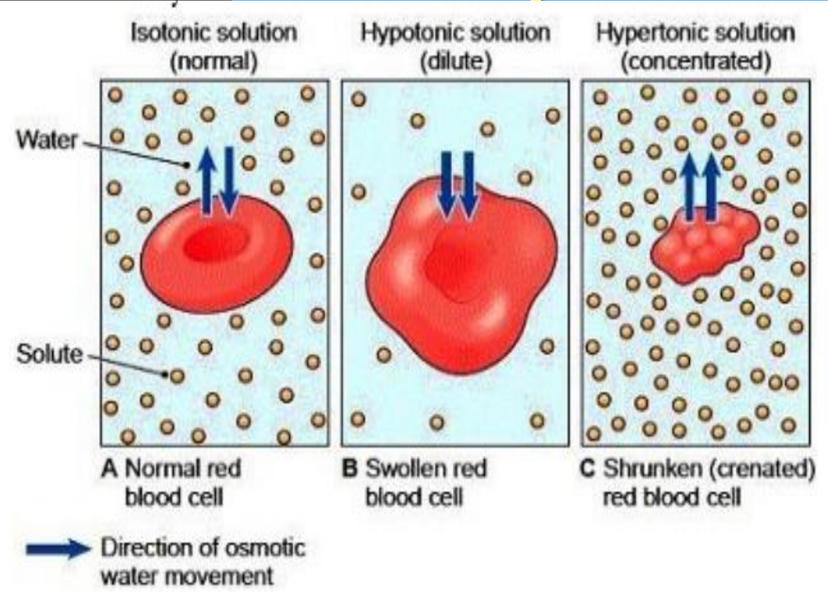
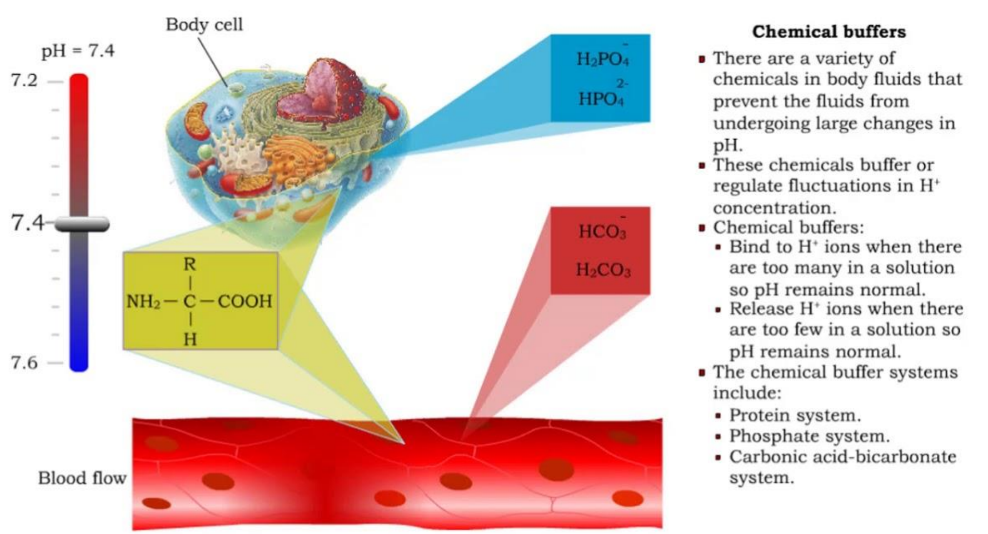
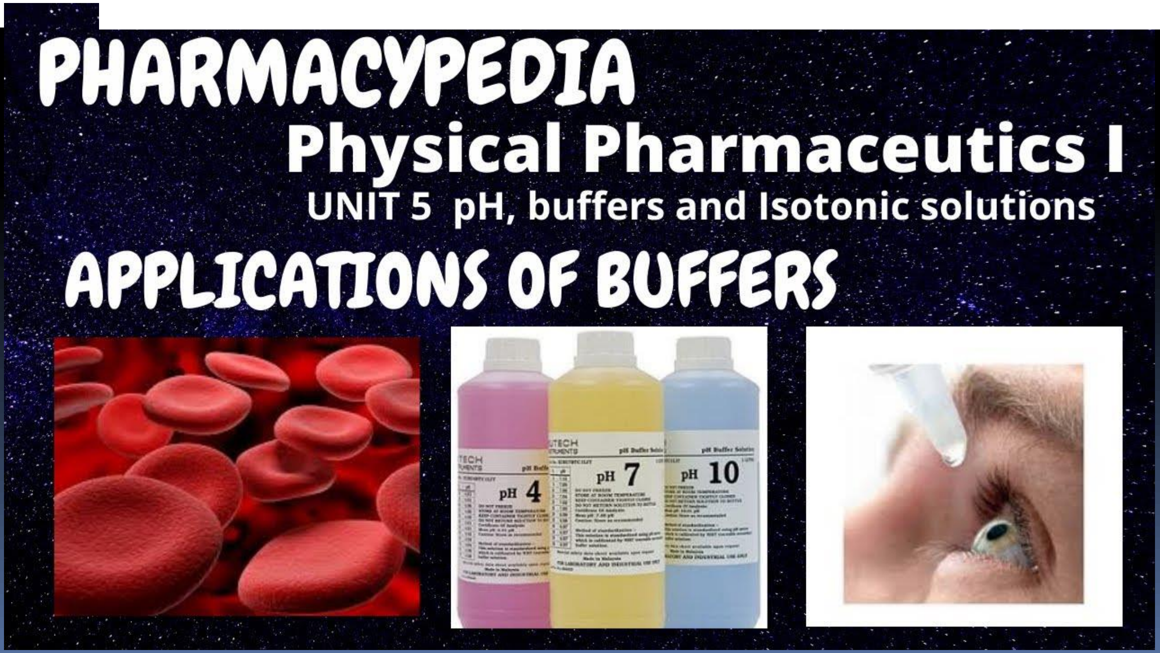
Buffers-definition, mechanism of action and importance of buffers in medicine







Aim: To revise the purpose of enzymes, how they work, and examine the factors which affect enzyme catalysed reactions.

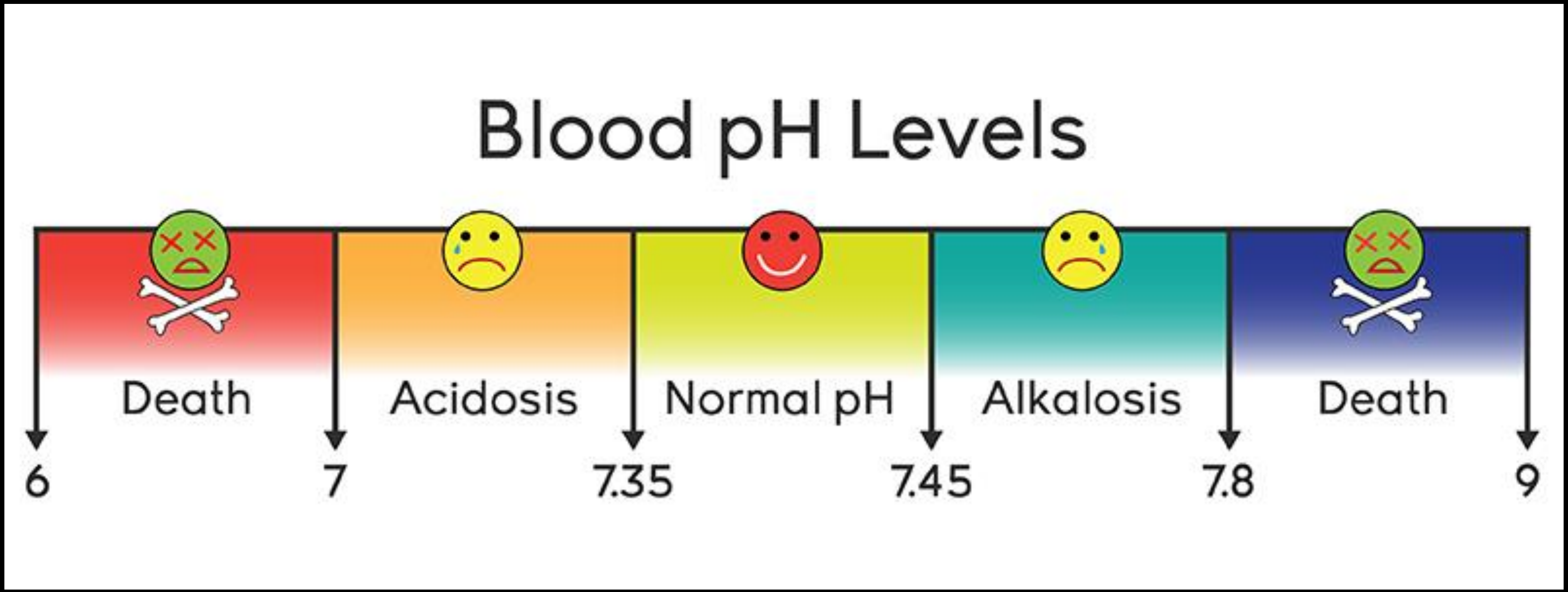
How many chemical reactions happen in our body?

