Acids and bases, pH and buffers

Buffers in Blood.
Acidosis and Alkalosis.

BUFFER SOLUTIONS

A guide for A level students

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CLINICAL BIOCHEMISTRY

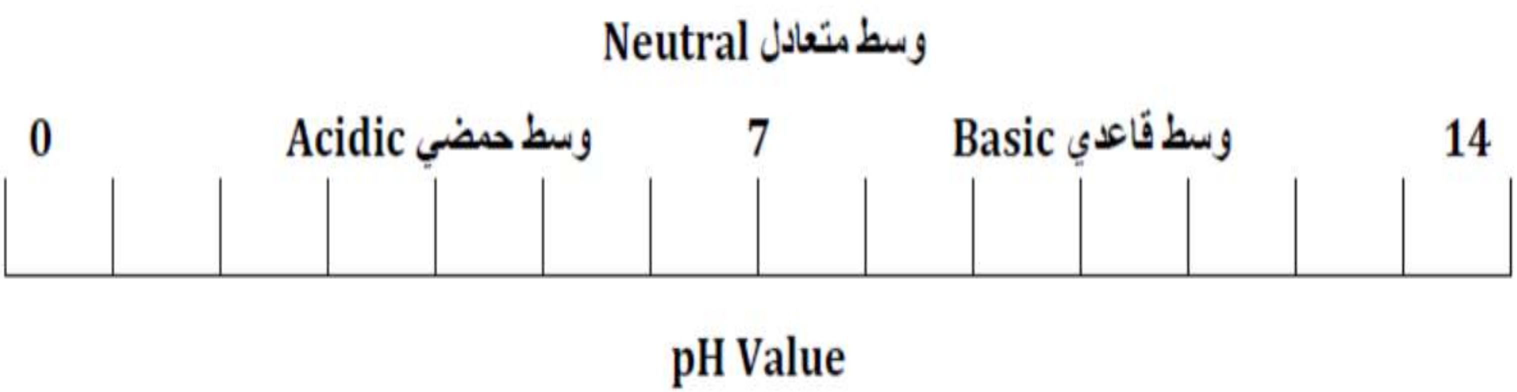
٢. المحاليل المنظمة Buffer Solutions

٢,١. الرقم الهيدروجيني pH

اقترح العالم سورنسن Sorensen طريقة للتعبير عن حموضة المحاليل باستخدام الرقم الهيدروجيني الذي يعرّف بأنه:
اللوغاريتم السالب لتركيز أيونات الهيدروجين $[H^+]$ في المحلول .

$$pH = -\text{Log}[H^+]$$

وبملاحظة أن الإشارة سالبة فإن قيمة الرقم الهيدروجيني ترتفع كلما انخفض تركيز أيونات الهيدروجين والعكس صحيح.



قياس الرقم الهيدروجيني :

لقياس الرقم الهيدروجيني للمحاليل المختلفة بدقة يجب أن نستخدم جهاز خاص يسمى **pH meter**. يتكون الجهاز من قطبين: الأول يسمى قطب مرجعي يحتوي على محلول مشبع من كلوريد البوتاسيوم يعمل اتصالاً كهربائياً بالمحلول، والثاني قطب زجاجي في أسفله غشاء رقيق على شكل انتفاخ حساس ونفّاذ لأيونات الهيدروجين. يقيس هذا الجهاز الفرق في الجهد بين القطبين، ويحوله إلى رقم هيدروجيني من 0 إلى 14.

جهاز قياس الرقم الهيدروجيني pH meter


CAL
CFM
Functions
MEM Memorize Reading
MR Memory Recall
RANGE Select pH/ORP
pH Calibration
CAL Start Calibration
Select 1st Buffer
CFM Confirm 1st Buffer
Select 2nd Buffer
CFM Confirm 2nd Buffer
RANGE
MEM
MR
HANNA instruments
pH 211
Microprocessor pH Meter
7.1

H+ concentration

Blood hydrogen ion concentration [H+] is maintained within tight limits in health. Normal levels lie between 35and 45 nmol/l . Values greater than or less than require urgent treatment;

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pH is defined as the negative log of the hydrogen ion concentration.

pH = -log[H]

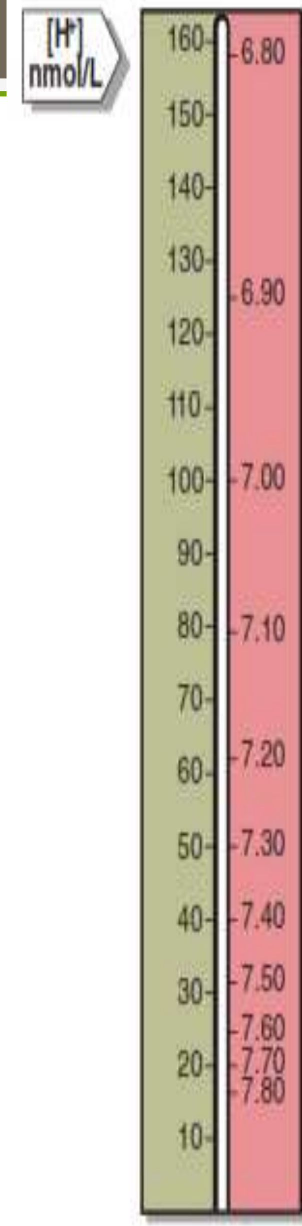


Fig 20.1 **The negative loga relationship between [H+] a**

H+ production

-Hydrogen ions are produced in the body as a result of metabolism, from the oxidation of the amino acids of protein ingested as food

-The total amount of H+ produced each day in this way is of the order of 60 mmol/l. If all of this were to be diluted in the extracellular fluid(≈ 14 L),

-As all the H+ produced are efficiently excreted in urine. Everyone who eats a diet rich in animal protein passes a urine that is profoundly acidic.

-Metabolism also produces CO2. In solution this gas forms a weak acid. Large amounts of CO2 are produced by cellular activity each day

-But under normal circumstances all of thisCO2 is excreted via the lungs, having been transported in the blood. Only when respiratory function is normal occur .

pH values in the organism

*pH values in the cell and in the extracellular fluid are kept constant within narrow limits. In the blood, the pH value normally ranges only between 7.35 and 7.45

*The pH value of cytoplasm is slightly lower than that of blood,at pH (4.5–5.5),

*(The H+ concentration is several hundred times higher than in the cytoplasm.)

** *values are found in the stomach (ca. 2) and in the small bowel (> 8). Since the kidney can excrete either acids or bases, depending on the state of the metabolism,*

*the pH of urine has a particularly wide range of variation

Buffering

#A buffer is a solution of a weak acid and its salt (or a weak base and its salt) that is able to bind H+ and therefore resist changes in pH.

