3. Henderson-Hasselbalch equation and its significance

Derivation of Henderson-Hasselbalch equation

A weak acid, HA, ionizes as follows:

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

The equilibrium constant for this dissociation is

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

Cross-multiplication gives

$$[H^+][A^-] = K_a[HA]$$

Divide both sides by [A−]:

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

Take the log of both sides:

$$\log[H^+] = \log\left(K_a \frac{[HA]}{[A^-]}\right)$$

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

Multiply through by −1:

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

Substitute pH and pKa for −log [H+] and −log Ka, respectively; then

$$pH = pK_a - \log\frac{[HA]}{[A^-]}$$

Inversion of the last term removes the minus sign and gives the Henderson-Hasselbalch equation

$$\mathbf{pH = pK_a + \log\frac{[A^-]}{[HA]}}$$

OR

$$pH = pK_a + \log\frac{[base]}{[acid]}$$

OR

$$pH = pK_a + \log\frac{[salt]}{[acid]}$$

NOTES FOR 6.7 & 6.8

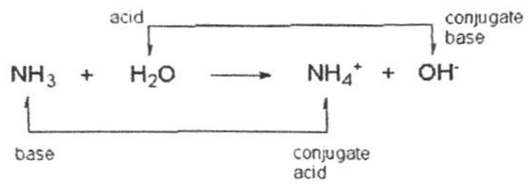
1. Acid, Bases & pH

Arrhenius concept of acids and bases – Acids dissociate in water to give hydrogen ions (H^+) and bases dissociate to give hydroxyl ions (OH^-).

Lewis concept – Acid accepts electron pair and base donates electron pair.

Bronsted-Lowry theory – Acid donates H+ ions and bases accept H^+ ions.

- The acid-base pair that differs only by one proton is called a conjugate acid-base pair.



- ***The conjugate base of a strong acid will be a weak base,*** and also vice versa.

pH scale (proposed by Sorenson)

- It is the H^+ concentration expressed as the negative logarithm of hydrogen ion concentration.

$$pH = -\log[H+] = \log\frac{1}{[H+]}$$

- Thus, the pH value is inversely proportional to the acidity.
- pH 7 indicates neutral pH.

2. Buffers and buffering capacity

Buffers – solutions which can resist changes in pH when acid or alkali is added.

Composition of a buffer – 2 types of buffers are there:
- Mixtures of weak acids with their salt with a strong base OR
- Mixtures of weak bases with their salt with a strong acid.

Examples:
- $H_2CO_3/NaHCO_3$ (Bicarbonate buffer – carbonic acid and sodium bicarbonate)
- CH_3COOH/CH_3COONa (Acetate buffer – acetic acid and sodium acetate)
- Na_2HPO_4/NaH_2PO_4 (Phosphate buffer)

Buffering capacity – defined as the number of moles of acid or base required to be added to one litre of the buffer solution so as to change its pH by one.

Buffer systems of the body are:

	Extracellular fluid	Intracellular fluid	Erythrocyte fluid
1.	$\frac{NaHCO_3}{H_2CO_3}$ (bicarbonate)	$\frac{K_2HPO_4}{KH_2PO_4}$ (phosphate)	$\frac{K^+Hb}{H^+Hb}$ (hemoglobin)
2.	$\frac{Na_2HPO_4}{NaH_2PO_4}$ (phosphate)	$\frac{K^+Protein}{H^+Protein}$ (protein buffer)	$\frac{K_2HPO_4}{KH_2PO_4}$ (phosphate)
3.	$\frac{Na^+Albumin}{H^+Albumin}$	$\frac{KHCO_3}{H_2CO_3}$	$\frac{KHCO_3}{H_2CO_3}$

Bicarbonate buffer system ($NaHCO_3/H_2CO_3$)

- Major extracellular buffer.
- Most important buffer system in the plasma.
- $NaHCO_3$ is the metabolic component regulated by the kidney.
- H_2CO_3 is the respiratory component regulated by the lungs.
- The ratio of HCO_3^- to H_2CO_3 at pH 7.4 is 20 under normal conditions. This is much higher than the theoretical value of 1 which ensures maximum effectiveness. But, under physiological conditions, the ratio of 20 (a high alkali reserve) ensures high buffering capacity against acids.