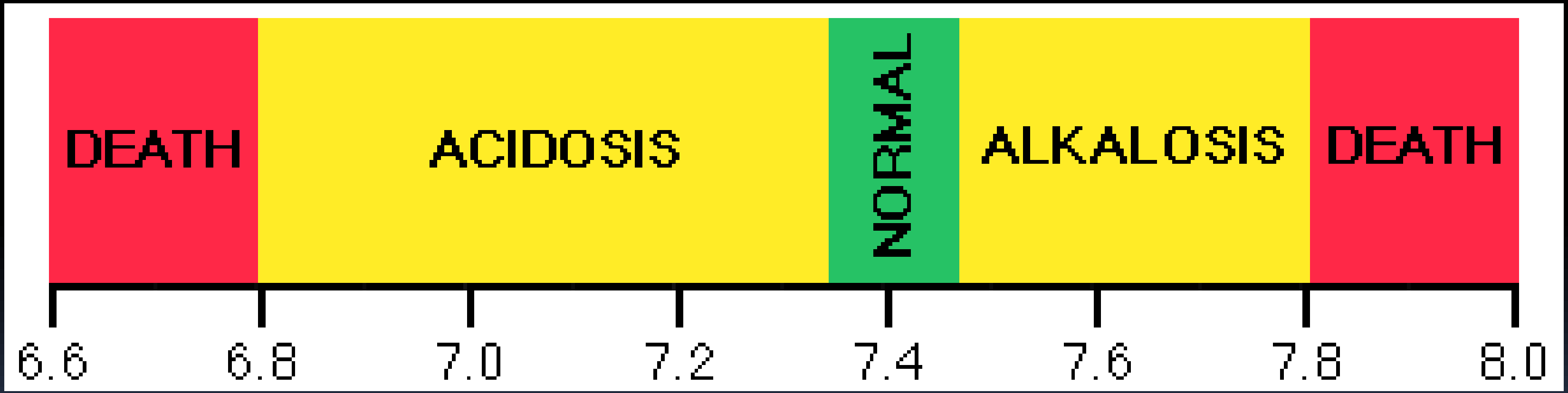
Since we have about 200 trillion cells and each one performs millions of chemical reactions, the total number of chemical reactions in the human body is about 400 billion per second every second of your life.

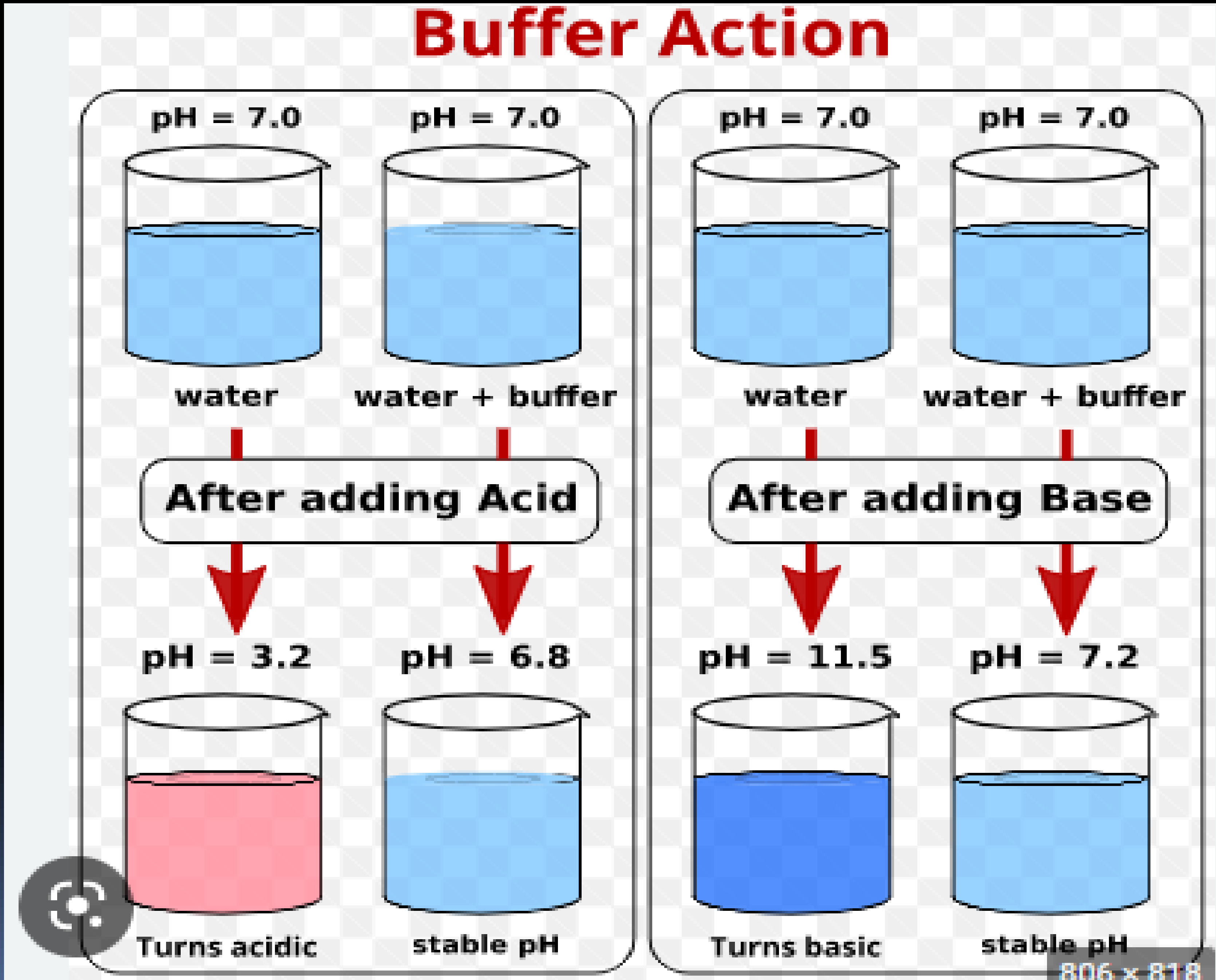
That's 4 times the amount of stars in our galaxy which is a mere 100 billion

