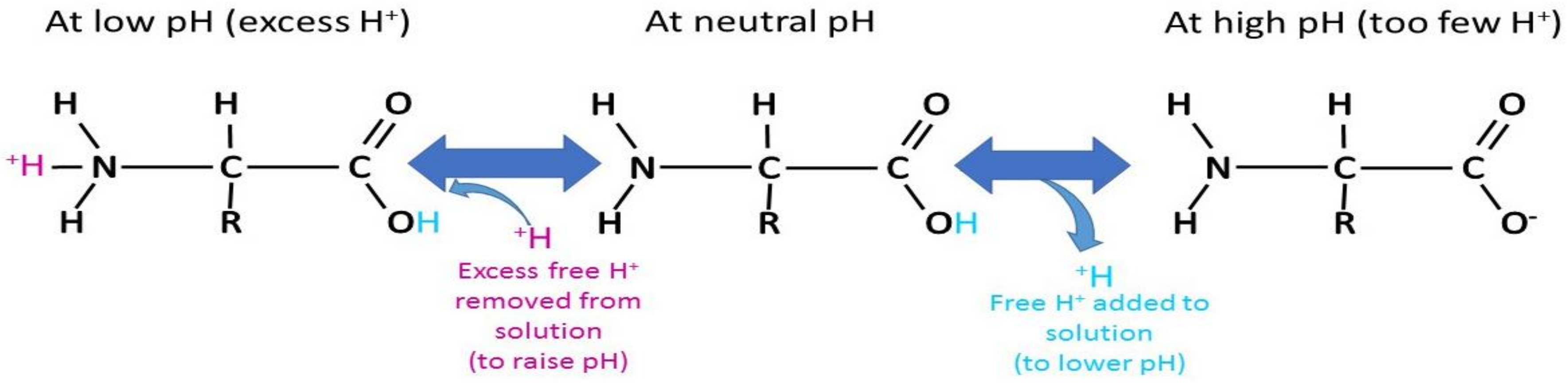
$$KH_2PO_4^- = 6,2\times10^{-8},\ pK_2=6.8$$



- **In blood:** the ratio $HPO_4^{2-} : H_2PO_4^- = 4:1$
- **In urine**: the ratio $HPO_4^{2-} : H_2PO_4^- = 1:4$

Protein buffer

- **Behaves as a buffer in both plasma and cells**
- Hemoglobin is by far the most important protein buffer.
- Most important intracellular buffer (**ICF**)
- The most plentiful buffer of the body
- Protein are excellent buffer because they contain both acid and base groups that can give up ot take up H^+
- **Proteins are extremely abundant in the cell**
- The more limited number of proteins in the plasma reinforce the bicarbonate system in the **ECF**



Regulation of Acid-Base Balance in Human Body

The concentration of H+ in the body fluids is reported as the pH. The body fluid pH is maintained between 7.35 and 7.45; any deviation from that range is life-threatening. Consequently, the mechanisms that regulate body fluid pH are critical for survival. **The pH of body fluids is mainly controlled by three factors: buffers, the respiratory system, and the kidneys.** When the pH of body fluids is not properly maintained, the result is acidosis or alkalosis.

