ACID BASE PHYSIOLOGY

(a) ***To explain and describe acid-base chemistry using the Henderson-Hasselbach equation.***

Definition of acid and base:

- "Bronsted-Lowry definition" is most commonly accepted in medicine:
  - Acid – Substance that donates a proton or hydrogen ion to another in solution
  - Base – Substance that accepts protons or hydrogen ions from another in solution

> Alternative definitions of acid-base include:
> - "Arrhenius definition":
>   - Acid – Substance that dissociates in H_2O to produce H^+
>   - Base – Substance that dissociates in H_2O to produce OH^-
> - "Lewis definition":
>   - Acid – Substance that can accept an electron pair
>   - Base – Substance that can donate an electron pair

Overview of Hydrogen ion (H^+):

- H^+ is a hydrogen atom without its orbital electron → essentially a "proton"
- In aqueous solution, H^+ is hydrated to form a "hydronium ion" (H_3O^+):

$$H^+ + H_2O \rightarrow H_3O^+$$

- "H^+ activity" (A_{H+}) is a measure of how many H^+ "seem" to be present in solution → This is determined by:
  - (i) Activity coefficient of H^+ (g)
  - (ii) Concentration of H^+ ($[H^+]$) – Quantity of H^+ "actually" present in solution

$$A_{H+} = g\,[H^+]$$

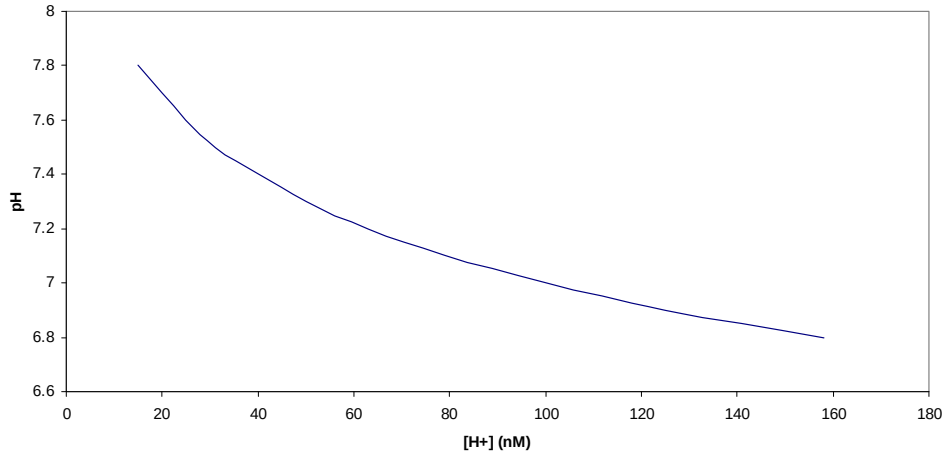
> Note: A_{H+} is synonymous with $[H^+]$ → because $[H^+] \approx A_{H+}$

Overview of pH system:

- pH is defined as the –ve base-10 logarithm of H^+ activity (A_{H+}) (where $[H^+] \approx A_{H+}$) → It serves as an indirect measure of H^+ activity (and $[H^+]$) in solution

$$pH = -\log_{10}(A_{H+}) \approx -\log_{10}[H^+]$$

- pH and A_{H+} (or $[H^+]$) are inversely related in a non-linear fashion due to the $\log_{10}$ scale (Ie. ↓ 1 unit pH = 10x ↑ A_{H+} or $[H^+]$)



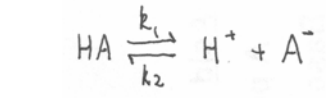
pH	$[H^+]$ (nmol/L)
7.8	16
7.7	20
7.6	25
7.5	31
7.44	**36**
7.4	**40**
7.36	**44**
7.3	50
7.2	62
7.1	80
7.0	**100**

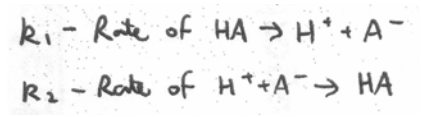
- "Neutral pH" is the pH at which $[H^+] = [OH^-]$ → this is temperature dependent

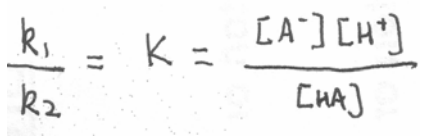
> Neutral point of H_2O is pH 7 at 25°C and 6.8 at 37°C (pH of neutral H_2O ↑0.017 unit for every 1 °C ↓ in temperature)

<u>Overview of Dissociation Constant (K) and pKa</u>:

- In solution, an acid (HA) will dissociate to a base (A^-) and H^+:







> "Dissociation constant" (Ka) is the proportion of relative reactions present at equilibrium → Ratio of k1:k2 (Ie. K>1 means k1>k2 such that more HA dissociates into H^+ and A^-, than HA reassociates from H^+ and A^-)

- pKa is calculated as the –ve base-10 logarithm of the dissociation constant (K) of a substance → It is defined as the pH at which a substance is 50% dissociated (or ionised) in solution

$$pKa = -\log_{10}(K)$$

- pKa is an indirect measure of the extent of dissociation of the substance in solution (Ie. $HA \rightarrow H^+ + A^-$) → in doing so, it determines its strength of acidity in solution

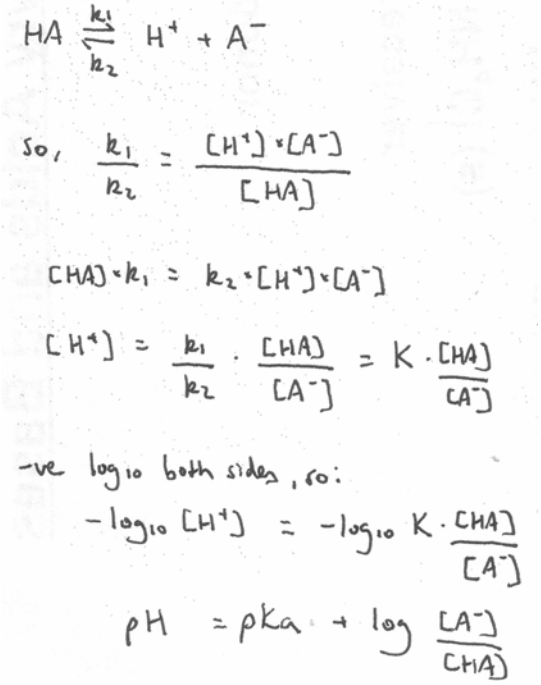
> ↓ pKa means ↑ dissociation and stronger acid
> ↑ pKa means ↓ dissociation and weaker acid

<u>Henderson-Hasselbach Equation</u>:

$$pH = pKa + \log([A^-]/[HA])$$

- This equation demonstrates that the ability of a substance to either donate a proton (Ie. act as an acid (HA)) or accept a proton (Ie. act as a base (A^-)) depends on two factors:
    - (i) pH of the solution
    - (ii) pKa of the substance

Derivation of Henderson-Hasselbach equation:



(b) ***To describe the chemistry of buffer mechanisms and to explain their relevant roles in the body.***

(c) ***To describe the regulation of acid-base balance.***

<u>H^+ Balance in the Body</u>:

- H^+ production in the body:
  - (1) "Volatile acids" (aka. "Respiratory acids")
    - CO_2 is produced by [O] metabolism of carbohydrates and triglycerides (Ie. decarboxylation in TCA cycle) → Majority (75%) is hydrated in plasma to form Carbonic acid (H_2CO_3)
    - H_2CO_3 produces 15000 mmol H^+/day
    - "Volatile acids" do NOT contribute to net acid balance in the body → because all H_2CO_3 in plasma is reformed as CO_2 in the lungs and eliminated
  - (2) "Non-volatile acids" (aka. "Metabolic acids" or "Fixed acids")
    - (a) Lactate production
      - Produced from anaerobic metabolism of glucose and glycogen in RBC, skin and skeletal muscle → Produces 1500 mmol H^+day
      - Does not contribute to net acid balance in the body → because lactate is oxidised in the liver to regenerate HCO_3^- (via Cori cycle) → Exception is with excessive production (Ie. lactic acidosis with tissue hypoxia)
    - (b) Sulphuric acid
      - Produced from metabolism of S-containing a.a (esp Cys and Met) → Produces of 45 mmol H^+/day
    - (c) Phosphoric acid
      - Produced from hydrolysis of phosphoproteins → Produces 13 mmol H^+/day
    - (d) Other acids (Eg. HCl produced from a.a. metabolism, ketoacids produced from fat metabolism, Etc.) → Produces 12 mmol H^+/day

  Note:
  - These acids are termed "Fixed acids" because they cannot be excreted by the lungs → Must be excreted by the kidneys (sulphuric acid, phosphoric acid and other acids) or metabolised by the liver (lactate)
  - They contribute to net acid balance in the body → Normally produced at 1-1.5 mmol H^+/kg/day (or ~ 70 mmol H^+/day) → Thus, to maintain acid-base balance, these "fixed acids" must be completely excreted!

- H^+ excretion from the body:
  - (1) Lungs
    - Eliminate all "volatile acids" (H_2CO_3) → 15000 mmol H^+/day
  - (2) Liver
    - Eliminates "fixed acids" (mainly lactate) → 1500 mmol H^+/day
  - (3) Kidney
    - Eliminates "fixed acids" (mainly phosphoric acid, sulphuric acid, other acids) as NH_4^+ and "titratable acids"
    - Accounts for at least 70 mmol H^+/day → 30 mmol/day as "titratable acids" and 40 mmol/day as NH_4^+

Summary of H^+ turnover:

Process	H^+ balance (mmol/day)
Production:	
CO_2 (as H_2CO_3)	15,000
Lactate	1500
Sulphuric acid	45
Phosphoric acid	13
Others (HCl, ketoacids)	12
Output:	
Lungs	15,000
Liver	1500
NH_4^+	40
Titratable acids	30

- Acid-base balance in the body:
  - To maintain acid-base balance (and pH) in the body → Daily acid production must EQUAL daily acid excretion → So "Net acid balance" must equal ZERO

> Net acid balance (NAB) = Net acid production (NAP) – Net acid excretion (NAE)

  - Net acid production (NAP):
    - Normally ~ 70 mmol of H^+ is produced daily from "fixed acids" (esp phosphoric acid, sulphuric acid, other acids)
  - Net acid excretion (NAE):

$$NAE = NH_4^+ + \text{"Titratable acids"} - HCO_3^-$$

    - Normally all "fixed acids" produced are excreted by the kidneys (~ 70 meq/day) → 30 mmol/day as "titratable acids" and 40 mmol/day as NH_4^+
    - Almost all filtered HCO_3^- is reabsorbed → HCO_3^- excretion ~ 0 mmol/day

<u>Overview of Acid-Base Homeostasis</u>:

- $[H^+]$ in body fluid is precisely regulated → maintained at low plasma [ ] of 40 nmol/L (normal range 35-45 nmol/L) to keep extracellular pH ~ 7.4 (normal range pH 7.35-7.44) and intracellular pH ~ 6.8

> Derangements of $[H^+]$ and pH can result in systemic effects (Eg. altered CNS reflexes, CVS depression, Etc.; See below) as a result of direct intracellular disturbances:
> - (i) Altered protein function (Eg. enzyme activity, transporter activity, Etc.)
> - (ii) Altered membrane excitability
> - (iii) Disruption in metabolic pathways (esp energy production)
> - (iv) Ion trapping of biological molecules in compartments, Etc.

- This tight regulation of $[H^+]$ and pH is achieved by the following processes:
  - (1) Buffering (1st line of defence) – Systems that immediately minimise changes in pH in the event that an acid or base is added to the body
  - (2) Compensation (2nd line of defence) – Physiological processes that attempts to normalise pH by restoring the HCO_3^-/PCO_2 ratio to normal → Either respiratory (rapid; mins-hrs) or renal (slow; hrs-days) → pH generally not completely restored to 7.4

- (3) Correction (3rd line of defence) – Mechanism that corrects acid-base derangement through correction of primary disorder

Acid-Base Homeostasis: Buffering

Overview of buffer systems:

- A "buffer" is a solution containing a weak acid and its conjugate base → Resists profound pH changes when exposed to a stronger acid or base by reversibly binding H^+

$$Buffer + H^+ \leftrightarrow H.Buffer$$

- Effectiveness of a buffer is dependent on:
  - (i) Amount of buffer present → ↑ [buffer] ↑ the effectiveness of the buffer
  - (ii) Buffer's pKa and pH of the carrier solution → Majority of buffering activity (80%) occurs within +/- 1 pH of the buffer's pKa, with the maximal effect at its pKa (thus buffer effectiveness ↑ if buffer pKa and solution pH within +/- 1 unit)
  - (iii) "Open" (physiological) vs "closed" (chemical) buffer system → Buffer effectiveness ↑ with open systems

Buffering systems of the body:

- There are two types of buffer systems in body:
  - (1) Bicarbonate buffers (H_2CO_3-HCO_3^- buffer system) → Can only buffer "fixed/metabolic" acids (because it cannot buffer itself!)
  - (2) Non-bicarbonate buffers (Hb, Phosphate and Protein buffer systems) → Can buffer both "respiratory" and "fixed/metabolic" acids
- Extracellular buffering:
  - (1) Blood – Mainly HCO_3^- and Hb (proteins and PO_4^- have minor roles)
    - (i) RBC – 1°ly Hb (35%; buffers 90% of H_2CO_3) and HCO_3^- (18%); proteins and PO_4^- are negligible
    - (ii) Plasma – 1°ly HCO_3^- (35%; buffers 70% of "metabolic" acids), protein (7%) and PO_4^- (2%)
  - (2) ISF – 1°ly HCO_3^- (ISF has ↑ capacity to buffer "metabolic" acids than blood because ISF volume (and HCO_3^- content) is 3x ↑ cf. blood)
- Intracellular buffering:
  - (i) 1°ly protein and PO_4^{3-} → because they occur at ↑ [ ] intracellullarly and have pKa's closer to intracellular pH (~ 6.8)
  - (ii) Fixed acid extrusion → Extrusion of IC H^+ in exchange for a strong electrolyte (Na^+, Cl^-, lactate) via an anti-port transporter
  - (iii) Organellar buffering → Sequester or release H^+ from IC organelle
  - (iv) Metabolic buffering → Alter production of acidic metabolites

> "Isohydric principle" – All buffer systems in solution in the body that participate in defence of acid-base changes are in equilibrium with each other (Ie. changing pH will affect all buffer pair ratios in solution)

Bicarbonate-carbonic acid (HCO_3^--H_2CO_3) buffer system:

- Consists of H_2CO_3 (weak acid) and HCO_3^- salt ($NaHCO_3$ in ECF; $KHCO_3$ or $Mg(HCO_3)_2$ in ICF):