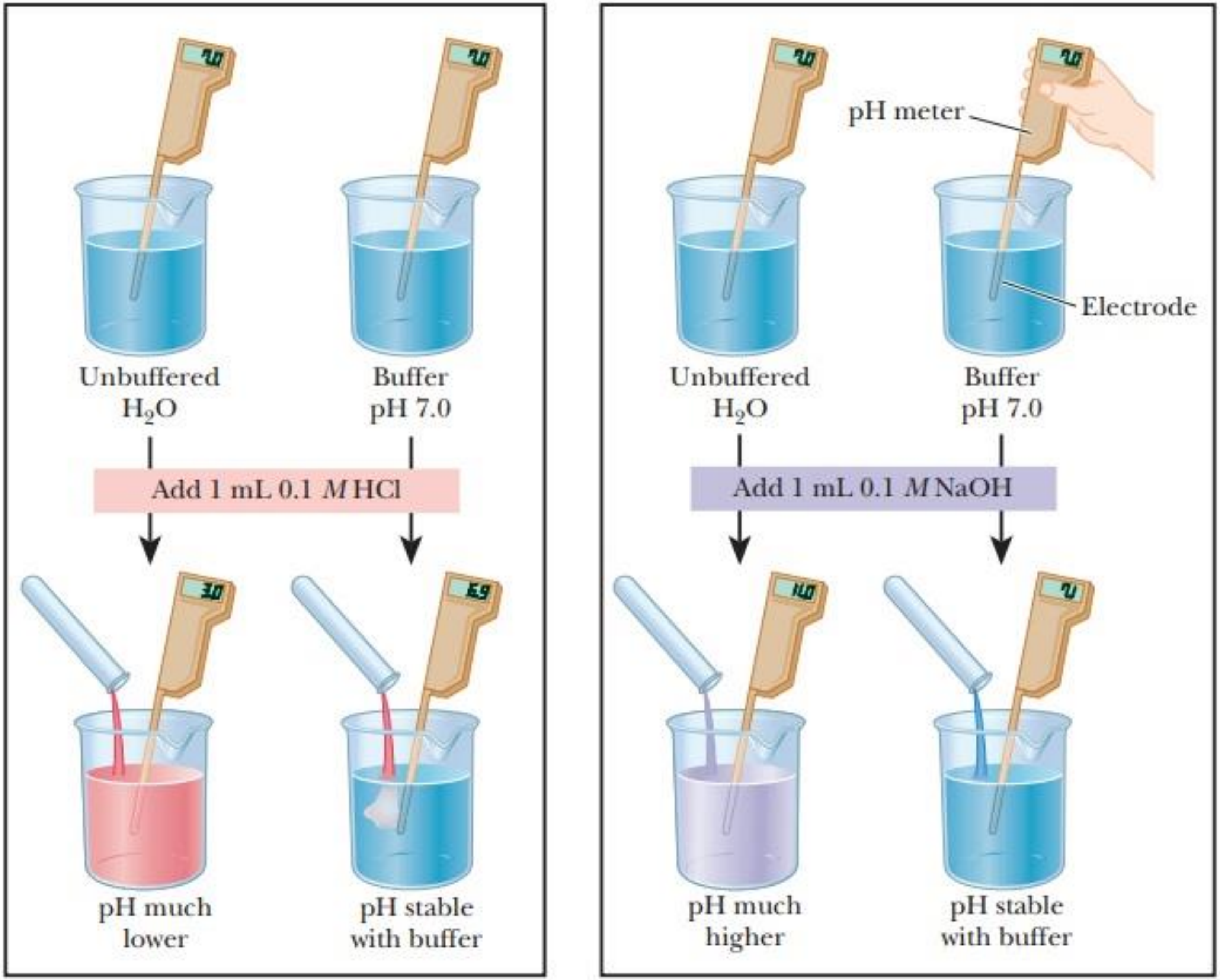
Importance of Buffers

- Control of pH is an essential property of biological systems
- pH of Human blood plasma is maintained between 7.35 – 7.45 within 0.2 pH. unit.
- Values outside not compatible with life
- Most enzyme catalyzing various intracellular and intercellular reaction, do so at some definite pH / within a very narrow range.