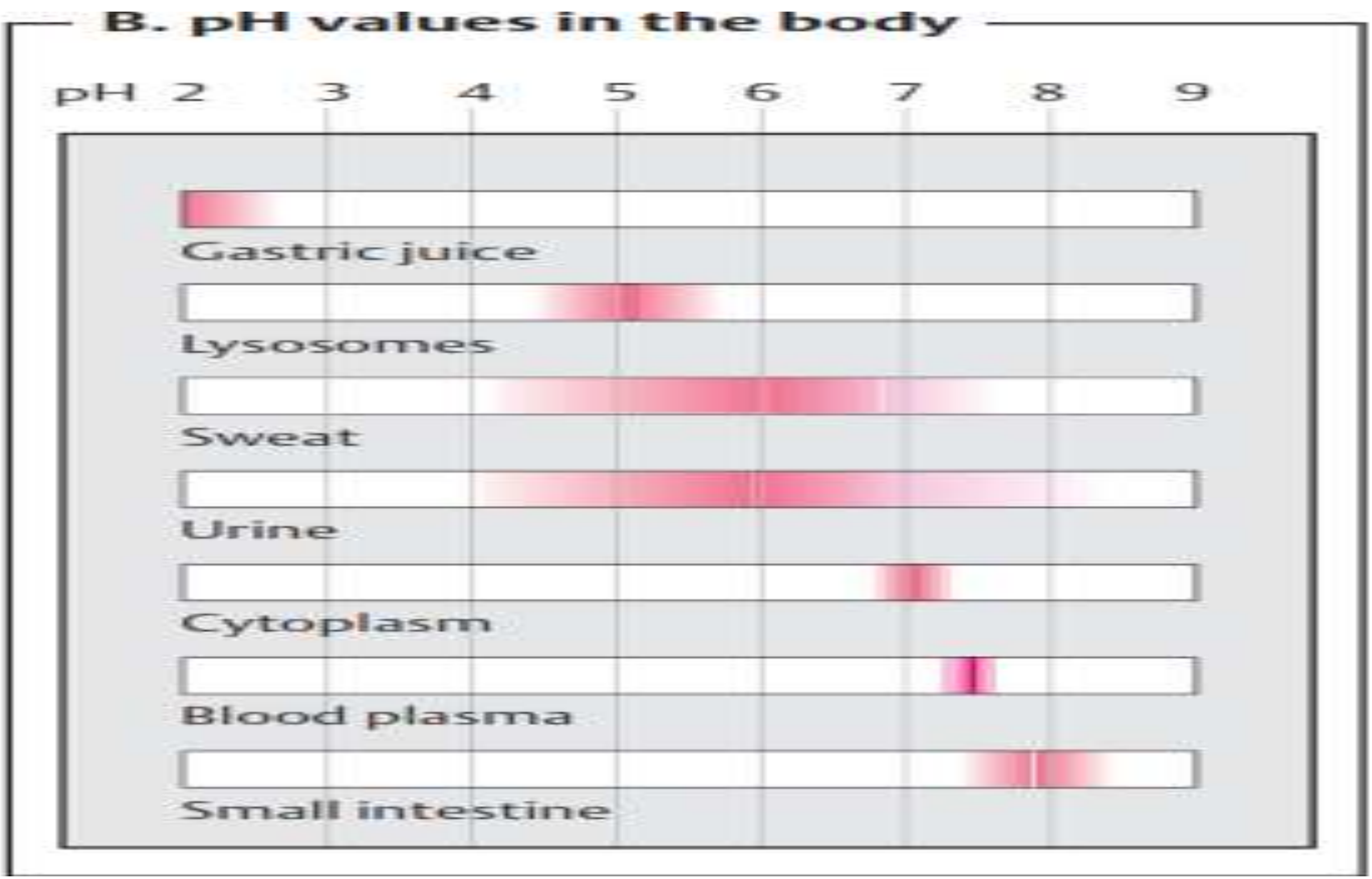
##Buffering does not remove H+ from the body. Rather, buffer mop up any excess H+ that are produced, in the same way that a sponge soaks up water.

###Buffering is only a short-term solution to the problem of excess H+. Ultimately, the body must get rid of the H+ by renal excretion.

Buffers

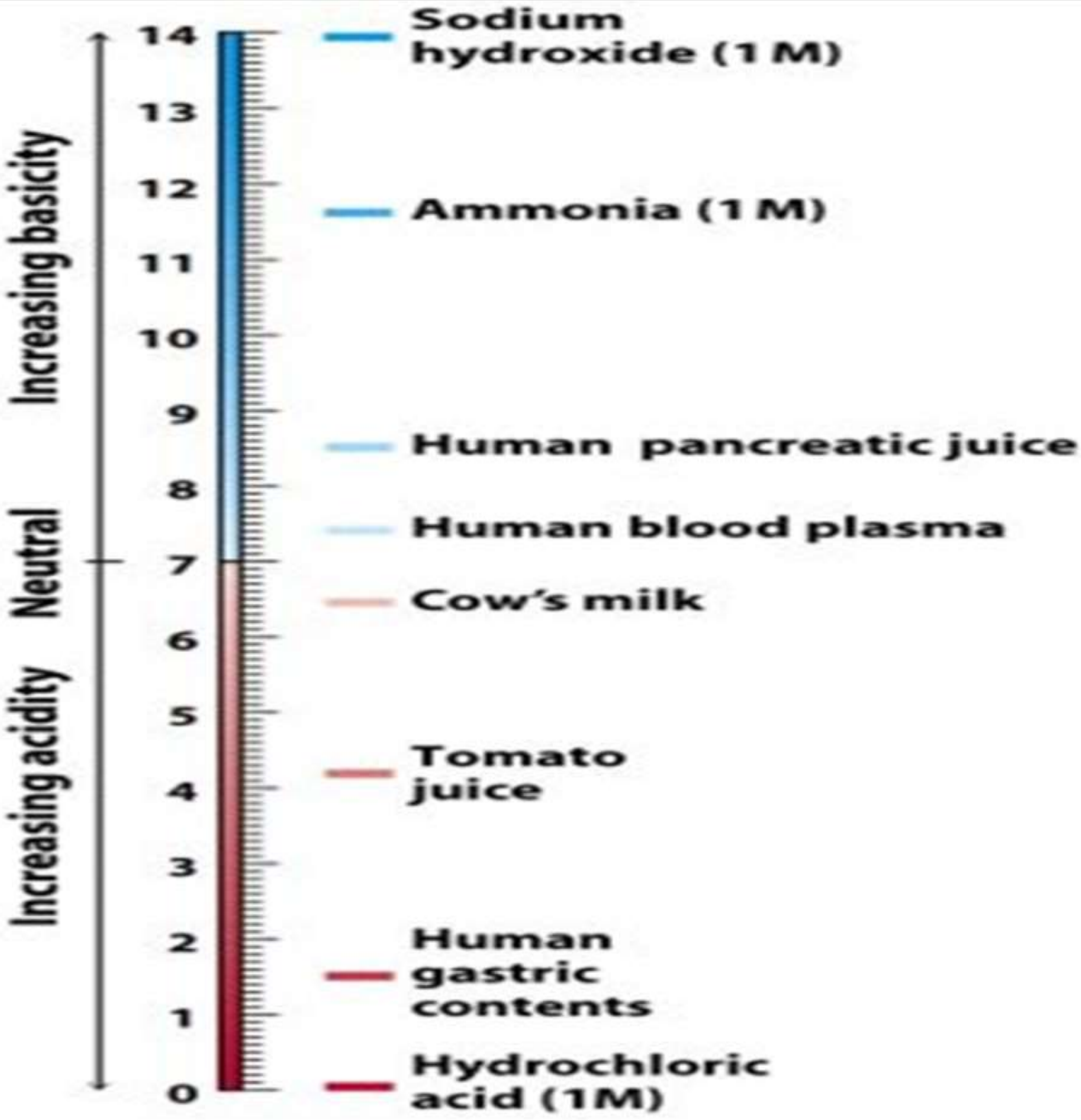
The body contains a number of buffers to changes in H+ production.

