Buffers, pH & Gastric Acid: An Overview

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How are acids and bases defined in biochemical systems?

- **Bronsted** and **Lowry** definition of Acids and Bases are the most convenient for the biochemical systems:
  - Conjugate Acid / Conjugate Bases concept
- **An acid is a Proton Donor;**
- **A base is a Proton Acceptor;**

- **Acid is always accompanied by Conjugate Base;**

- **Base is always accompanied by conjugate acid**

- **Examples**:
- Consider a weak acid: **HA,** it dissociates thus:

$$\mathbf{HA \Longleftarrow\!=\!=\!=\!=\!\Longrightarrow H^{+} + A^{-}}$$

  - **HA is undissociated acid,**
  - **A^- is its conjugate base; it is a proton acceptor,**

- Consider **H_2O**: It can act as a base and as an acid
- Water is Amphoteric thus:

$$\mathbf{H_2O + H_2O \Longleftarrow\!=\!=\!=\!=\!=\!\Longrightarrow H_3O^{+} + OH^{-}}$$

- Can you identify the conjugate base and conjugate acid is this expression?

Dissociation Constant; "Apparent Dissociation Constant"

- Dissociation of weak acid can be expressed thus:

  HA ⇐===⇒ H⁺ + A⁻

- **Ka** (moles/litre) is Dissociation constant for weak acid;

- "Apparent dissociation constant" (**Ka`)** is used in Biochemical and Clinical applications;

$$Ka = \frac{[A^-][H^+]}{[HA]} = Keq$$

$$Ka` = \frac{[A^-][H^+]}{[HA]}$$

What do you understand by “Concentration” of Acid or Base?

- Concentration of an acid or base depends on amount (in grams) of Acid or Base in 1000 ml of solution;
- 0.5 M HCl is less concentrated than 2.0 M HCl solution;
- 0.5 M Acetic acid is less concentrated than 2.0 M Acetic acid solution;

- 0.5 M HCl solution and 0.5 M Acetic acid solution have the same concentration, but the HCl adds more H^+ ions to the solution than the Acetic acid because HCl is a strong acid;
- Concentration is represented as **[ ],**
  - **Example: [HCl] = 0.5 M**

TAKE NOTE

- 1.0 molar (1.0 M) solution contains the molecular weight in grams of the compound in 1000ml of solution;
- 0.5 M NaCl solution will contain **0.5 x 58.4g** of NaCl in 1000ml of solution {Note: 58.4 is the mol wt of NaCl};

- 11.0mM solution of Glucose will contain 11 x 180mg of Glucose in 1000ml of solution {Mol wt of Glucose = 180}

How is Ka` relates to pKa` of a weak acid?

- Relative strengths of weak acids and weak bases are expressed quantitatively as their Apparent Dissociation Constants, which express their tendency to dissociate;
- **By definition: pKa` = $-\log_{10}$Ka`**

- For weak acid: **pKa`** is the **pH** at which the concentrations of Protonated and Unprotonated species are equal in solution {**HA = A^-**};
- Tendency of a weak acid to dissociate can be evaluated from the **Ka` or pKa`** value;

- **Ka`** is directly proportional to strength of the acid;

$$\mathbf{HA \Longleftrightarrow H^{+} + A^{-}}$$

$$\mathbf{Ka` = [A][H^{+}] / [HA]}$$

- The **smaller** the **Ka`** value, the lower the tendency of the acid to dissociate and the **weaker the acid**;
- The **larger** the **Ka`** value the higher the tendency of the acid to dissociate and the **stronger the acid**

- The **smaller** the **Ka`**, the **larger** the **pKa`;**

pKa` = -log Ka`

- **pKa`** is **inversely related to strength of the acid;**
- The **larger** the **pKa`** the **weaker the acid**;
- The **smaller** the pKa` the **stronger the acid;**

Some relevant weak acids and their conjugate bases

Acid	Conjugate base	pKa`
Ammonium ion (NH_4^+)	Ammonia (NH_3)	9.25
Carbonic acid (H_2CO_3)	Bicarbonate ion (HCO_3^-)	6.37
Dihydrogen phosphate ion ($H_2PO_4^-$)	Monohydrogen phosphate ion (HPO_3^{2-})	6.86
Lactic acid ($CH_3CHOHCOOH$)	Lactate ion ($CH_3CHOHCOO^-$)	3.86

HANDERSON – HASSELBALCH EQUATION

- Handerson-Hasselbalch equation is the relationship between **pH** of a solution containing a weak acid and the **Ka`** (or **pKa`**) of the acid;
- Equation for a weak acid can be expressed as follows:

$$pH = pKa + \log_{10} \frac{[\text{Conj Base}]}{[\text{Cong Acid}]}$$

What are some uses of Henderson-Hasselbalch equation?