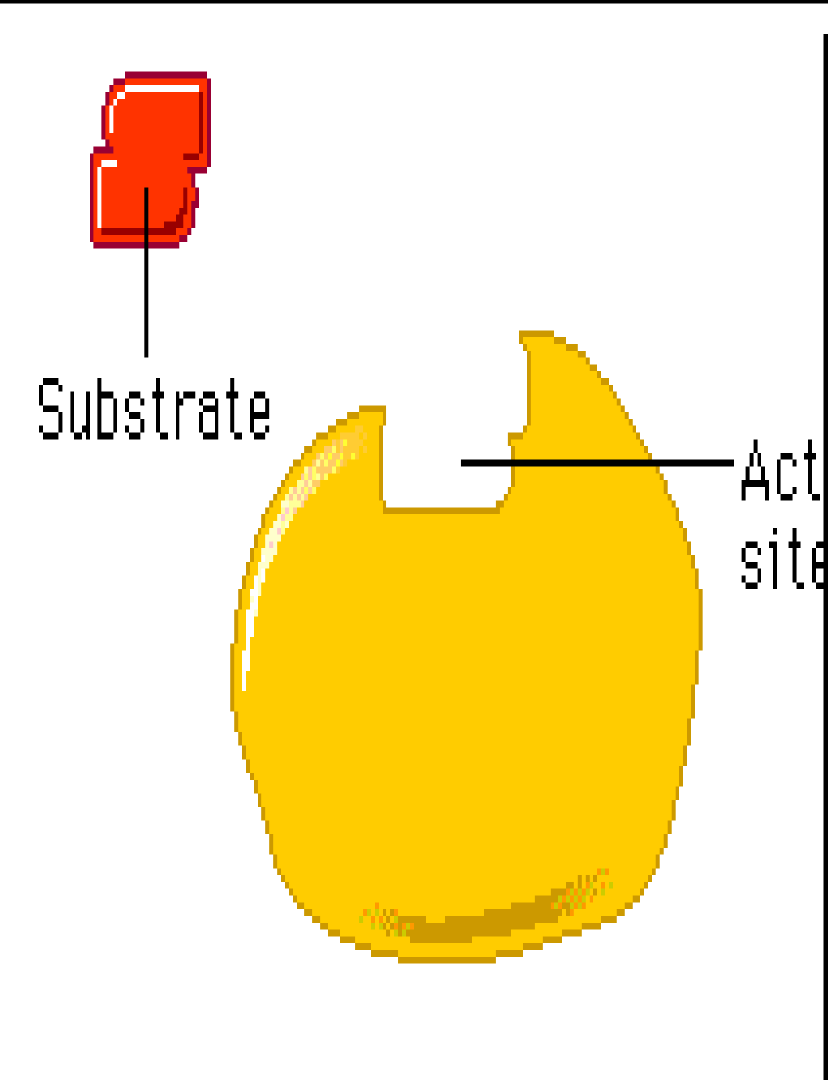

Blood pH Levels
Death
Acidosis
Normal pH
Alkalosis
Death
6
7
7.35
7.45
7.8
9

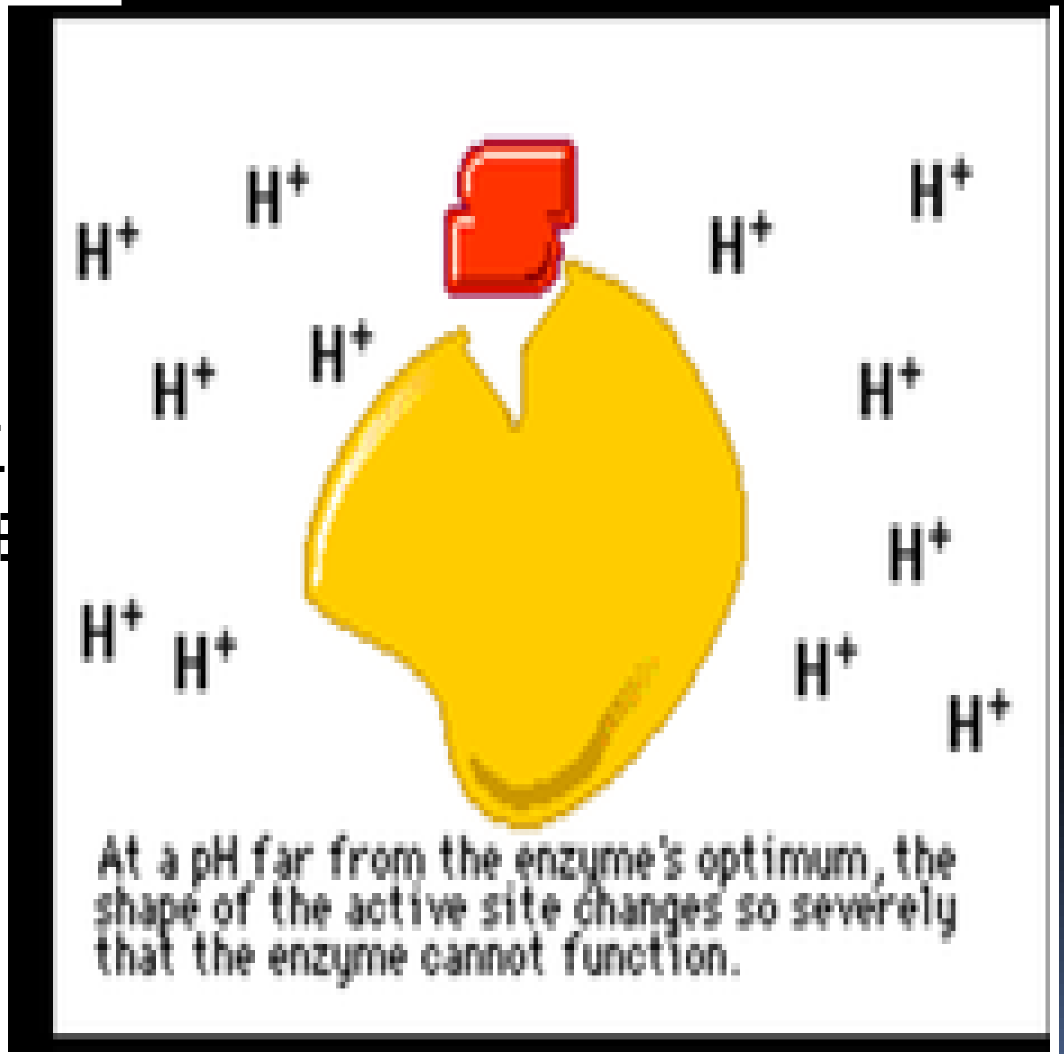

DEATH
ACIDOSIS
NORMAL
ALKALOSIS
DEATH
6.6
6.8
7.0
7.2
7.4
7.6
7.8
8.0


Buffer Action
pH = 7.0
pH = 7.0
water
water + buffer
After adding Acid
pH = 3.2
pH = 6.8
Turns acidic
stable pH
pH = 7.0
pH = 7.0
water
water + buffer
After adding Base
pH = 11.5
pH = 7.2
Turns basic
stable pH
806 × 818


pH meter
Electrode
Unbuffered H_2O
Buffer pH 7.0
Add 1 mL 0.1 *M* HCl
pH much lower
pH stable with buffer
Unbuffered H_2O
Buffer pH 7.0
Add 1 mL 0.1 *M* NaOH
pH much higher
pH stable with buffer

Animation of the inhibition of enzymes active site when in unbuffered acidic solutions





- **Buffer is system (solution) composed of two substances that effectively stabilise (limit the change of the $[H^+]$ ions** when H^+ ions are added or removed from a given solution.

- buffers **do not eliminate** H^+ from body – but **REVERSIBLY** bind H^+ until balance is re-established.

- General form of buffering reaction usually in form of conjugate acid-base pair:

$$HA \rightleftharpoons H^+ + A^-$$

HA = undissociated acid
A^- = conjugate base (any anion)

- Reaction direction (& dissociation rate) dependent on effective concentration of each chemical species.

- If $[H^+]\uparrow$ then equation moves leftwards and *vice versa* if $[H^+]\downarrow$ - ***minimises changes in $[H^+]$.***

Definition: Buffers are chemical systems (water solutions) that are composed of **WEAK ACID and SALT of the ANION of that weak acid**, or **WEAK BASE** and salt of the CATION of the weak base

For Example.

NH4OH+NH4CL

Let us illustrate buffer action by taking example of a common buffer system consisting of solution of acetic acid and sodium acetate.

$$CH3COOH \rightleftharpoons H^{+} + CH3COO^{-}$$

$$CH3COONa \longrightarrow Na^{+} + CH3COO^{-}$$

Since the salt is comletely ionized,it provides the common ion CH3COO in excess.

The biological buffer systems: REMEMBER!!!

- Biochemical reactions are especially sensitive to pH.
- Most biological molecules contain groups of atoms that may be charged or neutral depending on pH, and whether these groups are charged or neutral has a significant effect on the biological activity of the molecule.
- In all multicellular organisms, the fluid within the cell and the fluids surrounding the cells have a characteristic and nearly constant pH.
- This pH is maintained in a number of ways, and one of the most important is through buffer systems.
- **Two important biological buffer systems are:**
- The dihydrogen phosphate system.
- The carbonic acid system.

Biological Buffer

- In multicellular organisms, fluid within cells have a characteristic and nearly constant pH
- One important biological buffer system is **carbonic acid system**
- In blood plasma, the carbonic acid and hydrogen carbonate ion equilibrium buffers the pH

  -Carbonic acid (H_2CO_3) is the hydrogen-ion producer (acid)

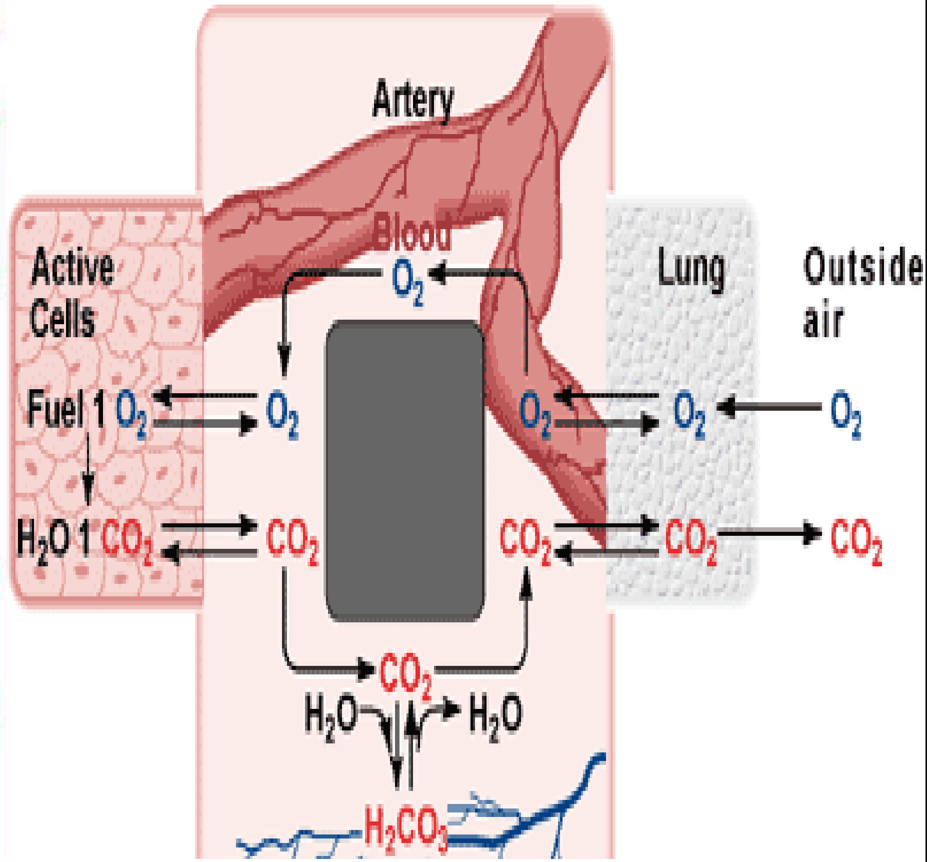
  -Hydrogen carbonate ion (HCO_3^-) is the hydroxide-ion producer (base)