1-Proteins can act as buffers,
2-and the hemoglobin in the erythrocytes has a high capacity for binding H+.
3-In the ECF, bicarbonate buffer is the most important.
4-In this buffer system, bicarbonate (HCO_3^-) combines with H+ to form carbonic acid(H_2CO_3).
5-This buffer system is unique in that the (H_2CO_3) can dissociate to water and carbon dioxide.


B. pH values in the body
pH 2 3 4 5 6 7 8 9
Gastric juice
Lysosomes
Sweat
Urine
Cytoplasm
Blood plasma
Small intestine

Buffer Solutions

- A buffer solution is a mixture that minimises
- pH changes on the addition of small amounts of acid or base.
- No buffer solution can cope with the addition of large concentrations of acid or alkali.
- pH changes are minimised as long as some of the buffer solution remains.
- Buffers can be acidic or basic but we only need to be concerned with acid buffers.



Increasing basicity
Neutral
Increasing acidity
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Sodium hydroxide (1 M)
Ammonia (1 M)
Human pancreatic juice
Human blood plasma
Cow's milk
Tomato juice
Human gastric contents
Hydrochloric acid (1M)

Acid strength	Acid				Base	Base strength
Strong	HCl	→	H^+	+	Cl^-	Negligible
Strong	H_2SO_4	→	H^+	+	HSO_4^-	Negligible
Strong	HNO_3	→	H^+	+	NO_3^-	Negligible
Strong	H_3O^+	→	H^+	+	H_2O	Very Weak
Medium	HSO_4^-	→	H^+	+	SO_4^{2-}	Very Weak
Medium	H_3PO_4	→	H^+	+	$H_2PO_4^-$	Very Weak
Medium	HF	→	H^+	+	F^-	Very Weak
Weak	CH_3COOH	→	H^+	+	CH_3COO^-	Weak
Weak	H_2CO_3	→	H^+	+	HCO_3^-	Weak
Weak	H_2S	→	H^+	+	HS^-	Weak
Weak	$H_2PO_4^-$	→	H^+	+	HPO_4^{2-}	Weak
Weak	NH_4^+	→	H^+	+	NH_3	Weak
Very Weak	HCO_3^-	→	H^+	+	CO_3^{2-}	Medium
Very Weak	HPO_4^{2-}	→	H^+	+	PO_4^{3-}	Medium
Very Weak	H_2O	→	H^+	+	OH^-	Strong
Negligible	HS^-	→	H^+	+	S^{2-}	Strong
Negligible	H_2	→	H^+	+	H^-	Strong

ACID STRENGTH ↑

BASE STRENGTH ↓

TABLE 9.4 K_a and pK_a Values for Selected Acids

Name	Formula	K_a	pK_a
Hydrochloric acid	HCl	1.0×10^{7}	−7.00
Phosphoric acid	H_3PO_4	7.5×10^{-3}	2.12
Hydrofluoric acid	HF	6.6×10^{-4}	3.18
Lactic acid	$CH_3CH(OH)CO_2H$	1.4×10^{-4}	3.85
Acetic acid	CH_3CO_2H	1.8×10^{-5}	4.74
Carbonic acid	H_2CO_3	4.4×10^{-7}	6.36
Dihydrogenphosphate ion	$H_2PO_4^-$	6.2×10^{-8}	7.21
Ammonium ion	NH_4^+	5.6×10^{-10}	9.25
Hydrocyanic acid	HCN	4.9×10^{-10}	9.31
Hydrogencarbonate ion	HCO_3^-	5.6×10^{-11}	10.25
Methylammonium ion	$CH_3NH_3^+$	2.4×10^{-11}	10.62
Hydrogenphosphate ion	HPO_4^{2-}	4.2×10^{-13}	12.38

ACIDS, ALKALIS, AND THE pH SCALE

The pH scale is a way of gauging the acidity or alkalinity of a solution. It is calculated using: $pH = -\log_{10}[H^+]$. Adding an acid to water increases the H^+ (H_3O^+) concentration, and decreases the OH^- concentration. An alkali does the opposite.

	pH	H^+ CONCENTRATION	OH^- CONCENTRATION	EVERYDAY EXAMPLE
ALKALINE (Turquoise → Blue → Purple)	14	1×10^{-14}	1	Drain Cleaner
	13	1×10^{-13}	0.1	Bleach
	12	1×10^{-12}	0.01	Ammonia
	11	1×10^{-11}	0.001	Soap
	10	1×10^{-10}	1×10^{-4}	Antacid Tablets
	9	1×10^{-9}	1×10^{-5}	Baking Soda
	8	1×10^{-8}	1×10^{-6}	Seawater
NEUTRAL (Green)	7	1×10^{-7}	1×10^{-7}	Pure Water
ACIDIC (Red → Orange → Yellow)	6	1×10^{-6}	1×10^{-8}	Urine (average)
	5	1×10^{-5}	1×10^{-9}	Black Coffee
	4	1×10^{-4}	1×10^{-10}	Tomato Juice
	3	0.001	1×10^{-11}	Soda
	2	0.01	1×10^{-12}	Lemon Juice
	1	0.1	1×10^{-13}	Stomach Acid
	0	1	1×10^{-14}	Battery Acid

Buffered Solutions Resist Changes in pH

If the pH of a solution remains nearly constant when small amounts of strong acid or strong base are added the solution is said to be buffered.

The ability of a solution to resist changes in pH is known as its buffer capacity. ***acetic acid and phosphoric acid*****,**

occurs when the concentrations of a weak acid and its conjugate base are equal in other words, when the pH equals the pKa.

The effective range of buffering by a mixture of a weak acid and its conjugate base is usually considered to be from one pH unit below to one pH unit above the pKa.