Different buffer systems assume dominant roles in different parts of the body

Extracellular Fluid

Major Buffer

- Bicarbonate buffer system

Minor Buffer

- Intracellular proteins
- Phosphate buffer system

Blood

Major Buffer

- Bicarbonate buffer system
- Hemoglobin

Minor Buffer

- Plasma proteins
- Phosphate buffer system

Intracellular Fluid

Major Buffer

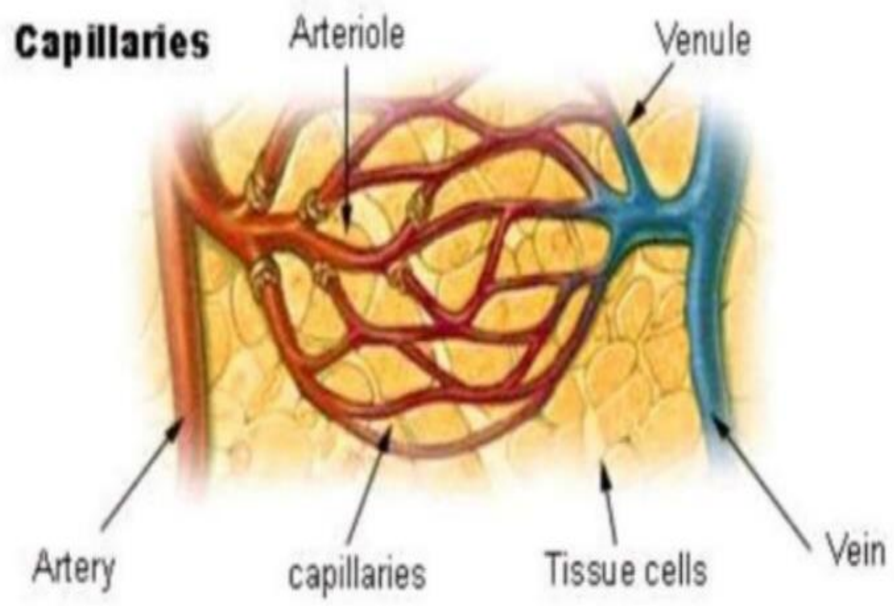
- Proteins
- Phosphate

Urine

Major Buffer

- Ammonia
- Phosphate

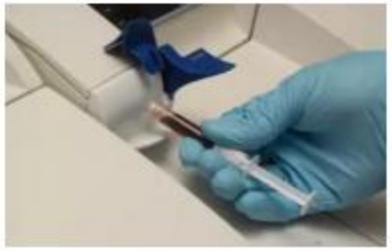
Blood Gas Testing



Blood collection under anaerobic conditions

- From the artery

- Capillary blood
  - Indications: to draw a small amount of blood
    - For infants and young children



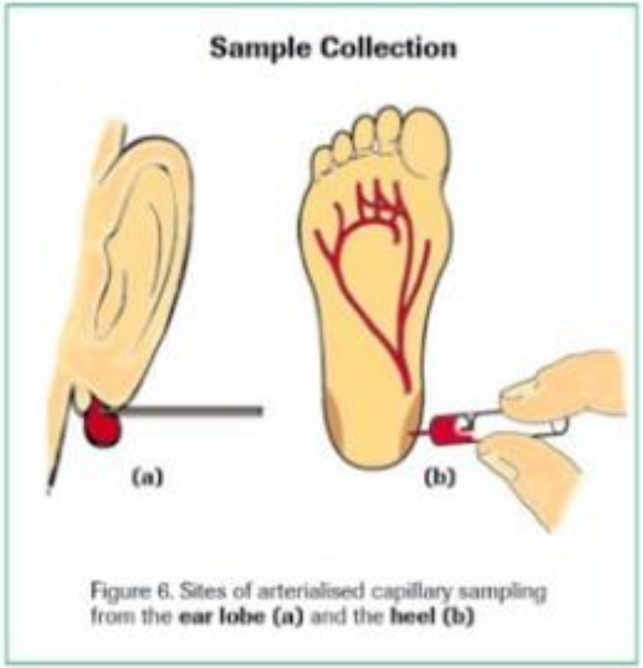


Figure 6. Sites of arterialised capillary sampling from the **ear lobe (a)** and the **heel (b)**

Normal Arterial and Venous values

	Arterial blood (interval)		Venous blood
pH	7.40	7.38 - 7.42	7.33 - 7.43
H^+ (nmol/l)	40	36 – 44	
pCO_2 (mmHg/kPa)	40 / 5.3	35 – 45 / 5.1 – 5.5	41 – 51
HCO_3^- (mmol/l)	25	22 - 26	24 - 28
BE	±2		
AG (mEq/l)	12	10 - 14	
Hb saturation (%)	95	80 – 95	70 – 75
pO_2 (mmHg)	95	80 – 95	35 – 49

pH of human blood 7.35-7.45 (or 7.38-7.42)