- $H_2CO_{3(aq)} \rightleftharpoons H^+_{(aq)} + HCO_3^-{}_{(aq)}$

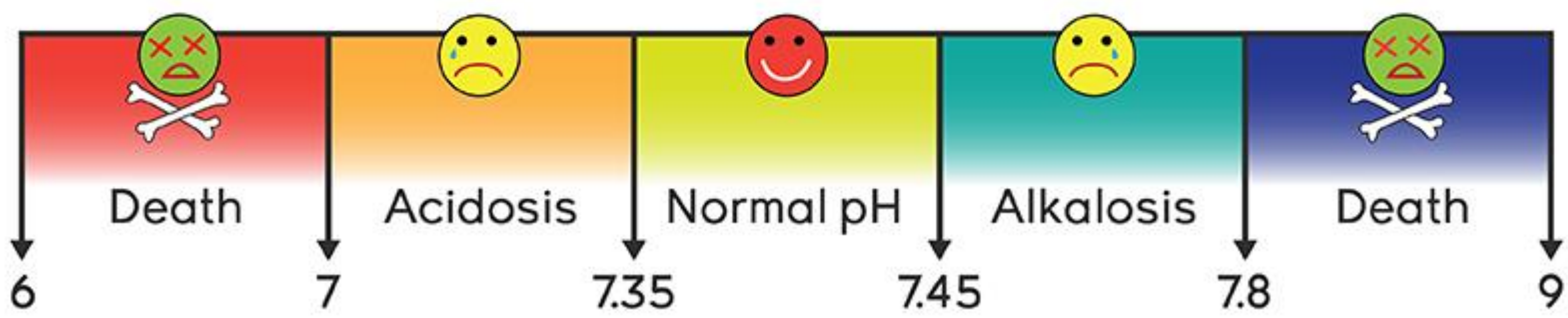
Carbonic acid — Hydrogen carbonate ion

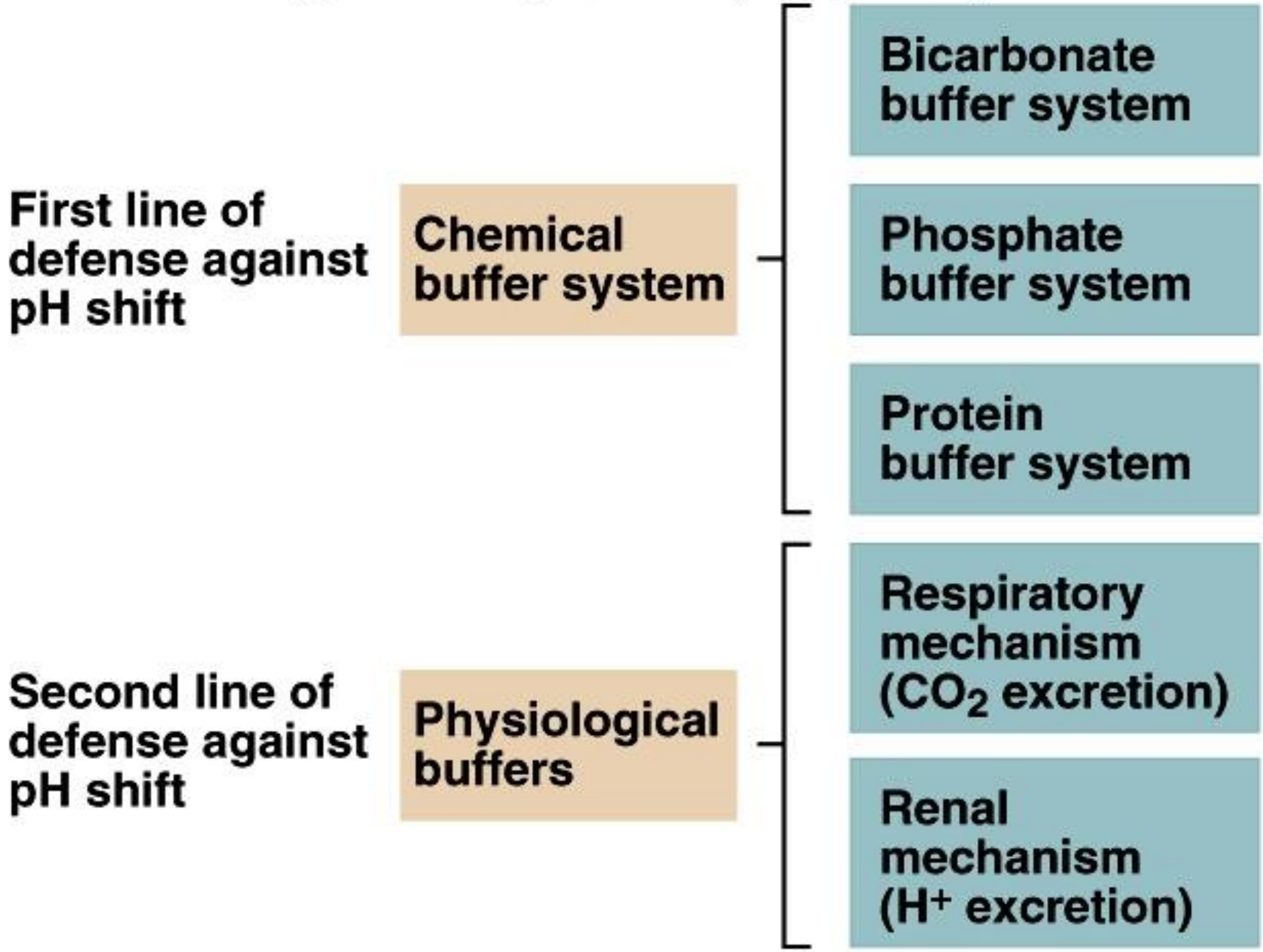
Table 1.4 - Buffer systems of the body fluids

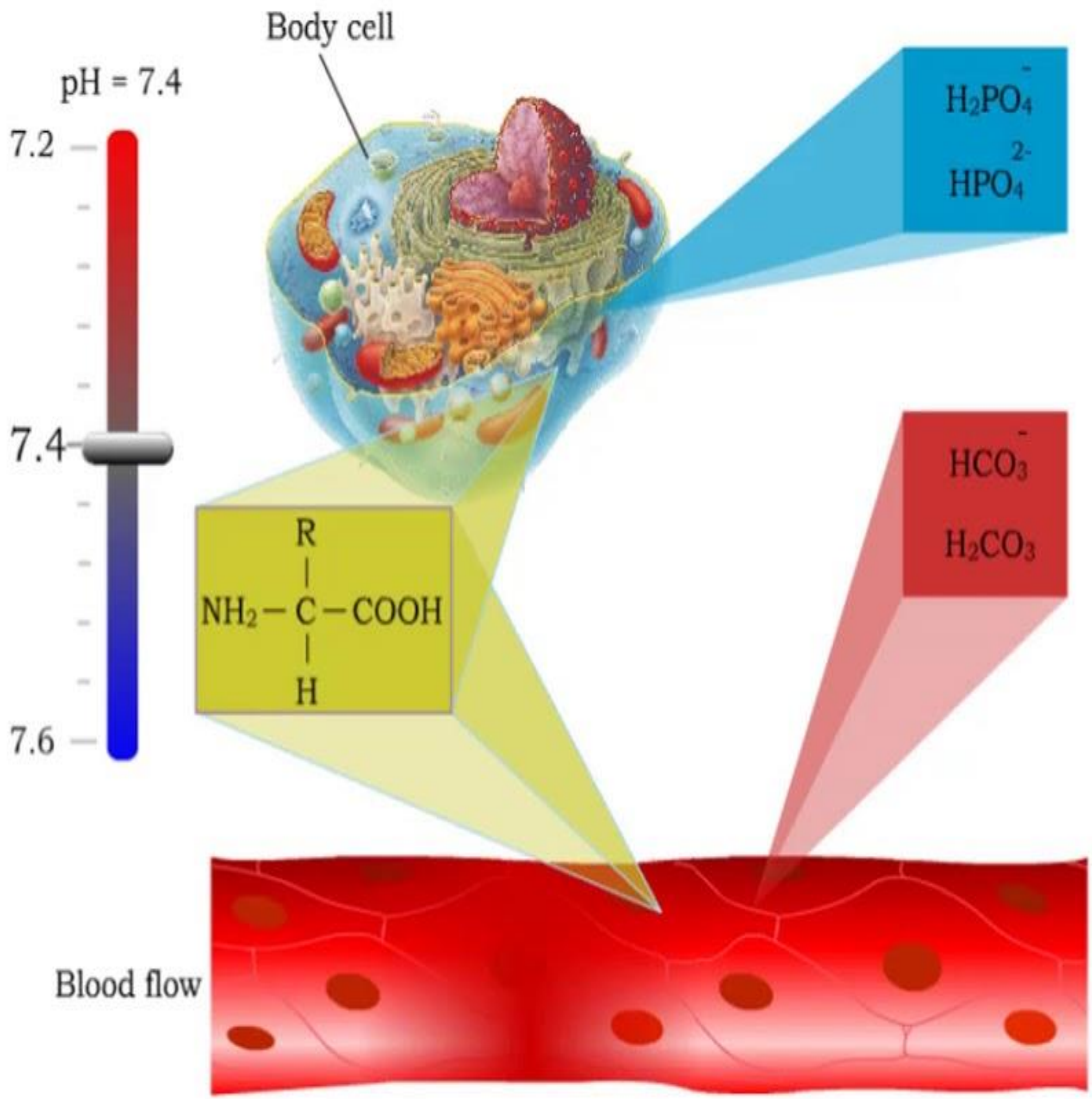
Sl. No	Body Fluid	Buffer system
1	Blood	Bicarbonate, Protein and Hemoglobin buffer system
2	Interstitial fluid	Bicarbonate buffer system
3	Intracellular fluid	Protein and Phosphate buf-