> * Carbonic anhydrase (CA) is found in – (i) RBC, (ii) Alveolar walls, (iii) Renal tubular cells, and (iv) Renal PCT brush border

- In presence of strong acid – H^+ from acid buffered by HCO_3^- → shifts to production of CO_2 production (via H_2CO_3) → CO_2 excreted from lungs
- In presence of strong base – Buffered by H_2CO_3 → Forms HCO_3^- which is excreted from kidneys

- Most important buffer in ECF (RBC, plasma and ISF) → 80% buffering capacity:
  - It's low pKa (6.1) relative to physiological pH and relatively small amounts in ECF ↓ its effectiveness as a buffer system in ECF
  - BUT this is offset by the fact that the system is "open" system → Can be controlled independently by the:
    - (i) Lungs – Excretion of CO_2 (or H_2CO_3) regulated rapidly by changes in minute ventilation
    - (ii) Kidneys – Excretion of HCO_3^- and H^+ regulated more slowly

Note: Henderson-Hasselbach equation can be modified to relate the pH of blood to constituents of this buffer system:

$pH = pKa + \log([A^-]/[HA]) \longrightarrow pH = pKa_{H2CO3} + \log [HCO_3^-]/[H_2CO_3]$

- pKa of H_2CO_3 is 6.1
- $[H_2CO_3]$ cannot be measured in blood due to its rapid dissociation to $HCO_3^- + H^+$ → Thus, $[H_2CO_3]$ is estimated by amount of dissolved CO_2

$[H_2CO_3]$ = solubility coefficient of CO_2 (in plasma) x PCO_2 = 0.03 x PCO_2

Thus: $pH = 6.1 + \log([HCO_3^-]/0.03xPCO_2)$

Haemoglobin (Hb) buffer system:

- Hb is an intracellular protein in RBCs BUT acts as the major non-HCO_3^- buffer in ECF
- Mechanisms for buffering capacity of Hb:
  - (1) ↑ [Hb] within RBCs (150 g/L) → Large amount of buffer present
  - (2) Structure of Hb
    - (a) Multiple (38) Histidine residues with Imidazole side chain (pKa 6.8) on globin chains of Hb → Anionic sites on Imidazole binds H^+ → Contributes to MAIN buffering capacity of Hb at physiological pH
    - (b) Amino acid groups on globin chains of Hb → CO_2 and H_2CO_3 can bind with terminal amino groups of amino acids of Hb to form "carbamino compounds"

$HbNH_2 + CO_2 \rightarrow HbNHCOO^- + H^+$ ←
$HbNH_2 + H_2CO_3 \rightarrow HbNH_3 + HCO_3^-$

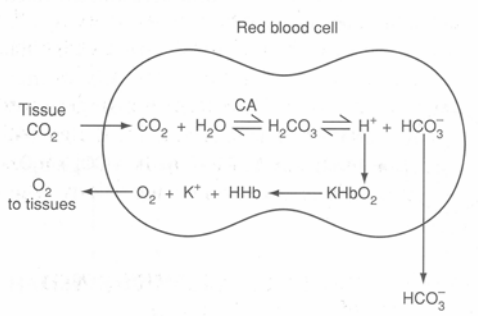
Nb. H^+ produced needs to be buffered too!

Nb. This accounts for 15% CO_2 transport in blood

  - (3) Hb is a weak acid
    - Hb exists as weak acid (HHb) and salt (KHb) with a pKa 6.8 → it is an even weaker acid cf. HCO_3^--H_2CO_3 buffer system (pKa 6.1)

$$\begin{array}{ccc} HCl + KHb & \rightleftharpoons & HHb + KCl \\ & & \uparrow\downarrow \\ & & H^+ + Hb^- \\ & & \uparrow\downarrow \\ H_2CO_3 + KHb & \rightleftharpoons & HHb + KHCO_3 \end{array}$$

        - Thus, in presence of ECF acid → Hb buffers extracellular H^+ → leads to HCO_3^- formation within RBC → HCO_3^- accumulates and diffuses down its electrochemical gradient out of RBC → ↑ plasma HCO_3^-
    - (4) RBC contains carbonic anhydrase and has ↑ solubility for CO_2:
        - Hb contributes to > 90% of blood capacity to buffer CO_2 (as H_2CO_3)
        - Tissue CO_2 diffuses into RBC whereby it is hydrated to form H_2CO_3 by CA → (i) H^+ produced then preferentially binds DeoxyHb (to form Reduced Hb), thus allowing offloading of O_2 to tissues, and (ii) HCO_3^- produced diffuses down its electrochemical gradient out of RBC → ↑ plasma HCO_3^-



    - (5) DeoxyHb is a better buffer than OxyHb (Haldane Effect)
        - DeoxyHb (pKa 8.2) is a better buffer than OxyHb (pKa 6.6) because it is a weaker acid and dissociates to a greater extent
        - In tissue capillaries → OxyHb unloads O_2 to form DeoxyHb → This facilitates:
            - Uptake and buffering of extracellular H^+ (as reduced Hb; HHb) produced from hydration of tissue CO_2 and dissociation of H_2CO_3 → 30% Haldane effect
            - Uptake of tissue CO_2 (as carbamino compounds) → 70% Haldane effect
        - Thus, pH venous blood is only slightly more acidic than arterial blood in spite of large tissue CO_2 produced daily!

> "Isohydric buffering" – For each mmol OxyHb that is reduced, 0.7mmol H^+ can be taken up by Hb, and 0.7mmol CO_2 can enter venous system without significantly changing pH

> Note: Despite being a protein, Hb has 6x ↑ buffering capacity than protein proteins because
> - (i) [Hb] is 2x of plasma protein
> - (ii) Hb has 3x buffering capacity gram per gram that of plasma protein → due to 3x ↑ content of imidazole-containing histidine residues

Protein buffer system:

- Amino acid side chains can buffer H^+:
    - Most amino groups (pKa 9) and carboxyl groups (pKa 2) have pKa very far from physiological pH → Contributes little to buffering
    - Imidazole group on Histidine (pKa 6.8) has the closest pKa to physiological pH → Most important amino acid in proteins for buffering (esp in Hb; See above)
- Much more important buffer in ICF (cf. ECF) because → (i) [proteins] intracellularly are much ↑, and (ii) intracellular pH is more acidic (Ie. closer to pKa of amino acid moieties)

Phosphate buffer system:

- Phosphoric acid is a tribasic acid:

$H_3PO_4 \leftrightarrow H^+ + H_2PO_4^-$ (pKa 2)
$H_2PO_4^- \leftrightarrow H^+ + HPO_4^{2-}$ (pKa 6.8)
$HPO_4^- \leftrightarrow H^+ + PO_4^{3-}$ (pKa 11.7)

- Main buffer intracellularly and in urine because → (i) [ ] are much ↑, and (ii) intracellular and urine pH are more acidic (Ie. closer to pKa of phosphate buffers)

> Note: Despite biphosphate ($H_2PO_4^-$) buffer having pKa very similar to physiological pH, it plays a minor role in extracellular buffering due to its low content in ECF and "closed" buffer system

- Important in "bone buffering" → $CaPO_4$ acts as "Alkali reserve" → During prolonged acidosis, it solubilises in plasma to ↑ $[PO_4^{3-}]$

Acid-Base Homeostasis: Compensation

Respiratory compensation:

- This compensatory response is characterised by a very rapid and high capacity excretion of "respiratory acids" (~ 15,000 mmol H^+/day as CO_2) by the lungs → Nb. it does NOT involve excretion of "metabolic/fixed acids"
- Compensatory response involves two steps:
  - (1) $PaCO_2$ is altered by changing minute ventilation

$$\text{Remember: } PaCO_2 = 0.83 \times \frac{VCO_2}{Va}$$

  - (2) Change in $PaCO_2$ normalises the $[HCO_3^-]/PaCO_2$ ratio → Minimises changes in pH caused by acid-base disturbance

$$\text{Remember: } pH = 6.1 + \log([HCO_3^-]/0.03 \times PCO_2)$$

> For example:
> - During metabolic acidosis (↓ pH, ↑ $[H^+]$, ↓ $[HCO_3^-]$) → Induces hyperventilation → Lowers $PaCO_2$ and normalises $[HCO_3]/PaCO_2$ ratio
> - During metabolic alkalosis (↑pH, ↓ $[H^+]$, ↑ $[HCO_3^-]$) → induces hypoventilation → Increases $PaCO_2$ and normalises $[HCO_3]/PaCO_2$ ratio

- Regulation of respiratory compensation:
  - (i) Peripheral chemoreceptors (located in aortic and carotid bodies)
    - Respond to ↓ arterial pH, ↓ PaO_2 and ↑ $PaCO_2$ → stimulate medullary ventilatory centres
    - Nb. Minor role in responding to ↑ $PaCO_2$ (only 20%) → BUT important for sensing acute changes in $PaCO_2$
  - (ii) Central chemoreceptors (located in medulla)
    - Responds to H^+ in adjacent brain ECF (which is formed from CO_2 traversing the BBB; and NOT plasma H^+ which is insoluble in BBB) → stimulate medullary ventilatory centres
    - Major role in responding to ↑ $PaCO_2$ (80%)
    - $[HCO_3^-]$ in brain ECF equilibrates slowly (over 24 hrs) → alters chemoreceptor sensitivity in the event of prolonged acidosis

> Note: MV ↑ 2L/min for every mmHg ↑ in $PaCO_2$ from normal

Renal compensation:

- This compensatory response is characterised by a very slow (7-10 days) and low capacity excretion of "fixed/metabolic acids" (~ 70 mmol H^+/day) → normalises the [HCO_3^-]/$PaCO_2$ ratio → Minimises changes in pH caused by acid-base disturbance

> Nb. This is the ONLY means of excreting "fixed acids" (Except for lactate → metabolised by liver)

- Renal regulation of acid-base balance involves tubular H^+ secretion:
  - At least 4390 mmol H^+ is actively secreted by kidneys each day:
    - (i) Majority is used to reabsorb filtered HCO_3^- → 4320 mmol H^+ is actively secreted by the tubular system each day to facilitate reabsorption of all 4320 mmol HCO_3^- filtered by the glomerulus (24 mmol HCO_3^-/L x 180 L/day = 4320 mmol) → this does not lead to net excretion of H^+ in urine or addition of new HCO_3^- to blood
    - (ii) Additional 70 mmol H^+ is actively secreted to excrete "fixed/metabolic" acids to achieve a "net acid balance" of zero → 30 mmol/day as filtered buffers (titratable acids) and 40 mmol/day as manufactured buffers (NH_4^+) → this leads to net excretion of H^+ in urine (and addition of new HCO_3^- to blood)
  - During acidosis:
    - (a) Tubular H^+ secretion leads to all filtered HCO_3^- being reabsorbed
    - (b) Excess H^+ is secreted and lost in urine bound to non-absorbable buffers (filtered buffers and manufactured buffers) → results in an additional 300 mmol H^+ excreted in urine per day (mainly bound to NH_4^+) and an additional HCO_3^- added to blood
  - During alkalosis, excess plasma HCO_3^- (> 28 mmol/L) is excreted in urine by:
    - (a) Loss of filtered HCO_3^- (Ie. not all is reabsorbed in tubular system)
    - (b) Secretion of HCO_3^- from "type B intercalated cells" in CCD

Summary of "net acid excretion":

$$NAE = NH_4^+ + \text{"Titratable acids"} - HCO_3^-$$

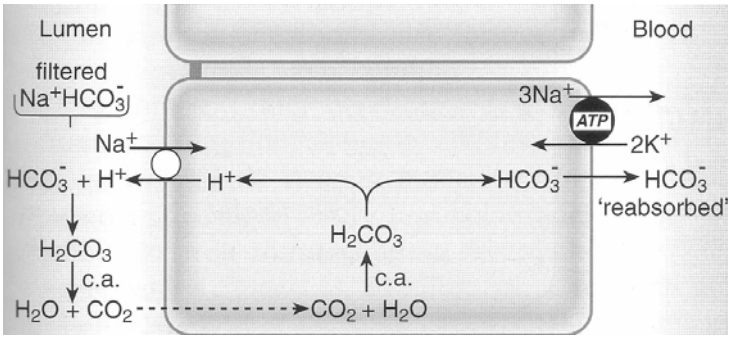
	Normal	Acidosis	Alkalosis
Titratable acids (mmol/day)	30	40	0
NH_4^+ (mmol/day)	40	160	0
HCO_3^- (mmol/day)	0	0	80
NAE / "New" HCO_3^- added (mmol/day)	+70	200	-80
Urine pH	6.0	4.6	8.0

> Note: Filtered H^+ at the glomerulus accounts for a very small % of excreted H^+ → 36 nM x 180 L/day = 6.48 umol of excreted H^+ per day

- Renal regulation of acid-base balance involves the following processes:
  - (1) Secretion of H^+ associated with HCO_3^- reabsorption by renal tubules:
    - Majority of HCO_3^- is reabsorbed by proximal tubular system → PCT (85%) and TAL (10%):
      - High capacity and low gradient system → Because 3900 mmol H^+ secreted/day (and 95% filtered HCO_3^- reabsorbed) BUT lowest urine pH achieved is only 7
      - In the brush border membrane of PCT tubular luminal cells:

- H^+ is secreted into the tubular lumen via apical Na^+/H^+ antiport → Secondarily active transport dependent on Na^+ gradient generated by basolateral Na^+/K^+ ATPase
- This H^+ then binds tubular luminal HCO_3^- filtered freely at the glomerulus to form H_2CO_3 → brush border CA then catalyses conversion of H_2CO_3 into CO_2 and H_2O, which are reabsorbed into tubular cells

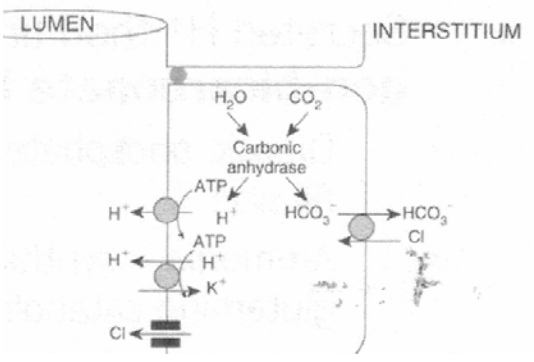
- Within tubular cells throughout the tubular system:
  - H_2O and CO_2 reabsorbed from the tubular lumen is catalysed by intracellular CA into H_2CO_3, which dissociates into H^+ and HCO_3^-
  - HCO_3^- produced is reabsorbed into the peritubular capillary
  - H^+ produced is then actively secreted back into tubular lumen where it can – (a) Aid in reabsorbing more HCO_3^-, or (b) Participate in net acid excretion once all HCO_3^- has been bound