Phosphate buffer system (Na_2HPO_4/NaH_2PO_4)

- Major intracellular buffer.
- The phosphate buffer is found to be effective at a wide pH range, because it has more than one ionizable group and the pKa values are different for these.

$$H_3PO_4 \xrightarrow{pKa = 1.96} H^+ + H_2PO_4^-$$

$$H_2PO_4^- \xrightarrow{pKa = 6.8} H^+ + HPO_4^= \ (Na_2HPO_4/NaH_2PO_4)$$

$$HPO_4^= \xrightarrow{pKa = 12.4} H^+ + PO_4^o$$

- Na2HPO4/NaH2PO4 is an effective buffer system in the body because its pKa value is nearest to physiological pH.

Protein buffer system (Haemoglobin, plasma proteins)

- Buffering capacity of protein depends on the pKa value of its ionizable side chains.
- The most effective group is histidine imidazole group with a pKa value of 6.1.

5. Respiratory regulation of blood pH

Introduction

- Respiratory regulation of blood pH is the second line of defence against acid load.
- This is achieved by changing the pCO_2.
- The respiratory system responds immediately, but it cannot proceed to completion.

Some observations of Henderson-Hasselbalch equation in various conditions:

i. When an acid is exactly half-neutralized (which means only half of the acid has become ionized or neutralized), $[A^-] = [HA]$

$$pH = pK_a + \log\frac{[A^-]}{[HA]} = pK_a + \log\left(\frac{1}{1}\right) = pK_a + 0$$

Therefore, at half-neutralization, $pH = pK_a$. This is the pH at which the buffer will be most effective. The effective range of a buffer is 1 pH unit higher or lower than pK_a ($pK_a \pm 1$).

ii. When the ratio $[A^-]/[HA] = 100:1$,

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

$$pH = pK_a + \log(100/1) = pK_a + 2$$

iii. When the ratio $[A^-]/[HA] = 1:10$,

$$pH = pK_a + \log(1/10) = pK_a + (-1)$$

Applications of Henderson-Hasselbalch equation

i. This equation can predict the pH of a buffer on the addition of a known quantity of acid and alkali.
ii. The concentration of salt or acid can be found out by measuring the pH.
iii. It can be used to calculate the amount of acid and conjugate base to be combined to prepare a buffer solution with a particular pH.
iv. It helps in assessing the acid-base status of the body.
v. Predicts the limits of compensation of body buffers.

ALWAYS REMEMBER THESE POINTS:

- pH of the buffer is dependent on the **relative proportion of the salt and acid** (according to the Henderson-Hasselbalch equation).
- Buffering capacity of a buffer is determined by the **absolute concentration of the salt and acid.**

4. Body buffers and their role in regulation of blood pH

Introduction

- Cellular metabolism predominantly generates acids, and body buffers avert imminent pH change by these acids.
- Thus, buffers are the first-line of defence against acid load.
- The important ones are:
  - bicarbonate buffer
  - phosphate buffer
  - proteins

6. Renal regulation of blood pH

Introduction

- Renal regulation of blood pH is the third line of defence against acid load.
- This is achieved by changing the pH of urine (acidification of urine).
- The renal compensation of acid-base imbalances occurs in a long term manner and is sustained.

Strategies used by renal regulation mechanism

The major renal mechanisms for regulation of pH are:

i. Excretion of free H^+
ii. Recovery of filtered bicarbonate

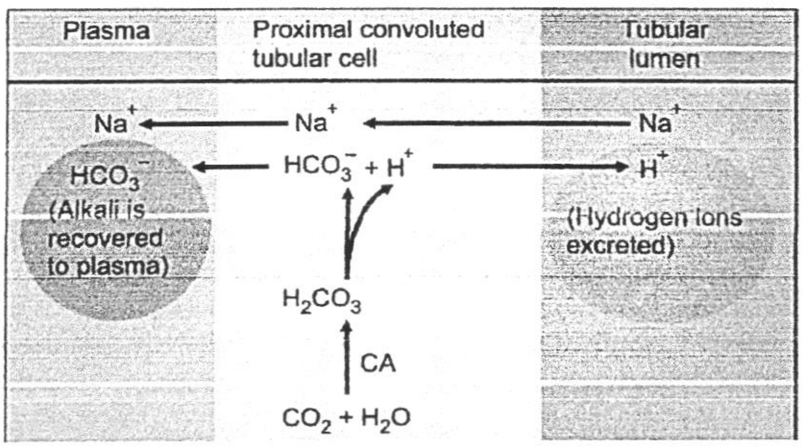
} Occurs in Proximal Convoluted Tubules

iii. Excretion of Titratable acids
iv. Excretion of NH_4^+ ions

} Occurs in Distal Convoluted Tubules / Collecting Duct

i. Excretion of free H^+ - Generation of bicarbonate (steps in the diagram below)