- Preparation of buffer solutions of known pH
- Calculating the pH of biochemical solutions;
- Representation of Acid – Base balance during metabolism;
- Helps to predict the effects of various alterations in Acid – Base balance;

What is a Buffer Solution?

- Buffer solution is a solution that resists change in pH when small amounts of acid or base are added
- **Two main types of buffer solutions**:
  - **Acidic buffer** solution:
    - Made up of a **weak acid** and **salt of the weak acid**;
  - **Basic buffer** solution:
    - Made up of a **weak base** and **salt the weak base;**

What are the major buffers in the metabolic system?

- Major buffers with Conjugate Acid/Conjugate Base pairs:
- **Bicarbonate buffer system: H_2CO_3 / HCO_3^- ;**
  - **NB: actual value of $[H_2CO_3] = \{[H_2CO_3] + [CO_{2\ dissolved}]\}$**
- **Haemoglobin buffer system: HHb / Hb^-**
- **Oxyhaemoglobin buffer system: $HHbO_2$ / HbO_2^-**
- **Phosphate buffer system: $H_2PO_4^-$ / HPO_4^{2-}**
- **Protein buffer system: RCOOH (NH_3^+) / RNH_2 (COO^-)**

- In RBC: main buffer systems are:
  - **Haemoglobin buffer,**
  - **Oxyhaemoglobin buffer,**
  - **Bicarbonate buffer;**

- Blood plasma: main buffer systems are:
  - **Bicarbonate buffer,**
  - **Protein buffer,**
  - **Phosphate buffer;**

How is the Bicarbonate Buffer system represented?

- An expression for Bicarbonate buffer system in blood is:

Carbonic Anhydrase

$$CO_2(gas) \Leftarrow\!==\!\Rightarrow CO_2(diss) + H_2O \Leftarrow\!==\!\Rightarrow H_2CO_3 \Leftarrow\!==\!\Rightarrow H^+ + HCO_3^-$$

- This expression can be separated into two:
- **Carbonic Anhydrase** (Zn activator) catalyses reaction:

$$CO_2 + H_2O \Leftarrow\!===\!\Rightarrow H_2CO_3$$

- Carbonic Acid rapidly dissociates thus:

$$H_2CO_3 \Leftarrow\!===\!\Rightarrow H^+ + HCO_3^-$$

- Combining the two expressions give:

$$CO_2 + H_2O \Leftarrow\!==\!\Rightarrow H^+ + HCO_3^-$$

- Equilibrium expression can be written as:
- CO_2 can be written as $\mathbf{Pco_2}$ (Partial Pressure CO_2 in solution)
- Concentration of dissolved CO_2 ($meqL^{-1}$) is obtained by multiplying Pco_2 by factor **α;**
- Thus, $\boldsymbol{\alpha Pco_2 = meq\ L^{-1}}$
- $\alpha = 0.03 meq\ L^{-1}\ mmHg^{-1}$ at 37^oC

$$Ka` = \frac{[H^+][HCO_3^-]}{[CO_2]}$$

- Substituting for $[CO_2]$ in the equilibrium expression gives

$$Ka` = \frac{[H^+][HCO_3^-]}{0.03\ Pco_2}$$

- Henderson – Hasselbalch equation for Bicarbonate buffer system becomes

$$PH = 6.1 + Log_{10} \frac{[HCO_3^-]}{0.03\ Pco_2}$$

- Note: in this equation $[HCO_3^-]$ is expressed in meq L^{-1}

GASTROINTESTINAL ACID PRODUCTION (GAP)