The physiological levels of the main gas parameters of the acid-base balance are:

pO_2: 70-95 mmHg

pCO_2: 35—45 mmHg

HCO_3^- concentration: 22-26 mmmol/L

Hb saturation by oxygen: 95-98%

$$pH = pK_a + \log\frac{HCO_3^-}{\propto \times pCO_2} = 6.1 + log\frac{22}{0.03 \times 35} = 7.42$$

$$pH = pK_a + \log\frac{HCO_3^-}{\propto \times pCO_2} = 6.1 + log\frac{26}{0.03 \times 45} = 7.38$$

α- solubility of CO_2 in water at 37 ℃

Acidosis

Acidosis occurs when the blood pH falls below 7.35.
The central nervous system malfunctions, and the
individual becomes disoriented and, as the condition worsens,
may become comatose. Acidosis is separated into two categories.

Respiratory acidosis results when the respiratory system
is unable to eliminate adequate amounts of CO_2.
Carbon dioxide accumulates in the circulatory system,
causing the pH of the body fluids to decline.

Metabolic acidosis results from excess production of acidic substances,
Such as lactic acid and ketone bodies, because of increased metabolism
or decreased ability of the kidneys to eliminate H+ in the urine.

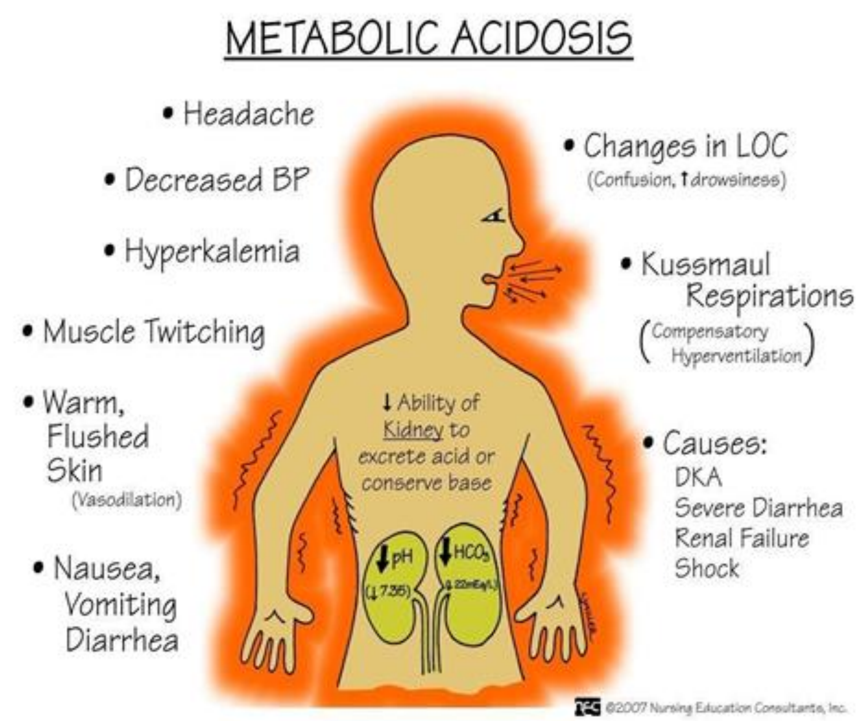
Alkalosis

Alkalosis occurs when the blood pH increases above7.45.
A major effect of alkalosis is hyperexcitability of the nervous system.
Peripheral nerves are affected first, resulting in spontane-ous nervous stimulation of muscles. Spasms and tetanic contrac-tions result, as can extreme nervousness or convulsions.
Tetany of respiratory muscles can cause death.

Respiratory alkalosis results from hyperventilation, as can occur in response to stress.

Metabolic alkalosis usually results from the rapid elimination of H^+ from thebody, as occurs during severe vomiting or when excess aldosterone is secreted by the adrenal cortex.