- Minority is reabsorbed by distal tubular system → DCT/CCD (5%):
  - Low capacity and high gradient system → Because 420 mmol H^+ secreted/day (and 5% filtered HCO_3^- reabsorbed) BUT urine pH created is as low as 4.5
  - Within "type A intercalated cells", CO_2 is hydrated to form H_2CO_3 (via intracellular CA) → dissociates into H^+ and HCO_3^-
  - H^+ derived from this reaction is secrete H^+ into the tubular lumen via apical H^+ ATPase and H^+/K^+ ATPase (primary active transports) → controlled by aldosterone
  - HCO_3^- produced via this reaction is absorbed into peritubular capillary

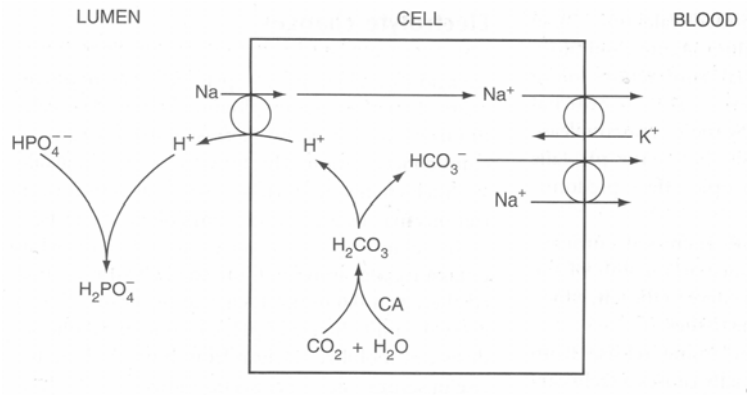
Note – Type B intercalated cells in CCD → secrete HCO_3^- produced from IC dissociation of H_2CO_3 via a luminal Cl^-/HCO_3^- exchanger → little importance because:

- (i) Very few type B intercalated cells (more type A cells in CD)
- (ii) Net tubular HCO_3^- handling is ALWAYS reabsorption (never secretion)



- (2) Formation of titratable acidity:

- Once all HCO_3^- in tubular fluid are reabsorbed by H^+ secretion, excess H^+ secreted in tubular fluid are bound to "filtered buffers" in the distal tubular system (DCT and collecting ducts) and excreted in urine:
    - (i) Mainly phosphate buffer system → H^+ combines 1°ly with HPO_4^{2-} to form $H_2PO_4^-$ (as urine acidity ~ 4.5 → close to HPO_4^{2-} buffer pKa of 6.8)
    - (ii) Organic buffer systems (Eg. creatinine, sulphate, β-hydroxybutyrate)
- Within distal tubular cells, CO_2 is hydrated to form H_2CO_3 (via intracellular CA) → dissociates into H^+ (which is secreted into tubular lumen via a secondarily active Na^+/H^+ anti-port → H^+ bind "filtered buffers") and a newly formed HCO_3^- (which is absorbed into peritubular capillary)

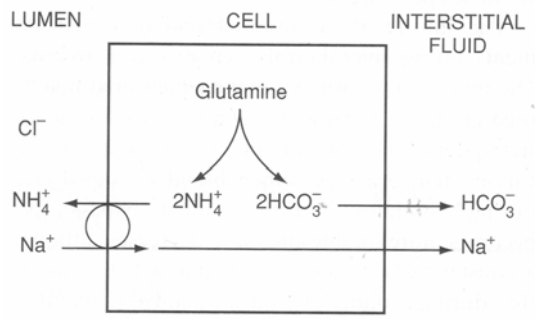


- "Filtered buffers" are vital for excretion of "fixed acids" under normal conditions → Leads to excretion of 30 mmol of "fixed acids" per day
- Note – They cannot be ↑ to excrete additional acid load during acidosis because:
    - (i) Availability of filtered buffers cannot be easily ↑ cf. "manufactured buffers" (Ie. phosphate buffer amount is dependent on diet and PTH levels)
    - (ii) Buffering capacity of filtered buffers maxed out at ↓ urine pHs

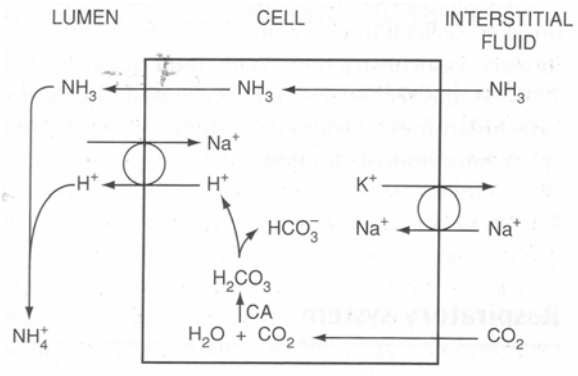
Aside: "Titratable acidity"
- Measured by the amount of alkali (as NaOH) that must be added to urine to return its pH to 7.4 (which is the pH of glomerular filtrate)
- Accounts for only a FRACTION of the H^+ secreted by the kidneys:
    - Accounts mainly for the H^+ that are bound to "filtered buffers" (phosphate and organic buffer systems) and excreted in urine
    - Does not account for HCO_3^- buffer system → because H_2CO_3 is converted and reabsorbed as CO_2 and H_2O → thus alters urine pH minimally
    - Ammonia buffer system contributes little to "titratable acidity" → because of high pKa of buffer system (9.1) (Ie. urine only titrated up to a pH of 7.4 and not any further)

- (3) Ammonia secretion:
    - Once all HCO_3^- in tubular fluid are reabsorbed by H^+ secretion, excess H^+ secreted in tubular fluid are also bound to "manufactured buffers" in the distal tubular system (medullary collecting ducts) and excreted in urine
    - Ammoniagenesis → PCT tubular cells produce NH_3:

- Filtered glutamine is taken up by PCT tubular cells and deaminated via Glutaminase (which is upregulated with chronic acidosis) into NH_4^+ and HCO_3^-
- NH_4^+ is secreted into the tubular lumen via a Na^+/ NH_4^+ antiport, while "newly formed" HCO_3^- is reabsorbed into peritubular capillary blood



- Ammonium cycling → In the TAL of LOH, 80% of tubular NH_4^+ is reabsorbed → this generates ↑ [ ] gradient of NH_4^+ in the medullary interstitium
- Ammonium excretion → Later in the medullary CD:
  - NH_3 diffuses from medullary interstitium into tubular cells of the CD, then into tubular fluid
  - Within the tubular cells of the CD, H^+ and HCO_3^- is formed by hydration of CO_2 using intracellular CA
  - H^+ secreted into tubular fluid by Na^+/H^+ antiport → combines with tubular NH_3 to form NH_4^+ → "ion trapped" in tubular fluid



- These "manufactured buffers" are vital for excretion of "fixed acids" under both:
  - (a) Normal conditions → Accounts for 40 meq of H^+ excretion per day
  - (b) Acidotic conditions → Can excrete an additional 300 mmol H^+ per day due to:
    - (i) ↑ transfer at low urine pH due to high pKa 9.2 of buffer system (Ie. allows additional H+ to be excreted even when tubular fluid has reached maximal acidity)
    - (ii) ↑ production of glutamine (very slow process)