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Most in *vitro* biochemical experiments involving purified molecules, cell extracts, or intact cells are performed in the presence of a suitable buffer to ensure a stable pH. A number of synthetic compounds with a variety of pKa values are often used to prepare buffered solutions but naturally occurring compounds can also be used as buffers.

For example,

Mixtures of acetic acid and sodium acetate (pKa= 4.8) can be used for the pH range from 4 to 6 ,

Mixtures of KH2PO4 and K2HPO4 (pKa = 7.2) can be used in the range from 6 to 8.

The amino acid glycine (pKa = 9.8) is often used in the range from 9 to 11.

Mixture of NaH2PO4 and Na2HHPO4 ----

Buffers in the Blood

- The pH of blood is 7.35 – 7.45
- Changes in pH below 6.8 and above 8.0 may result in death
- The major buffer system in the body fluid is H_2CO_3/HCO_3^-
- Some CO_2, the end product of cellular metabolism, is carried to the lungs for elimination, and the rest dissolves in body fluids, forming carbonic acid that dissociates to produce bicarbonate (HCO_3^-) and hydronium (H_3O^+) ions.
- More of the HCO_3^- is supplied by the kidneys.
- $CO_2 + H_2O \leftrightarrow H_2CO_3$
- $H_2CO_3 + H_2O \leftrightarrow H_3O^+ + HCO_3^-$

Carbonate buffer

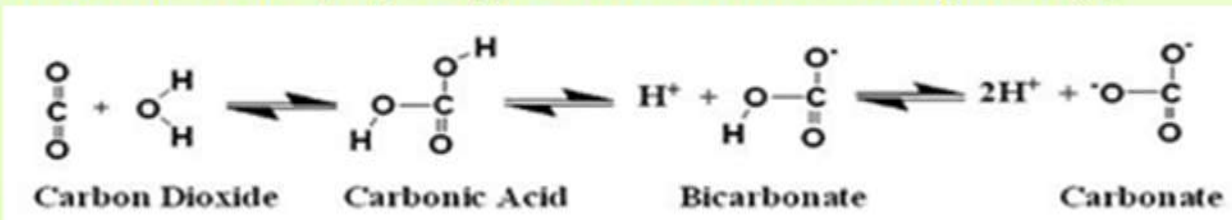
$$H_2CO_3 + H_2O \leftrightarrow H_3O^+ + HCO_3^-$$

- Excess acid (H_3O^+) in the body is neutralized by HCO_3^-
- $H_2CO_3 + H_2O \leftarrow H_3O^+ + HCO_3^-$
- Equilibrium shifts left
- Excess base (OH^-) reacts with the carbonic acid (H_2CO_3)
- $H_2CO_3 + OH^- \rightarrow H_2O + HCO_3^-$
- Equilibrium shifts right

Acid-base reactions

Acid + base → salt + H2O

- Exceptions:
- Carbonic acid (H_2CO_3)-Bicarbobate ion (HCO_3^-)



- Ammonia (NH_3)-

$$NH_3 + HCl \longrightarrow NH_4CL$$

pH of the blood buffer

- The concentrations in the blood of H_2CO_3 and HCO_3^- are 0.0024M and 0.024 respectively
- $H_2CO_3/ HCO_3^- = 1/10$ is needed to maintain the normal blood pH (7.35 – 7.45)

$$K_a = \frac{[H_3O^+][HCO_3^-]}{[H_2CO_3]}$$

$$[H_3O^+] = K_a \frac{[H_2CO_3]}{[HCO_3^-]} =$$

$$= 4.3x10^{-7} x \frac{0.0024}{0.024} = 4.3x10^{-7} x0.10 = 4.3x10^{-8}$$

$$pH = -\log(4.3x10^{-8}) = 7.37$$

The effectiveness of the blood buffer

- If the pH of 100 mL of distilled water is 7.35 and **one drop** of 0.05 M HCl is added, the pH will change to 7.00.
- To change 100 mL of "normal" blood from pH of 7.35 to 7.00, approximately **25 mL** of 0.05 M HCl is needed.
- With 5.5 L of blood in the average body, more than **1300 mL** of HCl would be required to make the same change in pH.

Regulation of blood pH

- The lungs and kidneys play important role in regulating blood pH.
- The lungs regulate pH through retention or elimination of CO_2 by changing the rate and volume of ventilation.
- The kidneys regulate pH by excreting acid, primarily in the ammonium ion (NH_4^+), and by reclaiming HCO_3^- from the glomerular filtrate (and adding it back to the blood).

Importance of the bicarbonate-carbonic acid buffering system

1. H_2CO_3 dissociates into CO_2 and H_2O, allowing H_3O^+ to be eliminated as CO_2 by the lungs
2. Changes in P_{CO_2} modify the ventilation rate
3. HCO_3^- concentration can be altered by kidneys

Other important buffers

- The phosphate buffer system ($HPO_4^{2-}/H_2PO_4^-$) plays a role in plasma and erythrocytes.
- $H_2PO_4^- + H_2O \leftrightarrow H_3O^+ + HPO_4^{2-}$
- Any acid reacts with monohydrogen phosphate to form dihydrogen phosphate

dihydrogen phosphate monohydrogen phosphate

- $H_2PO_4^- + H_2O \leftarrow HPO_4^{2-} + H_3O^+$
- The base is neutralized by dihydrogen phosphate

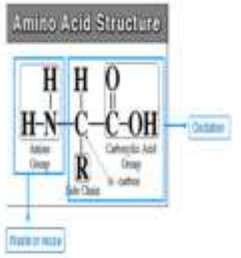
dihydrogen phosphate monohydrogen phosphate

- $H_2PO_4^- + OH^- \rightarrow HPO_4^{2-} + H_3O^+$

Proteins act as a third type of blood buffer

- Proteins contain – COO^- groups, which, like acetate ions (CH_3COO^-), can act as proton acceptors.
- Proteins also contain – NH_3^+ groups, which, like ammonium ions (NH_4^+), can donate protons.
- If acid comes into blood, hydronium ions can be neutralized by the – COO^- groups
- $-COO^- + H_3O^+ \rightarrow -COOH + H_2O$
- If base is added, it can be neutralized by the – NH_3^+ groups
- $-NH_3^+ + OH^- \rightarrow -NH_2 + H_2O$