GASO	pH	$PaCO_2$	HCO_3
Acidose Respiratória	↓	↑	normal
Alcalose Respiratória	↑	↓	normal
Acidose Metabólica	↓	normal	↓
Alcalose Metabólica	↑	normal	↑


METABOLIC ACIDOSIS
• Headache
• Decreased BP
• Hyperkalemia
• Muscle Twitching
• Warm, Flushed Skin (Vasodilation)
• Nausea, Vomiting Diarrhea
• Changes in LOC (Confusion, ↑drowsiness)
• Kussmaul Respirations (Compensatory Hyperventilation)
↓Ability of Kidney to excrete acid or conserve base
↓pH (↓7.35)
↓HCO3 (↓22mEq/L)
• Causes:
DKA
Severe Diarrhea
Renal Failure
Shock
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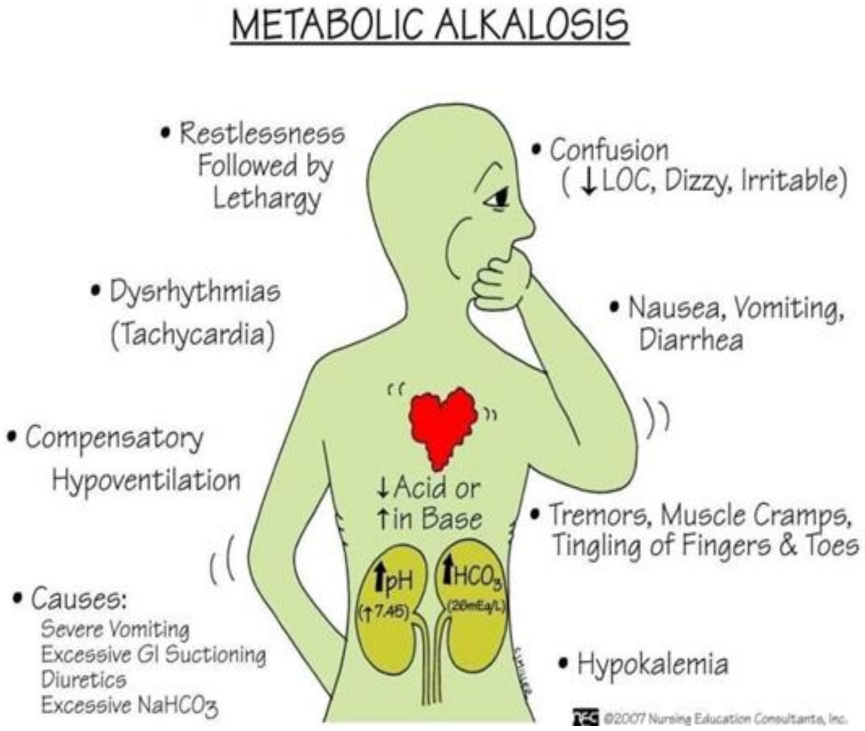
METABOLIC ALKALOSIS
• Restlessness Followed by Lethargy
• Dysrhythmias (Tachycardia)
• Compensatory Hypoventilation
• Causes:
Severe Vomiting
Excessive GI Suctioning
Diuretics
Excessive NaHCO3
• Confusion (↓LOC, Dizzy, Irritable)
• Nausea, Vomiting, Diarrhea
↓Acid or ↑in Base
↑pH (↑7.45)
↑HCO3 (26mEq/L)
• Tremors, Muscle Cramps, Tingling of Fingers & Toes
• Hypokalemia
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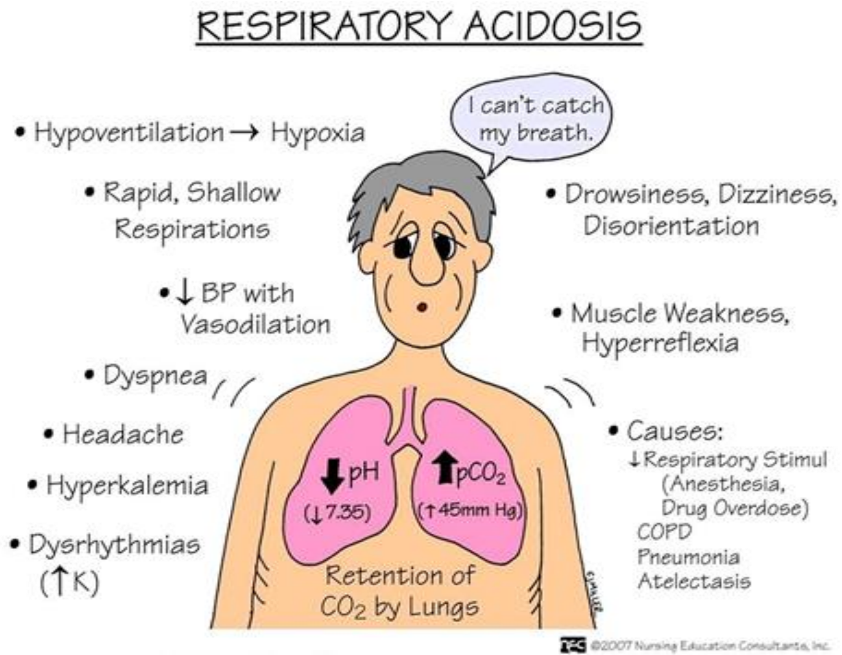