- Very negligible amount of H+ is lost through this mechanism.
- The sodium-hydrogen antiport mechanism is present at the apical membrane of both PCT and ascending limb of the loop of Henle. When Na+ enters the cell, H+ ions from the cell are secreted into the luminal fluid. Hence, factors affecting sodium, such as the aldosterone hormone, influence H+ secretion.
- 3 HCO_3^- ions and one Na^+ ion are absorbed by a symport system.
- Carbonic anhydrase inhibitors like acetazolamide can inhibit this process.
- *Under conditions of alkalosis, the excess bicarbonate can be excreted.*
- There is net excretion of H+ ions and net generation of HCO3-. So, this mechanism has the potential to increase the alkali reserve.



(*) Respiration regulation – 2nd line of defence against acid Base
– This is done by changing the pCO_2
– Respiratory system responds immediately but does not proceed to completion

(*) Tools used by respiratory regulation mechanism

i. **Hyperventilation / hypoventilation process**

- These processes are triggered when the central and peripheral chemoreceptors sense the pH changes.
- The below two diagrams will make it clear how do these mechanisms operate differently in acidosis and alkalosis.

Respiratory regulation in acidosis

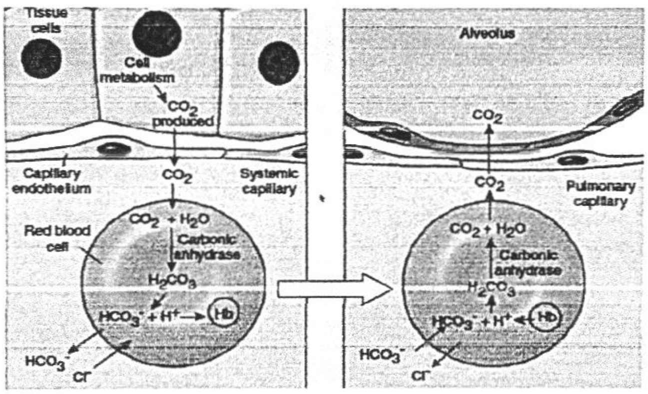
- Acids rise
- H+ rises
- pH falls
- Central chemoreceptors sense this
- Hyperventilation occurs to pump out CO_2

Respiratory regulation in alkalosis

- Alkali rise
- H+ falls
- pH rises
- Central chemoreceptors sense this
- Hypoventilation occurs to retain CO_2

ii. **Hemoglobin**

- It helps in the removal of CO_2 by *"Reversal of Chloride shift"*.



In tissues, these reactions occur...

Carbonic anhydrase

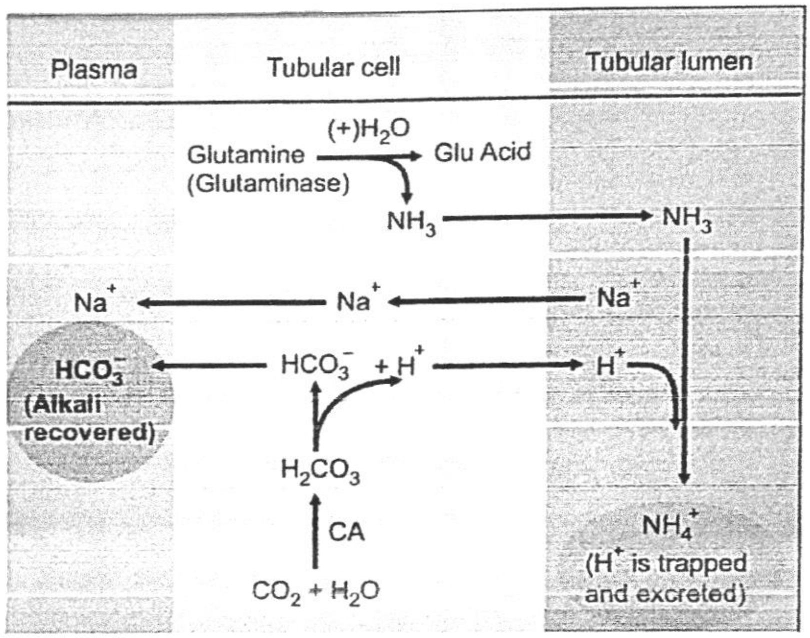
$$CO_2 + H_2O \longrightarrow H_2CO_3$$
$$H_2CO_3 \longrightarrow HCO_3^- + H^+$$
$$H^+ + Hb^- \longrightarrow HHb$$