Amino acid oxidation and the production of urea



ACIDIC AND BASIC PROPERTIES OF AMINO ACIDS

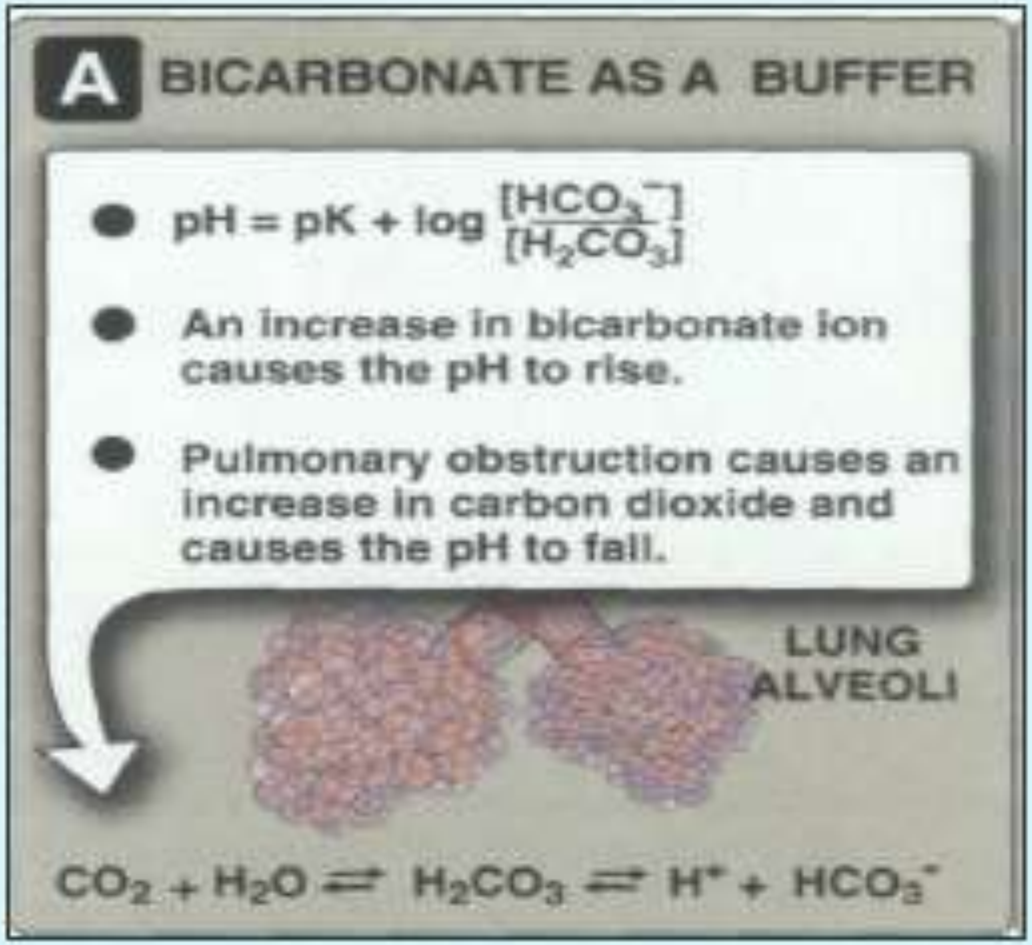
- Amino acids in aqueous solution contain weakly acidic α-carboxyl groups and weakly basic α-amino groups.
- Each of the acidic and basic amino acids contains an ionizable group in its side chain.
- Thus, both free and some of the combined amino acids in peptide linkages can act as **buffers.**
- The concentration of a weak acid (HA) and its conjugate base(A^-) is described by the **Henderson-Hasselbalch equation.**

Derivation of the equation

- For the reaction ($HA \rightleftharpoons A^- + H^+$)
- $$K_a = \frac{[H^+]\,[A^-]}{[HA]} \quad \text{------} (1)$$
- By solving for the $[H^+]$ in the above equation, taking the logarithm of both sides of the equation, multiplying both sides of the equation by -1, and substituting $pH = -\log [H^+]$ and $pK_a = -\log [K_a]$ we obtain:
- $$pH = pK_a + \log \frac{[A^-]}{[HA]} \quad \text{------} (2)$$

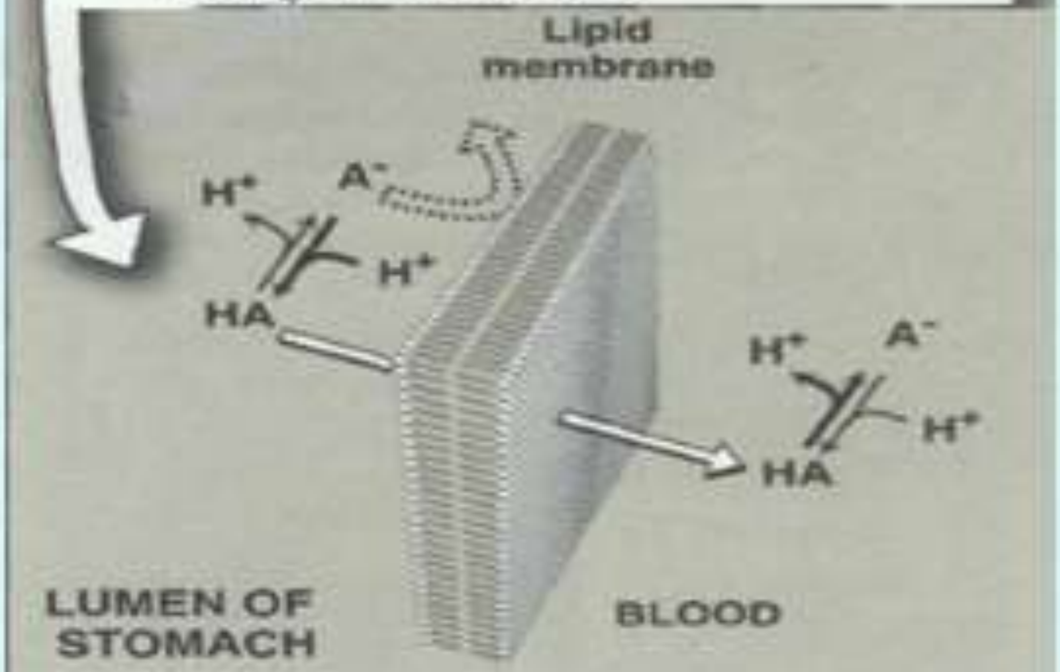
It is the <u>(Henderson-Hasselbalch equation)</u>

Other applications of the Henderson-Hasselbalch equation



B DRUG ABSORPTION

- $pH = pK + \log \frac{[Drug^-]}{[Drug\text{-}H]}$
- At the pH of the stomach (1.5), a drug like aspirin (weak acid, pK = 3.5) will be largely protonated (COOH) and, thus, uncharged.
- Uncharged drugs generally cross membranes more rapidly than charged molecules.



Normal Values for Blood Buffer in Arterial Blood.