Note: Control of H^+ secretion and excretion is ↑ by the following factors:
- (1) Raised $PaCO_2$
- (2) High EC $[H^+]$ → Stimulates glutamine production
- (3) K^+ and Cl^- depletion → ↑ renal HCO_3^- reabsorption
- (4) Inhibition of CA → H^+ secretion is inhibited
- (5) Aldosterone (and cortisol) → Upregulates H^+ and H^+/K^+ ATPases
- (6) ECF volume depletion → ↑ renal HCO_3^- reabsorption
- (7) ↓ GFR → ↓ filtration of HCO_3^-

- Minimum urinary pH:
  - Only a small amount of H^+ can be excreted in its free form → because active transport of H^+ secretion is inhibited at high urinary $[H^+]$ → thus lowest urinary pH achieved is 4.4
  - As a result, H^+ secretion and excretion in urine is dependent on binding to "urinary buffers" → (i) HCO_3^- buffer (pKa 6.1), (ii) HPO_4^{2-} buffer (pKa 6.8), and (iii) NH_3 buffer (pKa 9.1)
  - In the absence these urinary buffer systems → urine pH of 4.4 would be reached very rapidly and any further H^+ secretion would cease

Clinical Effects of Acid-Base Changes:

System	Acidosis	Alkalosis

CVS	- Direct –ve inotropic effect:
	  - Due to ↓ slow inward Ca^{2+} current and ↓ Ca^{2+} release from SR
	  - Initially opposed by medullary catecholamine response → until pH 7.2 then –ve inotropy
	- ↑ SNS activity (medullary catecholamine release):
	  - Offsets –ve inotropy
	  - BUT ↑ cardiac arrhythmias, ↑ SVR and renal/splanchnic vasoconstriction
	- Cardiac arrhythmias:
	  - Due to ↓ IC $[K^+]$ (↑ RMP of pacemaker cells) and ↑ adrenal medulla catecholamine release
	- Vascular effect
	  - Mild acidosis → ↑ SVR and renal/splanchnic vasoconstriction (due to medullary SNS response)
	  - ↑ acidosis → Vasodilation (skin, skeletal muscle, heart) and ↓ SVR
	- Pulmonary vasoconstriction → HTN
		- ↑ coronary VC and ↑ SVR

Respiratory	- ↑ MV → ↑ response with respiratory acidosis cf. metabolic acidosis b/c CO_2 more permeable than H^+ at BBB - Bronchodilation (due to hypercapnoea) - Right shift of O_2 HDC → ↑ O_2 tissue delivery	- Opposite effects to acidosis
CNS	Impairs LOC due to changes in CBF and ICP	Epilepsy
GIT	- Splanchnic vasoconstriction - ↓ GIT motility	
Electrolyte	- ↑ free ionised serum Ca^{2+} → due H^+ competing for –ve binding on albumin (chronically, 2° to Ca^{2+} mobilisation from bone) - ↑ serum K^+ → EC H^+ exchanged for IC K^+ (0.6 mmol ↑ $[K^+]$ per 0.1 ↓ pH)	- Opposite effects to acidosis

(d) ***To explain the principles of blood gas and acid-base analysis.***

(e) ***To interpret blood gas analysis and its management in clinical situations.***

Definitions in Acid-Base Disorders:

Term	***Definition***
Acidaemia	Arterial blood pH < 7.36 and $[H^+]$ > 44 nmol/L
Alkalaemia	Arterial blood pH > 7.44 and $[H^+]$ < 36 nmol/L
Acidosis	Abnormal process that tends to ↓ blood pH (Ie. cause acidaemia) if there are no secondary changes in the response to the primary disease
Alkalosis	Abnormal process that tends to ↑ blood pH (Ie. cause alkalaemia) if there are no secondary changes in the response to the primary disease
Primary aetiological disorder can be either:	
Respiratory	Changes in CO_2 → lead to changes in respiratory acid (H_2CO_3)
Metabolic	Changes in fixed/metabolic acids or HCO_3^-
Primary aetiological disorder can also be either:	
Simple	Single primary aetiological acid-base disorder present
Mixed	> 2 primary aetiological disorders present simultaneously

Assessment of Acid-Base Disorders:

- Assessing an acid-base disorder involves determining the (i) type of primary disorder (acidaemia vs alkalaemia; respiratory vs metabolic; simple vs mixed), (ii) degree of compensation present, and (iii) possible aetiology of the disorder
- This requires information derived from:
    - (1) History and physical examination
    - (2) ABG
        - (a) pH
            - Measured directly using pH electrodes (See below)
            - Assesses acidity or alkalinity of blood
        - (b) $PaCO_2$
            - Measured directly using CO_2 electrode (See below)
            - Assesses respiratory component of pH shift
        - (c) $[HCO_3^-]$
            - Assesses metabolic component of pH shift
            - Measured two ways:
                - (i) Plasma HCO_3^- – Measured indirectly with Henderson-Hasselbach equation (using known pH and $PaCO_2$ measurements) → thus levels inaccurate since pKa used for H_2CO_3 does not account for fluctuations in temperature and $PaCO_2$
                - (ii) Standard HCO_3^- – Defined as $[HCO_3^-]$ in whole blood which is fully oxygenated with $PaCO_2$ 40 mmHg and temp 37°C (normally 24 +/- 2 mmol/L) → preferred over plasma HCO_3^-
        - (d) Base excess (or deficit)
            - Defined as amount of strong acid or base required to titrate fully saturated whole blood at 37 °C and $PaCO_2$ 40 mmHg to a pH of 7.4 (normally 0 +/-2 mEq/L)

- Buffer base – Sum of buffer anions [ ] in blood (Hb, HCO_3^-, protein, phosphate) in fully oxygenated blood (normally 45-60 mmol/L)
- Base excess – Increase in buffer base → indicates ↑ buffering capacity (Ie. due to ↓ metabolic acids or ↑ in buffer systems)
- Base deficits – Decrease in buffer base → indicates ↓ buffering capacity (Ie. due to ↑ metabolic acids or ↓ buffer systems)

- Can be also used to measure metabolic component of pH shift → preferred over plasma HCO_3^-
- Assumptions:
  - (i) When pH of blood is normal, ratios and total [ ] of non-carbonic buffers are normal
  - (ii) Blood behaves as simple HCO_3^- solution (Ie. controlled by bicarbonate-carbonic acid buffer system) → so all buffering (and changes in blood pH/$[H^+]$) is achieved by changes in HCO_3^- levels → thus changes in $[HCO_3^-]$ reflects amount of acid/base added to blood
  - (iii) It is NOT affected by respiratory acid-base disturbances → because changes in $PaCO_2$ involves equal changes in plasma levels of H^+ and HCO_3^- from H_2CO_3
- Clinical limitations:
  - (i) It is an in vitro system with a set of assumptions
  - (ii) It does not account for extravascular buffers or interactions of blood buffers with ISF/ICF buffers
  - (iii) Assess only blood buffers (33% of total body buffering capacity)
  - (iv) Tends to overestimate acid-base changes of whole body