In lungs, these reactions occur...

$$HHb + O_2 \longrightarrow HbO_2 + H^+$$
$$HCO_3^- + H^+ \longrightarrow H_2CO_3$$
$$H_2CO_3 \longrightarrow H_2O + CO_2$$

iv. <u>Excretion of NH_4^+ ions (steps in the diagram below)</u>

- This mechanism helps trap hydrogen ions in the urine, so that a large quantity of acid may be excreted.
- Here, instead of sodium-hydrogen antiport, sodium-ammonium ion antiport is present.
- Net loss of H+ ions in urine
- Net gain of HCO_3- is present



7. Anion gap

<u>Definition</u>

- It's the difference between measured cations (positively charged ions like Na^+ and K^+) and measured anions (negatively charged ions like Cl^- and HCO_3^-).
- The unmeasured anions constitute the anion gap.

<u>Explanation for Anion gap</u>

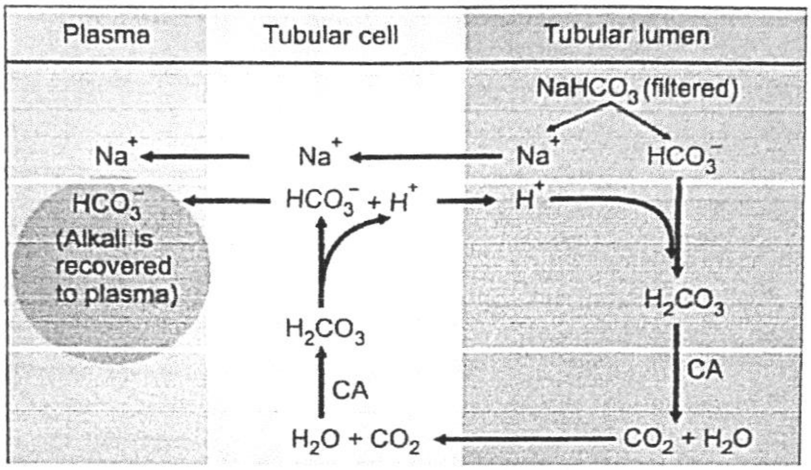
- The sum of cations and anions in ECF is always equal in order to maintain electrical neutrality. Still, there is a gap (anion gap) between the measured cations and anions.
- This is due to the presence of protein anions, sulfate, phosphate and organic acids.

<u>Equation for Anion gap</u>

$$AG = \left[Na^+\right] + \left[K^+\right] - \left[Cl^-\right] + \left[HCO_3^-\right]$$

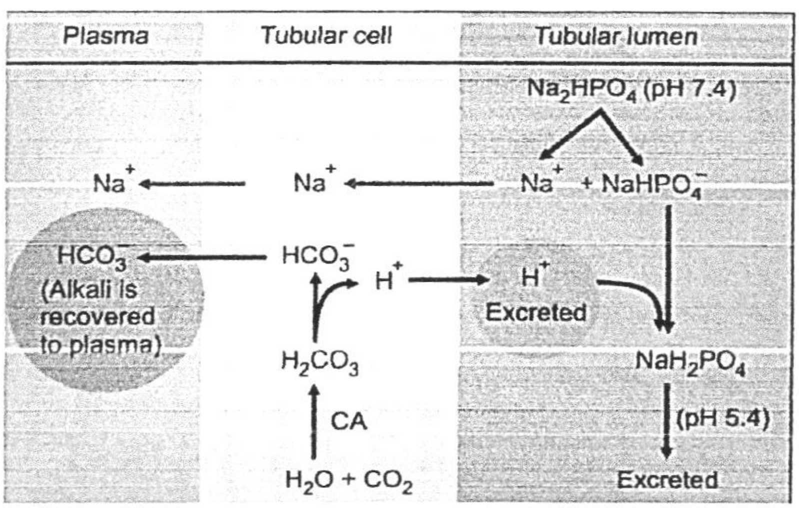
ii. Recovery of filtered bicarbonate (steps in the diagram below)

- The net effect of these processes is the reabsorption of filtered bicarbonate, which is mediated by the sodium-hydrogen exchanger. This mechanism prevents the loss of HCO_3^- through urine.
- This is mainly a mechanism to conserve the base.
- No net change in H^+ ions
- No net gain of HCO_3^-
- Cannot correct acidosis, but can maintain status quo.