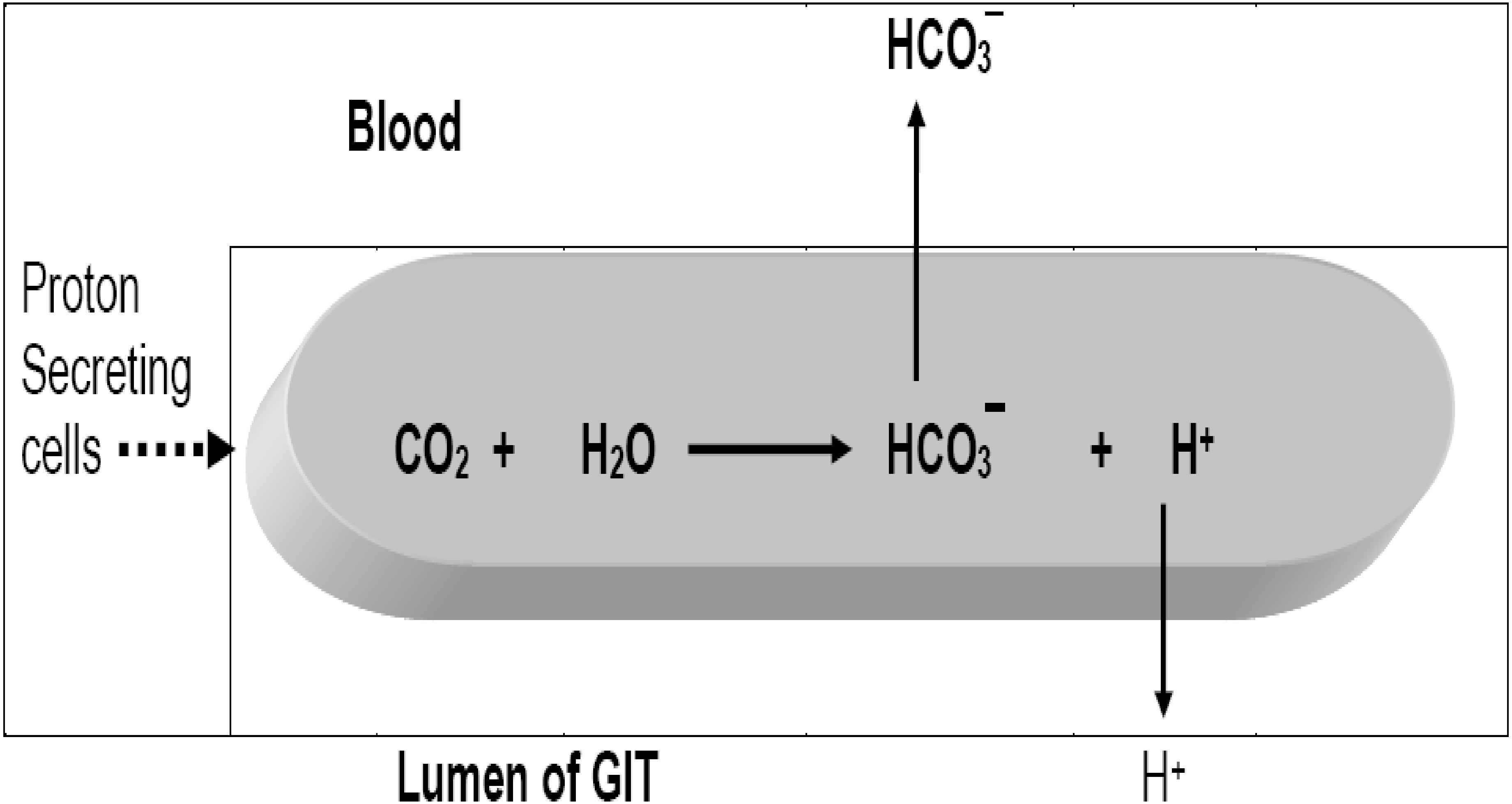
- **H^+ ions** or **HCO_3^- ions** are secreted into the gut lumen;
- Example:
  - **H^+ ions are secreted in the Stomach,**
  - **HCO_3^- ions are secreted in the Colon**,

- Production of HCO_3^- ions and H^+ ions inside Gut mucosal cells occur via the same net reaction:

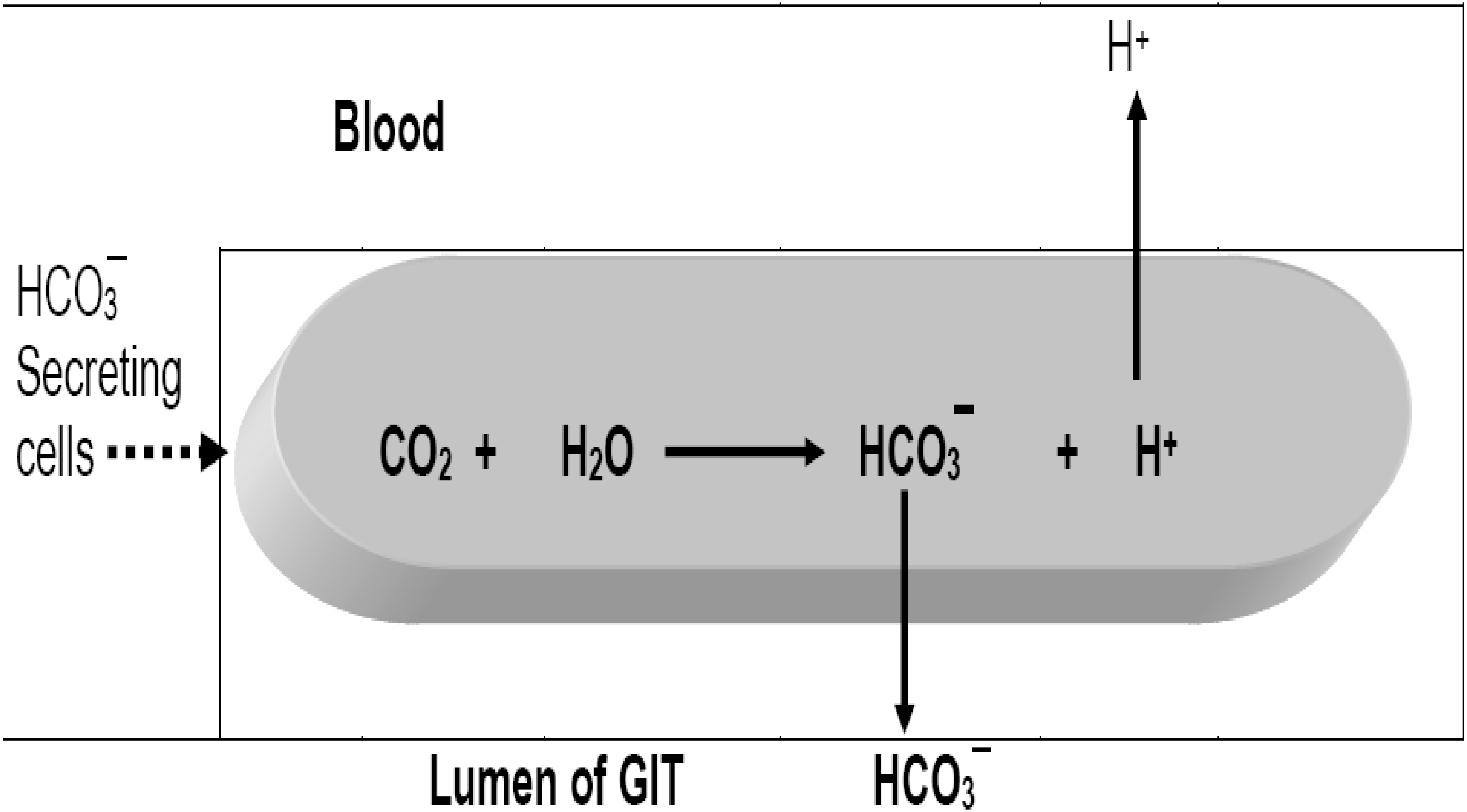
$$CO_2 + H_2O \Leftarrow\!=\!=\!=\!=\!=\!\Rightarrow HCO_3^- + H^+$$

Let us consider two different cases: **Fig. 1 & Fig. 2**

When **H^+ ions** are secreted into the Gut Lumen, HCO_3^- ions are also produced (**Fig. 1**)



When **HCO_3^- ions** are secreted into the Gut Lumen **H^+ ions** are also produced (Fig. 2)



- In Fig. 1, the H^+ ions produced do not stay in the Gut lumen because they are not needed;
- In Fig. 2, the HCO_3^- ions produced also do not stay in the Gut lumen because they are not needed;
- WHAT THEN HAPPENS TO THESE IONS IN BOTH CASES?