RESPIRATORY ACIDOSIS
I can't catch my breath.
• Hypoventilation → Hypoxia
• Rapid, Shallow Respirations
• ↓BP with Vasodilation
• Dyspnea
• Headache
• Hyperkalemia
• Dysrhythmias (↑K)
• Drowsiness, Dizziness, Disorientation
• Muscle Weakness, Hyperreflexia
• Causes:
↓Respiratory Stimul (Anesthesia, Drug Overdose)
COPD
Pneumonia
Atelectasis
↓pH (↓7.35)
↑pCO2 (↑45mm Hg)
Retention of CO2 by Lungs
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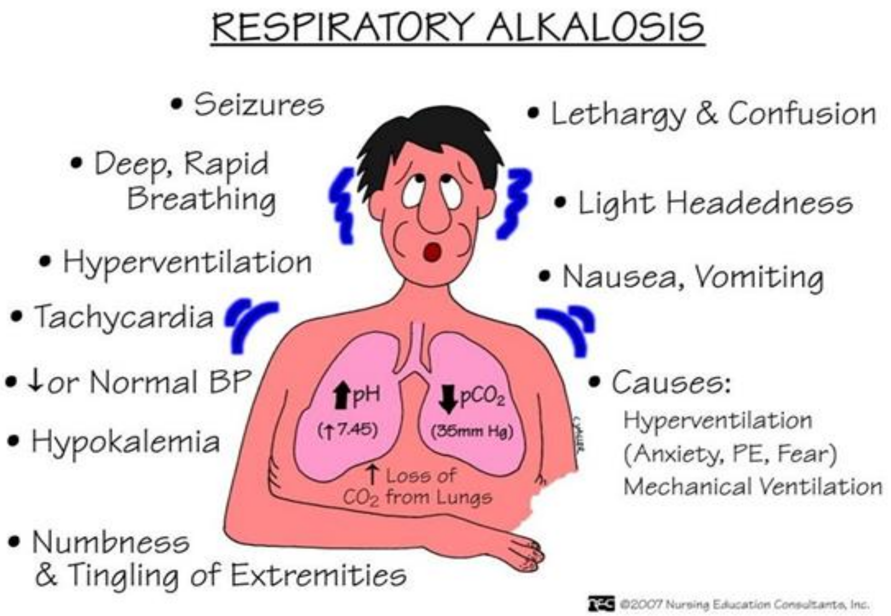
RESPIRATORY ALKALOSIS
• Seizures
• Deep, Rapid Breathing
• Hyperventilation
• Tachycardia
• ↓or Normal BP
• Hypokalemia
• Numbness & Tingling of Extremities
• Lethargy & Confusion
• Light Headedness
• Nausea, Vomiting
• Causes:
Hyperventilation (Anxiety, PE, Fear)
Mechanical Ventilation
↑pH (↑7.45)
↓pCO2 (35mm Hg)
↑Loss of CO2 from Lungs
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FINAL SLIDE

Thank you for your attention

This presentation has been prepared for educational purposes as part of the Medicinal Chemistry course for Students of Faculty Medicine Wroclaw Medicine University.