Blood pH Levels




First line of defense against pH shift
Chemical buffer system
Bicarbonate buffer system
Phosphate buffer system
Protein buffer system
Second line of defense against pH shift
Physiological buffers
Respiratory mechanism (CO_2 excretion)
Renal mechanism (H^+ excretion)



Chemical buffers

- There are a variety of chemicals in body fluids that prevent the fluids from undergoing large changes in pH.
- These chemicals buffer or regulate fluctuations in H^+ concentration.
- Chemical buffers:
  - Bind to H^+ ions when there are too many in a solution so pH remains normal.
  - Release H^+ ions when there are too few in a solution so pH remains normal.
- The chemical buffer systems include:
  - Protein system.
  - Phosphate system.
  - Carbonic acid-bicarbonate system.

II. Pharmaceutical Buffers

Buffer solutions are used in pharmaceutical formulation particularly in ophthalmic preparations

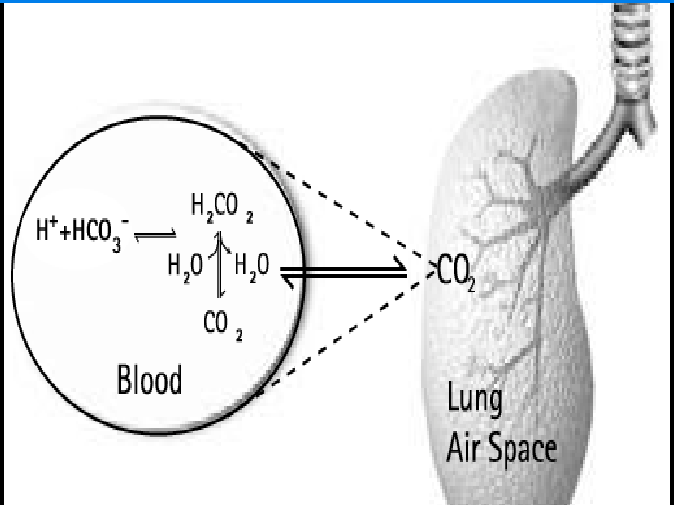
- **Gifford suggested two stock solutions of:**
  - boric acid and monohydrated sodium carbonate
  - mixed in various proportions to yield buffer solutions of pH values from about 5 - 9.

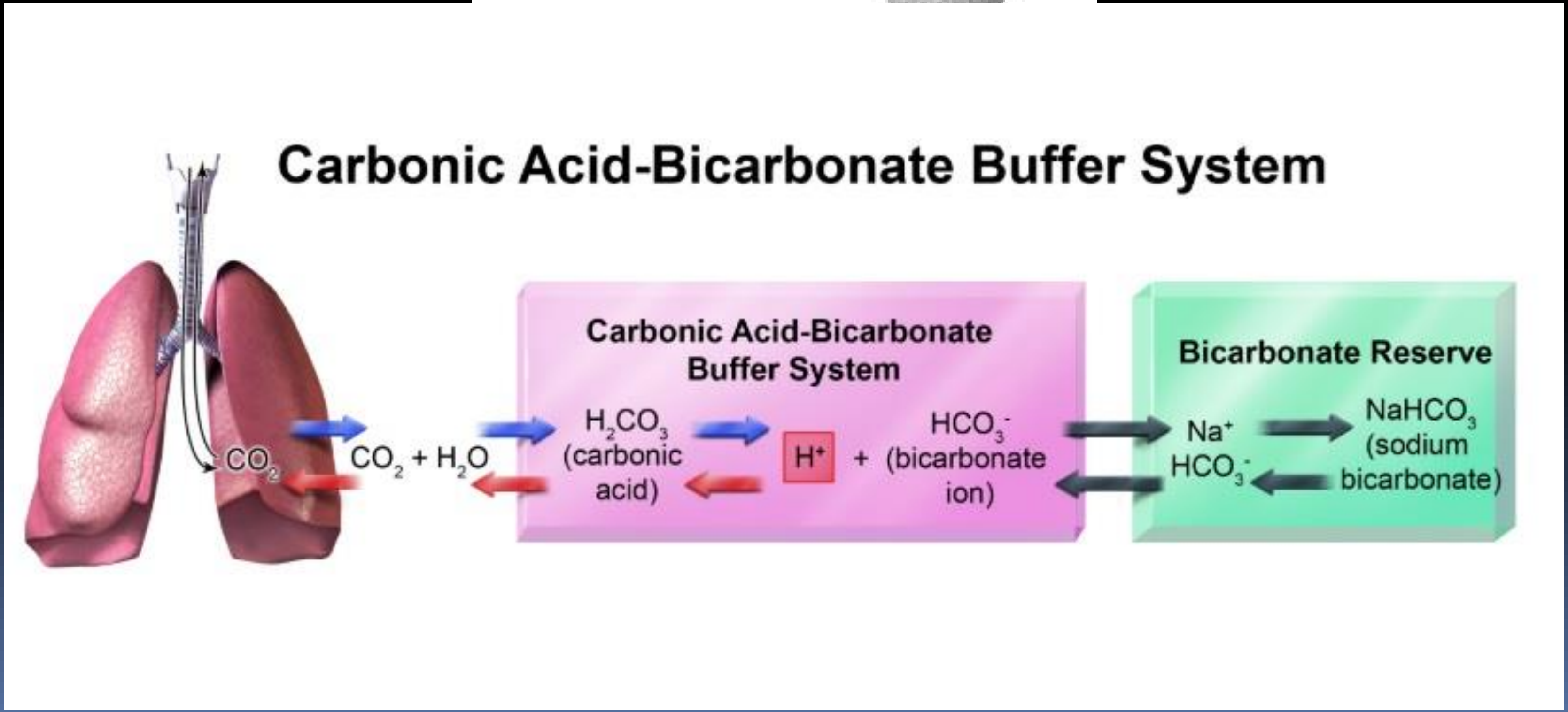
- **Sorensen proposed a mixture of the salts of:**
  - sodium phosphate for buffer solutions of pH 6 to 8.

- **The Clark-Lubs mixtures and their pH ranges**
  a. pH 1.2 to 2.2: HCl and KCl
  b. pH 2.2 to 4.0: HCl and potassium hydrogen phthalate
  c. pH 4.0 to 6.2: NaOH and potassium hydrogen phthalate
  d. pH 5.8 to 8.0: NaOH and KH_2PO_4
  e. pH 7.8 to 10 : H_3BO_3, NaOH and KCl

Sodium chloride is added to buffer mixture to make it isotonic with body.

Mechanism of how carbonate buffer is formed..