- Both ions are moved in the direction opposite to the Gut lumen;
- That is, into the Interstitial Fluid and Blood as shown in the respective figures; (Fig. 1 and 2);
    - If an organ secretes **H^+ ions** into the Gut Lumen, it must secretes **HCO_3^- ions** into the Blood;
    - If it secretes **HCO_3^- ions** into the Gut Lumen, it must secretes **H^+ ions** into the Blood;
- **Effects on Blood and Gut Lumen are equal and opposite because a One-to-one ratio of H^+ ion and HCO_3^- ion are produced by each reaction;**

What happens in different segments in the GIT?

STOMACH:

- Under fasting (basal) conditions: Parietal cells secrete H^+ ions into Gastric lumen at about 10mM per hour;
- Following meals (Postprandial): Rate of secretion can reach as much as 50mM per hour;
  - Secretion of H^+ ions lowers pH to 1.0 in Chyme;
- Stomach releases HCO_3^- in Blood at rest and during meals
- Resulting in very slight Postprandial Rise in **[HCO_3^-]** in plasma, which often cause, after a delay, Renal Spillage of HCO_3^- ions;
- Rise in **[HCO_3^-]** that occurs in urine after a meal is called the "Alkaline tide";

DUODENUM AND ASSOCIATED ORGANS

- HCO_3^- are secreted into Duodenal Chyme from **3** sources:
  - Pancreas,
  - Gall Bladder,
  - Duodenal Mucosa,
- **[HCO_3^-]** in Pancreatic Fluid is about 25mM (Basal) and 150mM (Postprandial);
- In 24 hours, **[HCO_3^-]** Pancreatic secretion is about 200mM
- **[HCO_3^-]** in Bile from Gall Bladder is about 40mM;
- Duodenal Mucosa also generates and secretes HCO_3^- ions;
- In all cases equi-molar amount of **[H^+]** is release in blood

JEJUNUM, ILEUM, and COLON

- Jejunum secretes small quantities of H^+ ions into the Gut lumen, thereby Alkalinising the Blood;
- Ileum secretes HCO_3^- ions, thus Acidifying the Blood;
- Colon secretes over 200mM HCO_3^- ions per day into Lumen; thus has a major Acidifying effect on blood;

GENERAL CONCEPT

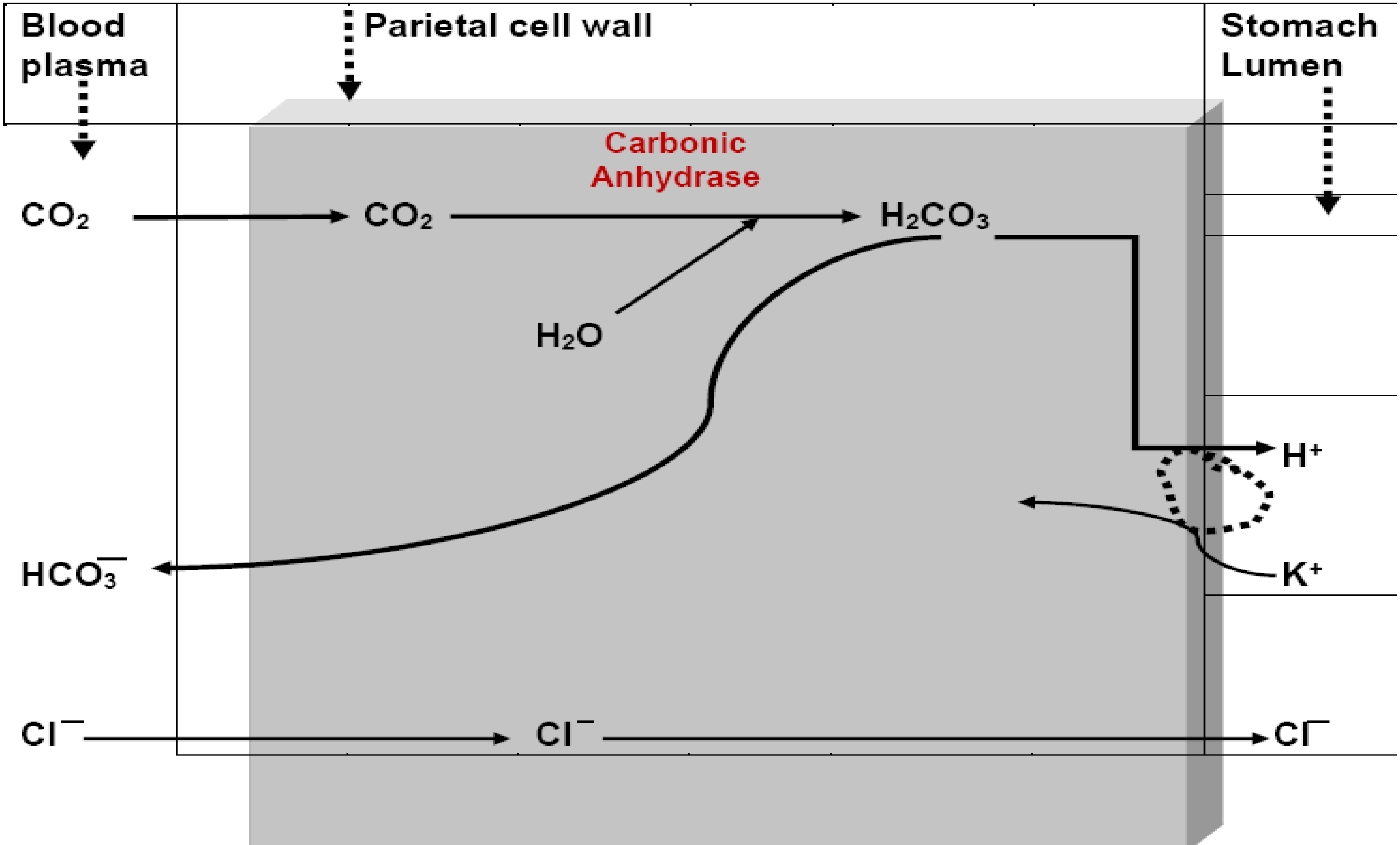
General view is as follows:

- Stomach secretes:
  - **H^+ ions into Gut lumen, thus Acidifying its contents,**
  - **HCO_3^- ions into the Blood, thus Alkalinising it;**
- Almost all the segments below the Pylorus have the opposite effect: They secrete
  - **HCO_3^- ions into the Gut lumen, Alkalinising it;**
  - **H^+ ions into the blood, thus Acidifying it;**

Simplified mechanism for Production of Gastric Acid

- Parietal (Oxyntic) cells are major source of Gastric HCl
- Source of H^+ is Carbonic Anhydrase formation of H_2CO_3 from H_2O and CO_2 (**Fig. 4**)
- Alkaline urine follows Ingestion of Meals ("Alkaline tide"), as a result of formation of HCO_3^- ions in the process of HCl secretion;
- Secretion of H^+ into lumen is an active process driven by a membrane-located H^+- K^+ ATPase;
- Parietal cells contain numerous Mitochondria needed to generate ATP used for H^+ - K^+ ATPase to function;
- HCO_3^- ions pass into Plasma in exchange for Cl^-, which is coupled to secretion of H^+ into lumen;

Fig. 4: Production of Gastric Acid (HCl) in Parietal cell using H^+ - K^+ ATPase (H^+ - K^+ – Pump)



What are some of the effects of Gastric acid?

- Gastric acid caused proteins to denature, making them more accessible to the action of Proteases;
- Low pH has the effect of destroying most microorganisms entering the GIT;
- Some clinical conditions may arise from defects in digestive processes, such as ulceration by Gastric HCl or diminished secretion of HCl causing **Achlorhydria;**
- Parietal cells may secrete HCl at concentration of 160 mM (equivalent to pH of 0.8);
- Despite the high acidity the epithelium of the stomach is intrinsically resistant to damage by Gastric acid;
- Excessive secretion of Gastric acid may lead to Gastritis, Gastric ulcers and Peptic acid disease;

REFERENCES

- Textbook of Biochemistry, with clinical correlations, Ed. By T. M. Devlin, 4th Ed.
- Harper's Illustrated Biochemistry 26th Edition; 2003; Ed. By R. K. Murray et. al.
- Biochemistry, By V. L. Davidson & D. B. Sittman. 3rd Edition.
- Hames BD, Hooper NM, JD Houghton; Instant Notes in Biochemistry, Bios Scientific Pub, Springer; UK.
- VJ Temple Biochemistry 1001: Review and Viva Voce Questions and Answers Approach; Sterling Publishers Private Limited, 2012, New Delhi-110 – 020.