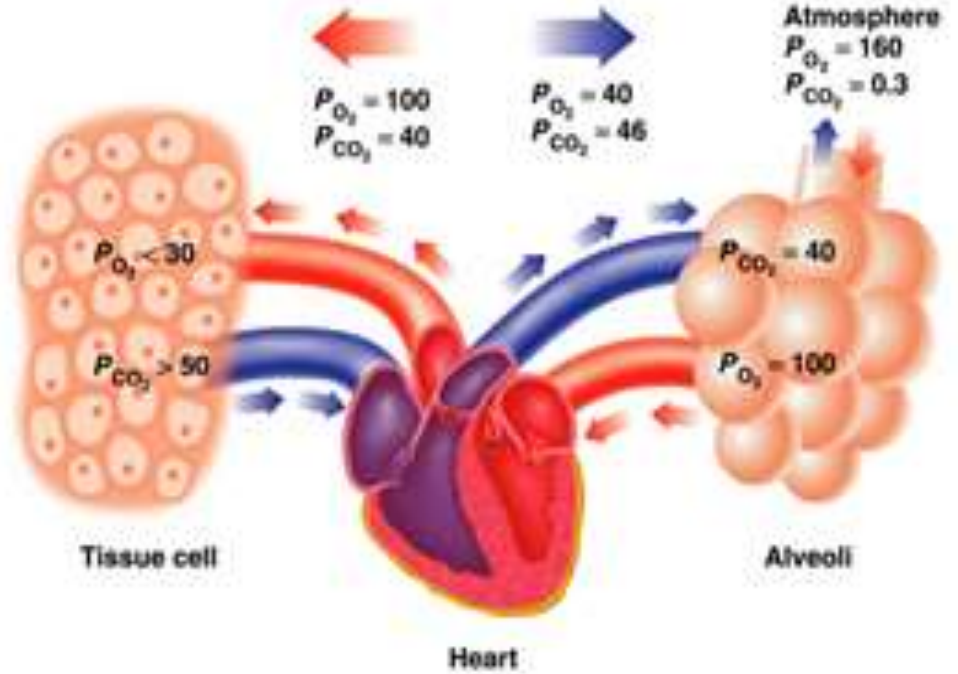
- The following values are determined by blood gas analyzer:
- pH 7.35 – 7.45
- P_{CO_2} 35 – 45 mm Hg
- H_2CO_3 2.4 mmoles/L of plasma
- HCO_3^- 24 mmoles/L of plasma
- P_{O_2} 80 – 110 mm Hg

Blood Gases

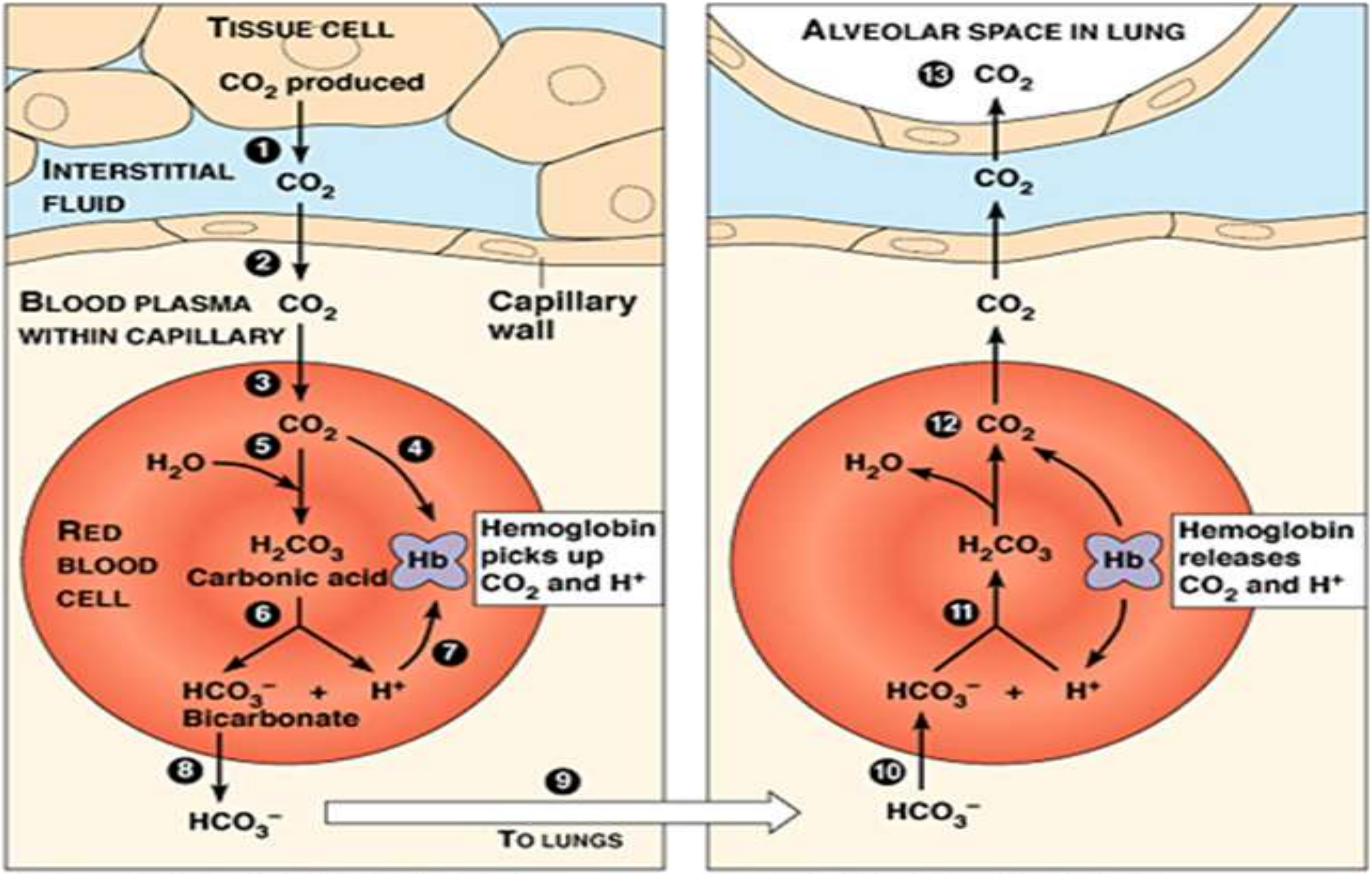
- In the body, cells use up O_2 and give off CO_2.
- O_2 flows into the tissues because the partial pressure of O_2 is higher (100 mm Hg) in oxygenated blood, and lower (<30 mm Hg) in the tissues.
- CO_2 flows out of the tissues because the partial pressure of CO_2 is higher (>50 mm Hg) in the tissues and lower (40 mm Hg) in the blood.

Blood Gases

- In the lungs, O_2 enters the blood, while CO_2 from the blood is released.
- In the tissues, O_2 enters the cells, which release CO_2 into the blood.