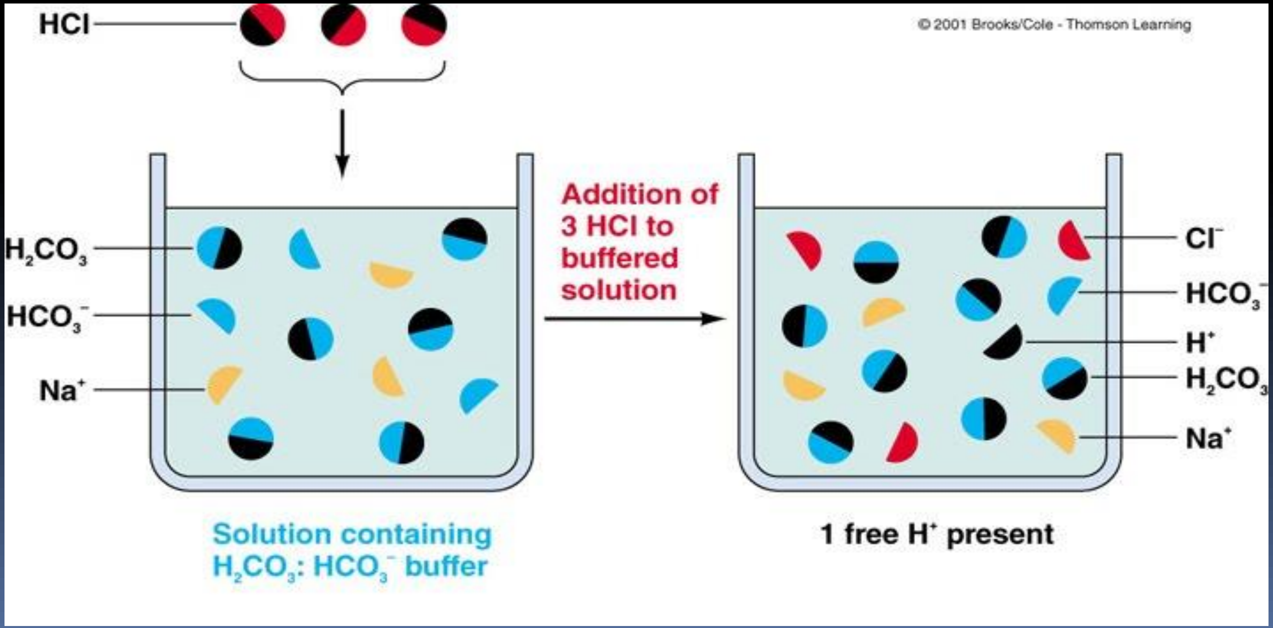
Control of $[H^+]$ - Buffers

What buffer systems exist in the body?

1) Bicarbonate buffer system

- Most important buffering system. Works by acting as proton acceptor for carbonic acid.

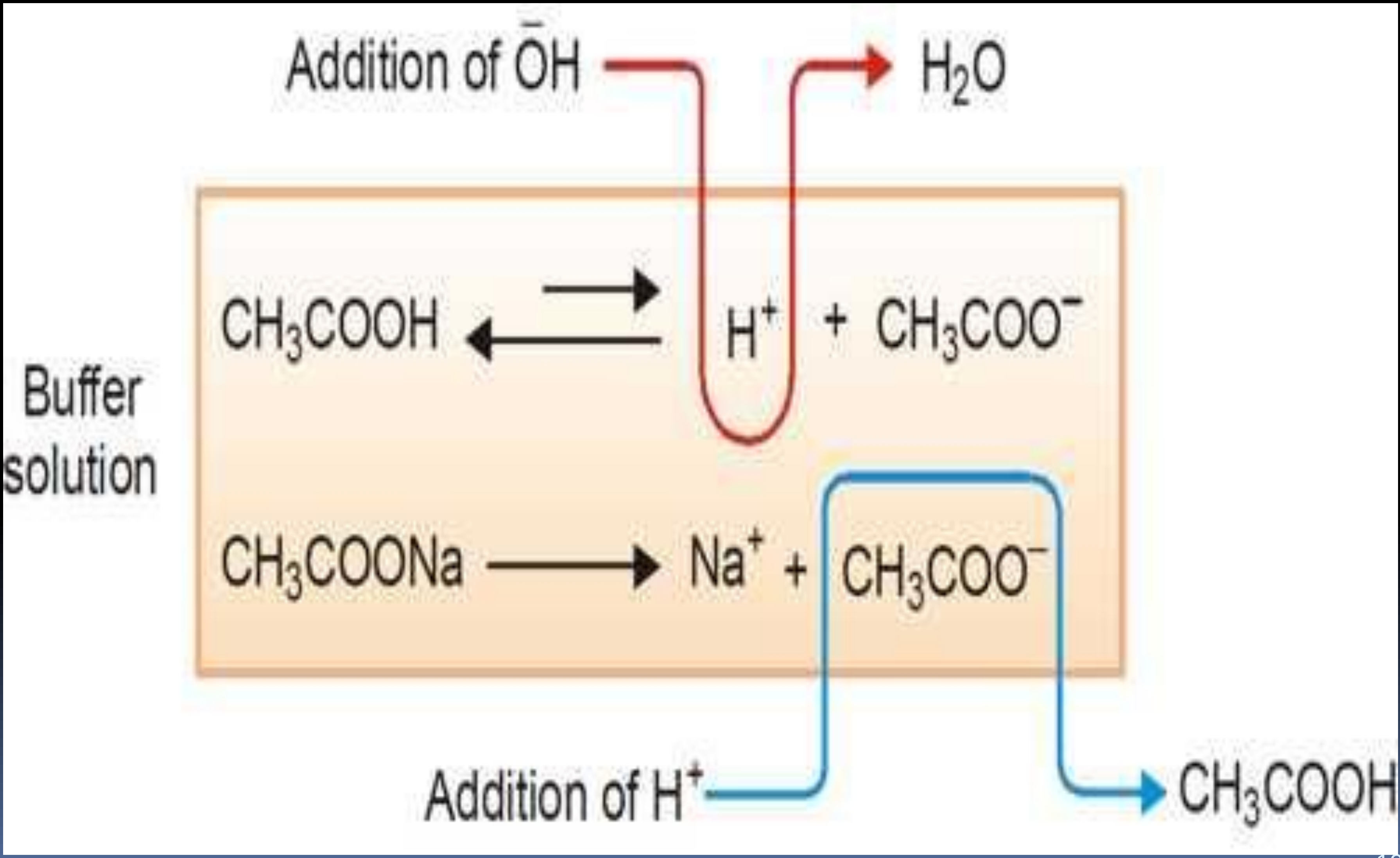
$$CO_2 + H_2O \underset{}{\overset{CA}{\rightleftharpoons}} \underset{\text{carbonic acid}}{H_2CO_3} \rightleftharpoons H^+ + \underset{\text{bicarbonate}}{HCO_3^-}$$



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.

Example of how one buffer works when Strong ACID (H+) or STRONG Base (OH-) Is added



Control of $[H^+]$ - Buffers

2) Phosphate Buffering System

- Phosphate buffer system not important as extracellular fluid buffer (concentration too low).
- However, major **INTRACELLULAR** buffer and important in **RENAL TUBULAR FLUID**.
- Main components are HPO_4^{2-} and $H_2PO_4^-$

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

(Strong acid converted to weak acid ∴ less effect on pH)

$$OH^- + H_2PO_4^- \leftrightarrow H_2O + HPO_4^{2-}$$

(Strong base converted to weak base ∴ less effect on pH)

PHOSPHATE BUFFER SYSTEM

- 1) **Phosphate buffer system**

$$Na_2HPO_4 + H^+ \rightleftharpoons NaH_2PO_4 + Na^+$$

  - Most important in the intracellular system
  - Alternately switches **Na^+** with **H^+**

H^+

Click to animate

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Control of $[H^+]$ - Buffers

3) Protein Buffers

- Proteins among most plentiful buffers in body, particularly highly concentrated **INTRACELLULARLY.**

- ~ 60 - 70% of total chemical buffering of body fluids is located intracellularly, mostly due to intracellular proteins.

- Carboxyl and amino groups on plasma proteins are effective buffers;

$$RCOOH \leftrightarrow RCOO^- + H^+$$

$$RNH_3^+ \leftrightarrow RNH_2 + H^+$$

Control of [H^+] - Buffers

3) Protein Buffers

- Most important non-bicarbonate buffering proteins are titratable groups on **HAEMOGLOBIN**
  (Hb also important for buffering CO_2).

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

$$\downarrow$$

(DeoxyHb a better buffer than OxyHb)

$$H^+ + Hb^- \Leftrightarrow HHb$$

- pH of cells changes in proportion to pH of extracellular fluid – CO_2 can rapidly traverse cell membrane.

Control of [H^+] - Buffers

4) Bone

- Probably involved in providing a degree of buffering (by ionic exchange) in most acid-base disorders.