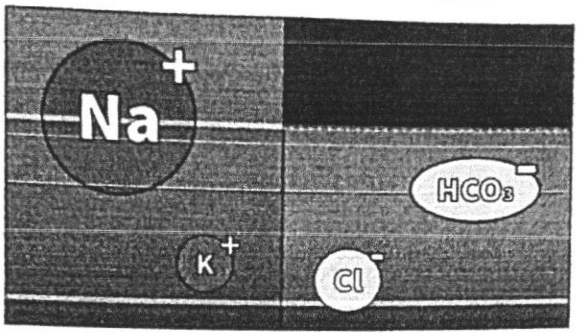


iii. Excretion of Titratable acids (steps in the diagram below)

- **Hydrogen ions are secreted by the distal tubules and collecting ducts by Hydrogen ion-ATPase located in the apical cell membrane.**
- **"Titratable acidity"**: Number of millilitres $\frac{1}{10}$ N NaOH required to titrate 1 litre of urine to pH 7.4. This is a **measure of net acid excretion** by the kidney.
- The major titratable acid in urine is sodium acid phosphate.
- Net loss of H+ ions in urine occurs.
- Net gain of HCO3- is present.



- *Normal Anion Gap Metabolic Acidosis (NAGMA)* → HCO3- being lost



  - Suppose the blue area is the anion gap here.
  - If a condition like diarrhoea occurs, it will lead to loss of HCO3- from the body, i.e., from the red area of measurable anions.
  - But the body, to maintain electrical neutrality, will absorb an equal amount of chloride via the kidney.
  - Thus, the size of the red area does not change. Therefore, the blue area (unmeasured anions) also does not change.
  - This is also known as **hyperchloremic acidosis** as chloride level increases as a compensation for bicarbonate loss.

Causes of metabolic acidosis

Elevated Anion Gap[a]	Normal Anion Gap[a]
M—Methanol, metformin	U—Ureteral diversion
U—Uremia	S—Saline infusion
D—Diabetic (or alcoholic) ketoacidosis	E—Exogenous acid
P—Paraldehyde, phenformin	D—Diarrhea
I—Isoniazid, iron	C—Carbonic anhydrase inhibitors
L—Lactic acidosis	A—Adrenal insufficiency
E—Ethylene glycol, ethanol	R—Renal tubular acidosis
S—Salicylates	

Especially Type I, Type II and Type IV RTA

Associated hyperkalemia

- There is H^+-K^+ antiporter in many of the cells in the body.
- As the H^+ ions increase in metabolic acidosis, many of them will diffuse into the cells thus leading to diffusion of K+ outside the cells, thereby resulting in hyperkalemia.
- Hence, care should be taken while correcting the acidosis, which may lead to sudden hypokalemia. This is more likely to happen in treating diabetic ketoacidosis by giving glucose and insulin (insulin can drive K^+ ions back into the cells) together.

Clinical features of metaboic acidosis

- To compensate for the primary decrease of the metabolic component (HCO_3^-), body will try to decrease the respiratory component (pCO_2) also as compensations always occur in the direction of the primary disturbance.
- So, to flush out CO_2 from the body, the body will try to hyperventilate.
- The marked increase in respiratory rate and depth of respiration will lead to a particular respiratory pattern known as **Kussmaul respiration**.

Normal range of anion gap = 12 ± 2 mmol/L

Use of anion gap

- Used to find out the type of metabolic acidosis (discussed in metabolic acidosis) – HAGMA (High Anion Gap Metabolic Acidosis) and NAGMA (Normal Anion Gap Metabolic Acidosis).

8. Acid base disturbance – Metabolic acidosis

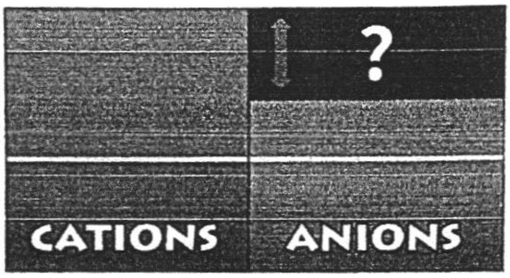
Definition

- This happens due to a **primary deficit of bicarbonate**.
- This can either be due to the HCO_3^- getting used up or the HCO_3^- being lost from the body.