Hemoglobin as a Buffer



(a) CO_2 transport from tissues

(b) CO_2 transport to lungs

Acidosis and alkalosis

- Can be either metabolic or respiratory
- Acidosis:
  - Metabolic: production of ketone bodies (starvation)
  - Respiratory: pulmonary (asthma; emphysema)

- Alkalosis:
  - Metabolic: administration of salts or acids
  - Respiratory: hyperventilation (anxiety)

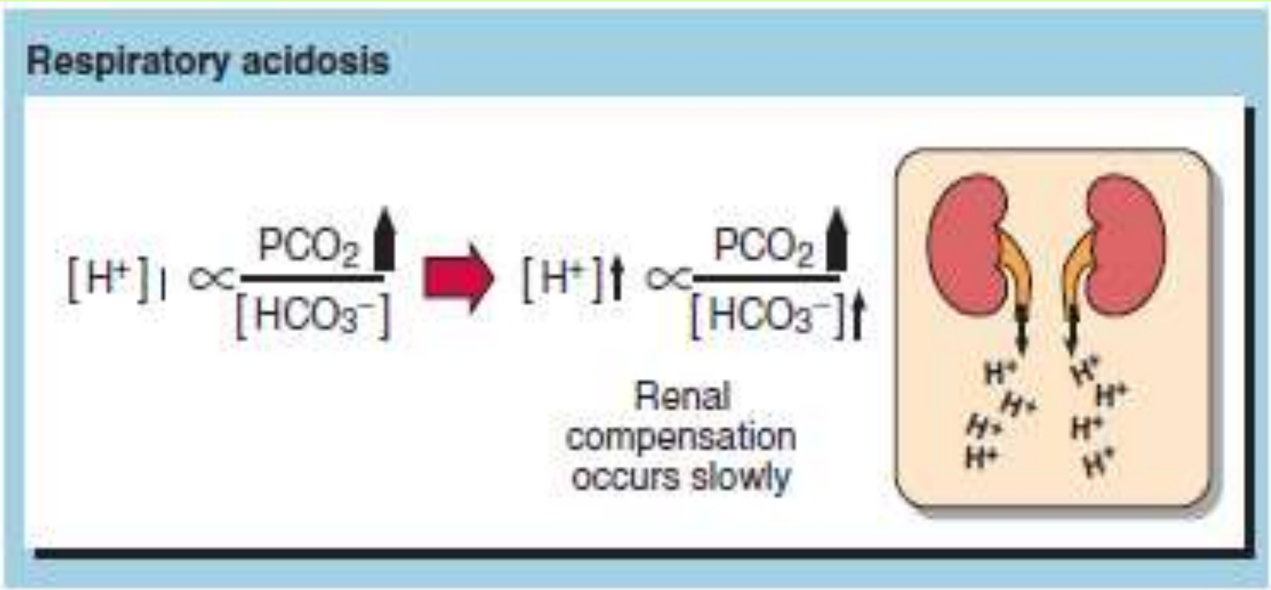
Acid-Base Imbalances

- pH< 7.35 acidosis

- pH > 7.45 alkalosis

Respiratory Acidosis

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

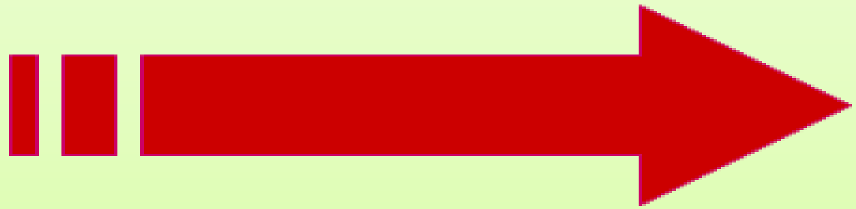


Respiratory Acidosis: CO_2 ↑ pH ↓

- Symptoms: Failue to ventilate, suppression of breathing, disorientation, weakness, coma
- Causes: Lung disease blocking gas diffusion (e.g., emphysema, pneumonia, bronchitis, and asthma); depression of respiratory center by drugs, cardiopulmonary arrest, stroke, poliomyelitis, or nervous system disorders
- Treatment: Correction of disorder, infusion of bicarbonate

Respiratory Alkalosis

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

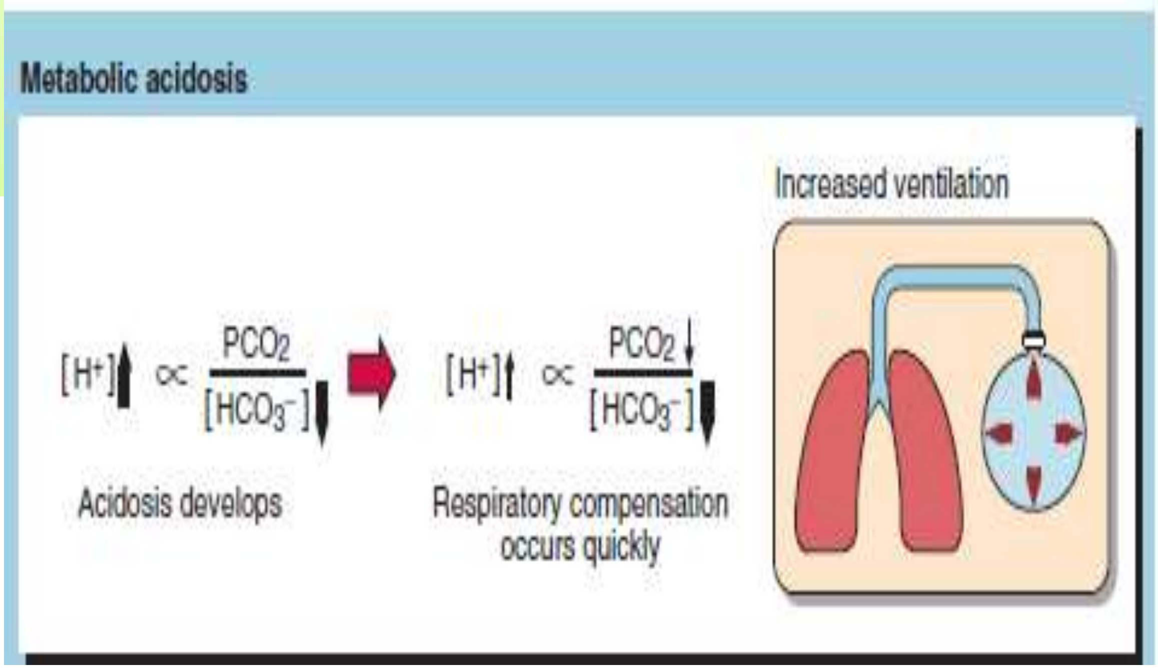


Respiratory Alkalosis: CO_2 ↓ pH ↑

- Symptoms: Increased rate and depth of breathing, numbness, light-headedness, tetany
- Causes: hyperventilation due to anxiety, hysteria, fever, exercise; reaction to drugs such as salicylate, quinine, and antihistamines; conditions causing hypoxia (e.g., pneumonia, pulmonary edema, and heart disease)
- Treatment: Elimination of anxiety producing state, rebreathing into a paper bag