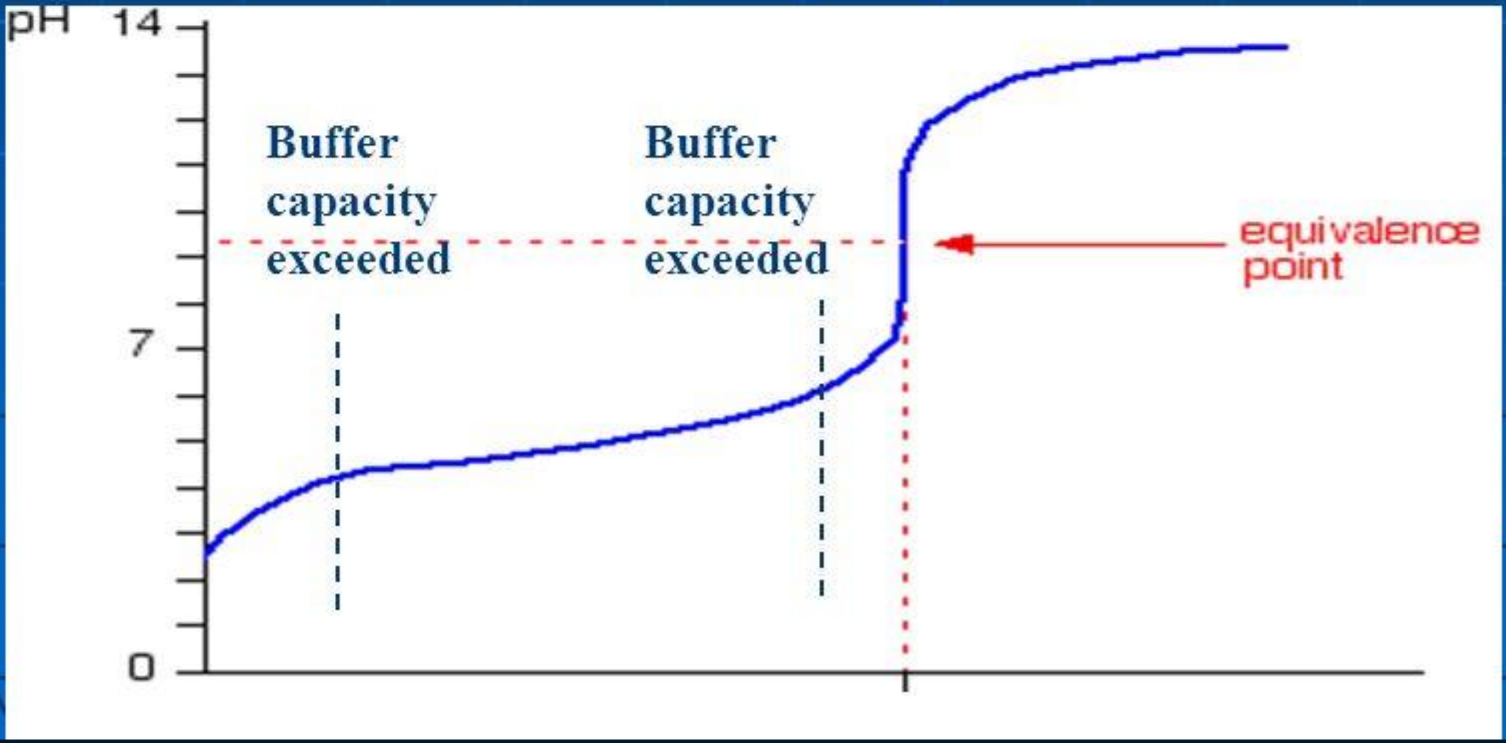
- However, important source of buffer in **CHRONIC** metabolic acidosis (*i.e.* renal tubular acidosis & uraemic acidosis).

- $CaCO_3$ (base) is most important buffer released from bone during metabolic acidosis.

- Results in major depletion of skeletal mineral content (*e.g.* Chronic metabolic acidosis that occurs with renal tubule acidosis (RTA) can lead to development of Rickets / osteomalacia).

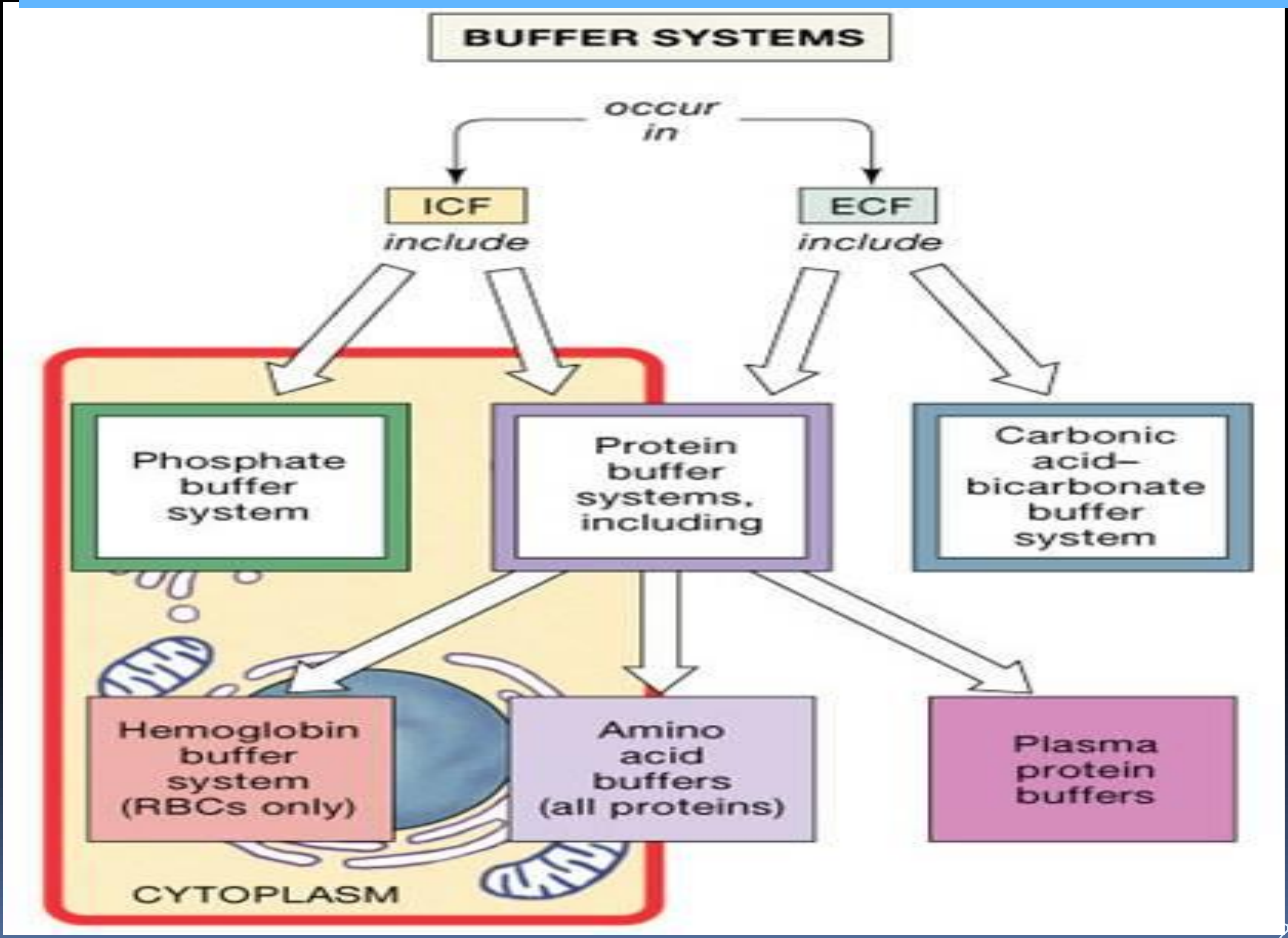
Buffer Capacity

- Buffer Capacity :The amount of acid or base that can be added before a significant change occurs in pH.



Remember: Buffer capacity is HIGHEST if we use EQUIMOLAR concentrations of weak acid (or weak base) and their salts

REMEMBER THESE BUFFER SYSTEMS in the BODY



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