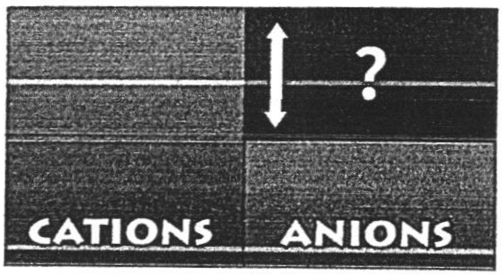
Types of metabolic acidosis

Depending on the anion gap, it can be divided into 2:

- *High Anion Gap Metabolic Acidosis (HAGMA)* → HCO3- getting used up



  - Suppose the blue area is the anion gap here.
  - If an acid like salicylic acid accumulates in the body, it will dissociate into salicylate anion and H^+.
  - The H+ ions thus formed will combine with the HCO3- ion in measurable anions (red area) and will lead to decrease in the number of measurable anions.
  - At the same time, salicylate anions (being unmeasurable) accumulate in the blue area and thus the unmeasurable anions increase in amount thereby resulting in HAGMA.



10. Acid base disturbance – Respiratory acidosis

Definition

- This happens due to a **primary excess of carbonic acid**.

Causes of respiratory acidosis

Affecting the respiratory centre

- Narcotics, barbiturates
- CNS trauma, tumors, infections, degeneration
- Comatosed patients
- Primary central hypoventilation

Affecting the respiratory apparatus

- COPD
- Asthma
- Lung infections
- Acute Respiratory Distress Syndrome (ARDS)
- Chest wall deformities
- Inability to breathe due to pneumothorax or pleural effusion

Others

- Pickwickian syndrome
- Sleep apnea

Treatment of respiratory acidosis

- Acute cause → Tracheal intubation, assisted mechanical ventilation, oxygen supply should be ***strictly monitored***
- Chronic cause → measures to improve lung function

11. Acid base disturbance – Respiratory alkalosis

Definition

- This happens due to a **primary deficit of carbonic acid**.

Causes of respiratory alkalosis

Affecting the respiratory centre

- Anxiety, hysteria
- Hypoxia
- CNS infections, stroke
- Pregnancy
- Hyperthyroidism

Affecting the respiratory apparatus

- Pneumonia
- Congestive Heart Disease
- Interstitial Lung Disease

Others

- Ventilator-induced hyperventilation

Betsy
Dr. BETSY THOMAS
MD, FRCOG, DNB, MICOG
[illegible]
AMALA INSTITUTE OF MEDICAL SCIENCES
AMALA NAGAR, THRISSUR-680 555

Treatment of metabolic acidosis

- Stop the production of acid or loss of bicarbonate / treat the underlying cause.
- Give HCO_3^- intravenously by calculating the exact amount.
- Always keep an eye on K^+ while correcting the acidosis.

9. Acid base disturbance – Metabolic alkalosis

Definition

- This happens due to a **primary excess of bicarbonate**.

Causes of metabolic alkalosis

Chloride-responsive

- Prolonged vomiting or nasogastric suction
- Prolonged diuretic use (loop diuretics)
- Dehydration

Chloride-resistant

- Mineralocorticoid excess (as in primary & secondary hyperaldosteronism)
- Glucocorticoid excess (as in Cushing's syndrome, Cushing's disease & exogenous cortisol therapy)
- Bartter Syndrome

Due to exogenous base

- Iatrogenic causes (bicarbonate IV fluid therapy, massive blood transfusion, antacids)
- Milk-alkali syndrome

Associated hypokalemia

- To fight the alkali buildup in the body, the kidney tries to conserve the H+ ions. In exchange, it gives out K^+ ions to maintain electrical neutrality.
- This loss of K^+ ions results in hypokalemia.

Clinical features of metabolic alkalosis

- Hypoventilation occurs as the body tries to correct the primary metabolic defect of increased bicarbonate by increasing the respiratory component (pCO_2).

Treatment of metaboic alkalosis

- Correction of the underlying cause
- Proton pump inhibitors (pantoprazole, omeprazole)
- Stop diuretics
- Saline infusion (in contraction alkalosis as in vomiting)
- Acetazolamide (but can worsen K+ loss)