Metabolic Acidosis

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

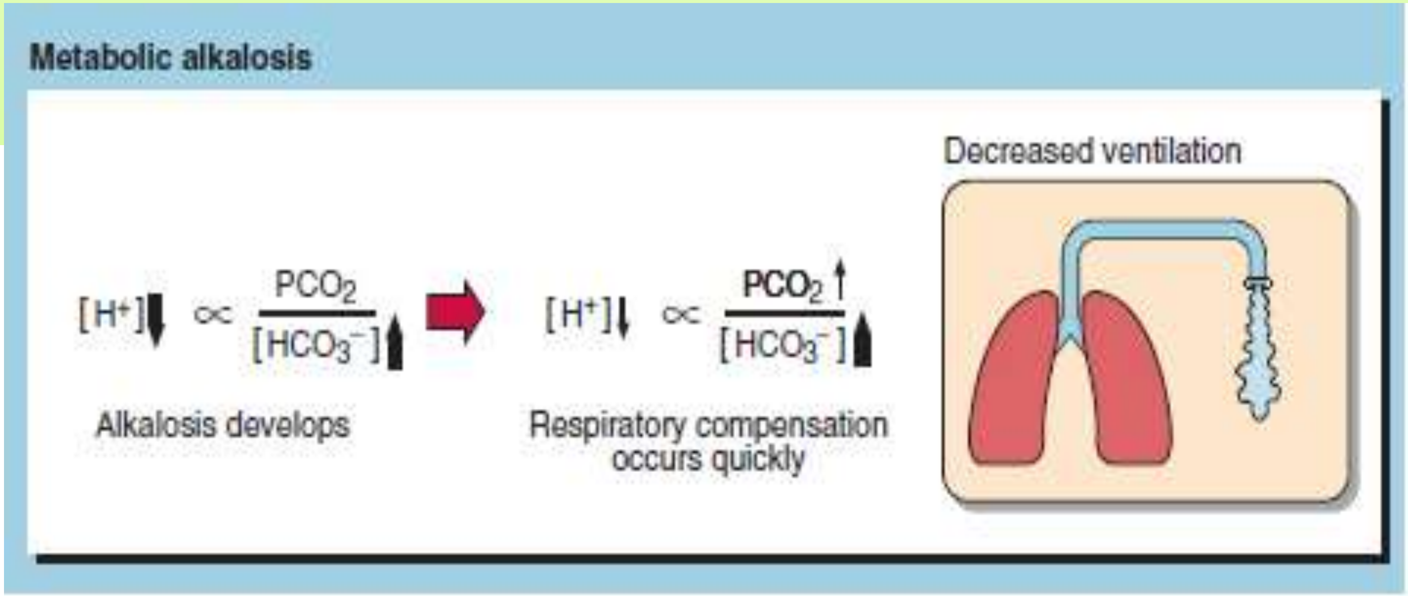


Metabolic (Nonrespiratory) Acidosis: $H^+ \uparrow$ pH $\downarrow$

- Symptoms: Increased ventilation, fatigue, confusion
- Causes: Renal disease, including hepatitis and cirrhosis; increased acid production in diabetes mellitus, hyperthyroidism, alcoholism, and starvation; loss of alkali in diarrhea; acid retention in renal failure
- Treatment: Sodium bicarbonate given orally, dialysis for renal failure, insulin treatment for diabetic ketosis

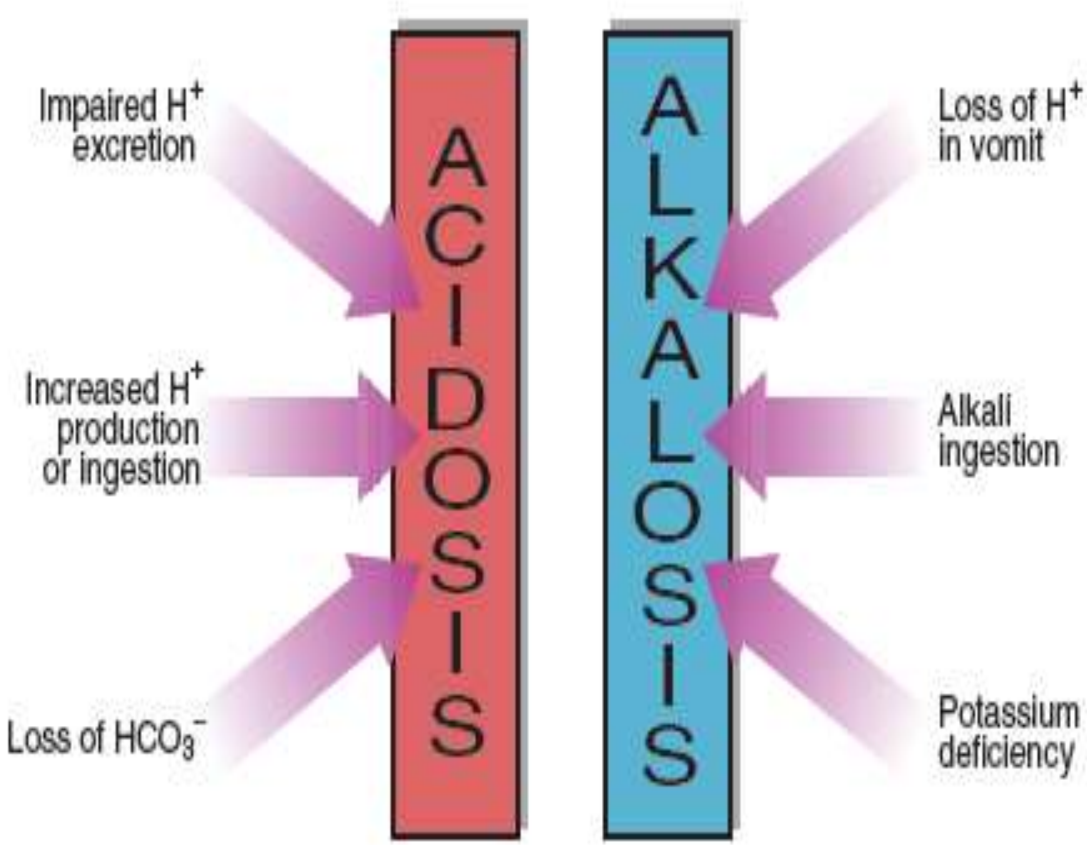
Metabolic Alkalosis

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



Metabolic (Nonrespiratory) Alkalosis: $H^+ \downarrow$ pH $\uparrow$

- Symptoms: Depressed breathing, apathy, confusion
- Causes: Vomiting, diseases of the adrenal glands, ingestions of access alkali
- Treatment: Infusion of saline solution, treatment of underlying diseases


Impaired H+ excretion
Increased H+ production or ingestion
Loss of HCO3−
ACIDOSIS
ALKALOSIS
Loss of H+ in vomit
Alkali ingestion
Potassium deficiency