- (e) PaO_2
  - Measured directly using Clark electrode (See below)
  - Assesses whether patient is hypoxaemic and has potential respiratory disease (Ie. ↑ A-a PO_2 gradient)
  - When FiO_2 is not known (Ie. assess if breathing supplemental O_2) → utilise Alveolar gas equation to determine PAO_2 → if $PaO_2 > PAO_2$, likely that patient is using supplemental O_2)
- (f) Temperature
  - With ↓ temperature:
    - (i) ↑ gas solubility (CO_2 and O_2) → ↓ $PaCO_2$ (↓ 4.5% per ↓ 1°C) and ↓ PaO_2
    - (ii) ↑ pH
      - Neutral H_2O: pH ↑ 0.017 unit per ↓ 1°C
      - Blood: pH ↑ 0.015 unit per ↓ 1°C (Rosenthal correction factor) → Different from neutral H_2O due to imidazole moieties of histidine in Hb
  - Consequence → Assessment of blood at 37°C from hypothermic patient can lead to falsely ↑ PO_2 and PCO_2 and falsely ↓pH → thus above "correction factors" are applied to avoid this

Note – There are two means to correct for temperature changes:

(i) α-stat hypothesis
- Refers to the theory that the degree of ionisation of imidazole groups should remains constant despite changes in temperature whilst keeping CO_2 stores constant (Ie. pH changes with temp)
- So with ↓ temp → pKa of imidazole groups on proteins will ↓ → % unprotonated imidazole groups remain constant → thus, no change in CO_2 stores
- Blood sample is heated to 37°C and is interpreted against values at that temperature irrespectively of what the patient's temperature is

(ii) pH-stat hypothesis
- Refers to the theory that pH should remain constant despite changes in temperature
- To overcome ↑ CO_2 with ↑ temp → CO_2 is added to system to maintain a constant PCO_2 ~ 40mmHg → thus, there is an overall ↑ in CO_2 stores
- Blood sample is measured against normalised values at 37°C regardless of what the patient's temperature is (as there is no change in pH with temp change)

- (g) FiO_2
- (h) SaO_2

- (3) Biochemistry laboratory results → used to determine:
  - (a) Anion gap (AG):
    - AG represents all the unmeasured anions in plasma (sulphates, phosphates, organic acids, proteins) → normally 12 +/-2 mmol/L

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

Nb. An AG arises because routine clinical electrolyte measurements include most cations (Na^+ and K^+) but only some anions (Cl^- and HCO_3^-) → thus, several anions in plasma remain unmeasured. Since the law of electroneutrality states that sum of +ve charges is balanced by sum of –ve charges, the AG is the "apparent" difference between the total measured cation [ ] and total measured anion [ ]

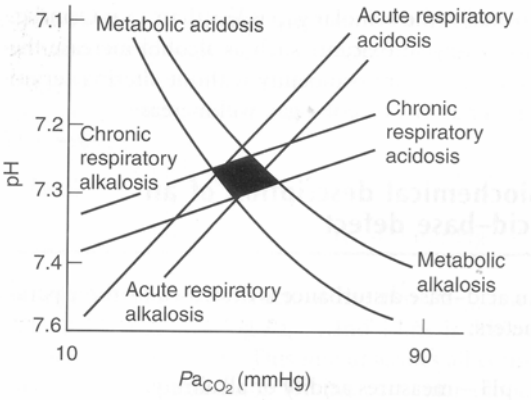
  - (b) Osmolar gap (OG):
    - OG is the difference between measured and calculated serum osmolality (normally < 15 mOsm/kg)
    - Assist in differentiating causes elevated AG metabolic acidosis (Ie. ↑ with presence of circulating intoxicants, such as methanol)

OG = "Measured" serum osmolality – "Calculated" serum osmolality

Where "calculated" serum osmolality = $2x[Na^+]$ + [urea] + BSL

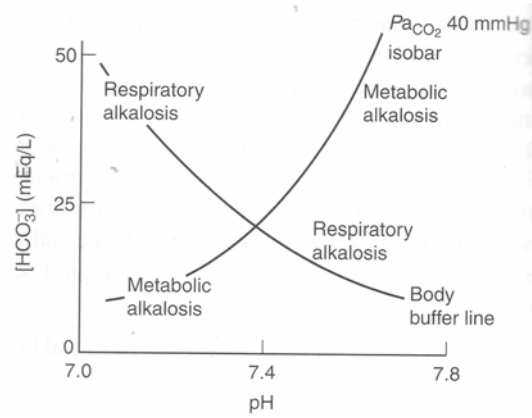
Graphical Assessment of Acid-Base Disturbances:

- pH/$PaCO_2$ diagram:



- Shaded square → limits of arterial pH and $PaCO_2$ in normal people
- 95% CI bands for acute and chronic respiratory acidosis/alkalosis and metabolic acidosis/alkalosis

- pH/HCO_3^- diagram:



Acid-base disturbances defined by:

- (i) $PaCO_2$ 40 mmHg isobar line → Obtained on titration of blood sample with HCl with PCO_2 maintained at 40 mmHg by constant CO_2 flow
- (ii) Body buffer line → Relates plasma $[HCO_3^-]$ to pH when oxygenated blood sample equilibrated with gas mixtures having varied PCO_2

Assessing acid-base disturbance:

- Respiratory disorders → Classified as acidosis vs alkalosis depending on whether they lie to left or right of isobar line (> or < 40 mmHg)
- Metabolic disorders → Classified as acidosis vs alkalosis depending on whether they lie above or below body buffer line (> or < 24 mmol/L standard HCO_3^-)

Types of Acid-Base Disturbances:

(I) *Respiratory acidosis*:

- Defined as a primary acid-base disorder due to rise in $PaCO_2$ to level higher than expected (Ie. ↑ H_2CO_3)
- Causes:
  - Alveolar hypoventilation (Ie. ↓ MV, ↑ dead space)
  - ↑ inspired PCO_2 (Eg. CO_2 absorber saturated)
  - ↑ CO_2 production (Eg. fever, TTX, MH)
- Buffering/compensatory response:
  - Acutely → Buffering (mainly IC buffers) and respiratory correction (↑ MV)
  - Chronic:
    - Renal compensation by retention of HCO_3^- – ↑ $PaCO_2$ causes proximal tubular cells to ↑ H^+ secretion → ↑ production of HCO_3^-
    - ↓ plasma Cl^- – Net renal H^+ excretion causes Cl^- to maintain electroneutrality → leads to +ve BE
- ABG findings:

	Acute	Chronic
pH	↓ (expect 7.25 @ $PaCO_2$ 60; 7.15 @ $PaCO_2$ 80)	Normalises (but < 7.4)
$PaCO_2$	↑	↑
BE	Normal	+ve
HCO_3^-	↑ (expect compensation if $[HCO_3^-]$	↑ (expect compensation if $[HCO_3^-]$ ↑ by 4

	↑ by 1 mmol/L for each 10 mmHg ↑ $PaCO_2$ above 40 mmHg	mmol/L for each 10 mmHg ↑ $PaCO_2$ above 40 mmHg

- Treatment → Correct underlying cause

(II) *Respiratory alkalosis*:

- Defined as a primary acid-base disorder due to fall in $PaCO_2$ to a level lower than expected (Ie. ↓ H_2CO_3)
- Causes:
  - Invariably due to alveolar hyperventilation (Ie. ↑ MV, ↓ DS)
  - ↓ CO_2 production (Eg. GA, hypothermia)
- Buffering/compensatory response:
  - Acutely → Buffering (mainly IC buffers) and respiratory correction (↓ MV → but limited by need to maintain oxygenation!)
  - Chronic → Renal compensation by ↑ HCO_3^- excretion and ↓ NH_4^+ excretion (Ie. net H^+ retention/HCO_3^- loss) → -ve BE and ↓ serum HCO_3^-
- ABG findings:

	Acute	Chronic
pH	↑ (expect 7.5 @ $PaCO_2$ 30; 7.6 @ $PaCO_2$ 40)	Normalises (but > 7.4)
$PaCO_2$	↓	↓
BE	Normal	-ve
HCO_3^-	↓ (expect compensation if $[HCO_3^-]$ ↓ by 2 mmol/L for each 10 mmHg ↓ $PaCO_2$ above 40 mmHg	↓ (expect compensation if $[HCO_3^-]$ ↓ by 5 mmol/L for each 10 mmHg ↓ $PaCO_2$ above 40 mmHg

- Treatment → Correct underlying cause

(III) *Metabolic acidosis*:

- Defined as a primary acid-base disorder that causes plasma HCO_3^- to fall to a level lower than expected due to either (i) an increase in metabolic/fixed acids, and/or (ii) loss of bases in blood
- Causes:
  - ↑ AG → Due to replacement of HCO_3^- with fixed/metabolic acids (which are unmeasured anions) → MUD PILES
  - Normal AG → Usually associated with hyperchloraemia → GI losses (diarrhoea, pancreatic fistula, external drainage of pancreatic/biliary secretions, uretero-enterostomy, obstructed ileal conduit), RTA, renal interstitial disease, mineralocorticoid deficiency, infusion of HCl/NH_4Cl or CAi (acetazolamide)
  - ↓ AG → Hypoproteinaemic states
- Buffering/compensatory response:
  - Acutely:
    - Buffering (60% IC buffers; 40% EC buffers)
    - Respiratory compensation
      - Initially – ↓ pH triggers peripheral chemoreceptors → hyperventilation → ↓ $PaCO_2$ to partly normalise pH
      - BUT this ↓ brain ECF $[H^+]$ and causes central chemoreceptors to limit the ↑ in MV
      - Full effect of respiratory compensation requires 12-24 hrs – HCO_3^- equilibrates across BBB and brain ECF $[H^+]$ ↑ → inhibition on MV by central chemoreceptors gradually removed
      - Nb. Respiratory compensation CANNOT excrete fixed acids!
  - Chronic → Renal compensation by excreting excess acid anions (equivalent to reabsorption of HCO_3^-/excretion of H^+)
- ABG findings:

	Acute	Chronic
pH	↓	Normalises (but < 7.4)
$PaCO_2$	Normal	↓ (expect compensation if $PaCO_2$ is

		$1.5x[HCO_3^-] + 8)$
BE	-ve	-ve
HCO_3^-	↓	↓↓

- Treatment:
  - (i) Eliminate causative factor
  - (ii) IV NaCl → allows kidneys to excrete sufficient HCl to correct acidosis (if acidaemia is not affecting C.O.)
  - (iii) IV HCO_3^- (if acidaemia depressing C.O. → avoid vicious cycle of worsening CVS depression with increasing lactic acidosis)
  - (iv) Dialysis

(IV) *Metabolic alkalosis*:

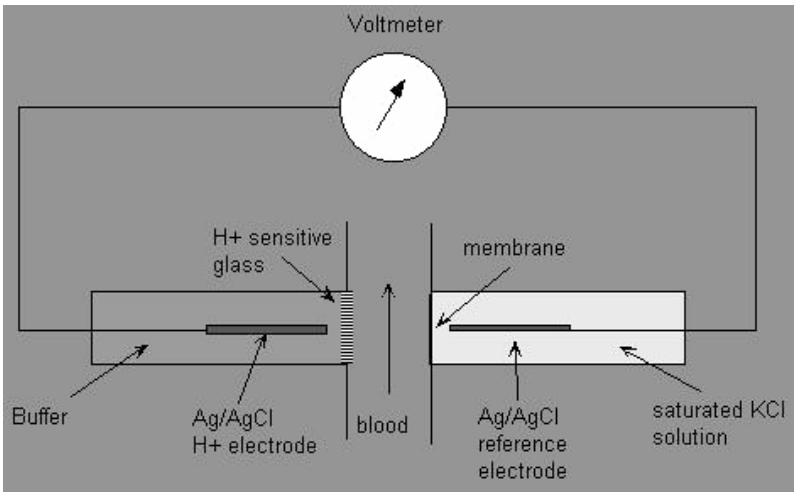
- Defined as a primary acid-base disorder that causes plasma HCO_3^- to rise to a level higher than expected due to either (i) an gain in bases, and/or (ii) loss of metabolic/fixed acids in blood
- Causes:
  - Loss of acids → Renal (hyperaldosteronism, Cushing's, thiazide diuretics, severe hypokalaemia, hypomagnesiuma, hypercalcaemia) or GIT (NG suctioning, severe vomiting)
  - Increased base intake → $NaHCO_3$ administration, metabolic conversion of exogenous organic ions (Eg. Lactate)
- Buffering/compensatory response:
  - Acutely → Buffering (70% EC buffers; 30% IC buffers) and respiratory compensation (↓ MV)
  - Chronic → Renal compensation by excretion of excess HCO_3^-/retention of H^+
- ABG findings:

	Acute	Chronic
pH	↑	Normalises (but > 7.4)
$PaCO_2$	Normal	↑ (expect compensation if $PaCO_2$ is $0.7x[HCO_3^-] + 20$)
BE	+ve	+ve
HCO_3^-	↑	↑↑

- Treatment:
  - (i) Eliminate causative factor
  - (ii) Replace volume deficit with NaCl → allows excretion of $NaHCO_3$
  - (iii) IV NH_4Cl (if very severe alkalosis or poor renal/cardiac function)
  - (iv) IV HCl avoided due to very low pH and need for administration by CVC only
  - (v) CAi (Eg. acetazolamide)
  - (vi) Spironolactone (to ↑ K^+)

Aside: Measurement of pH, PCO_2 and PO_2 from ABG

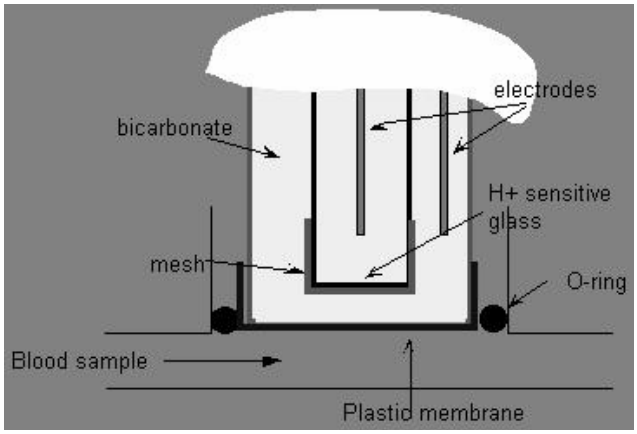
Measurement of pH: pH electrode (Glass-Calomel electrode)



- Consists of two ion-selective electrodes:
  - (i) Measuring electrode
    - Ag/AgCl surrounded by buffer solution in glass casing → glass bulb at electrode tip made of special pH-sensitive glass (-vely charged glass that is permeable to H^+) that is in direct contact with arterial blood sample
    - H^+ diffuses from blood sample through the glass membrane into buffer solution → buffer solution allows a pH (and $[H^+]$) gradient to form across the glass membrane → produces an electrical potential difference that is dependent on the pH (and $[H^+]$) gradient
  - (ii) Reference electrode
    - Hg/Hg_2Cl_2 (calomel) surrounded by saturated 0.1 M KCl solution ("salt bridge") that is separated from the arterial blood sample via a semi-permeable membrane
    - Maintains constant potential despite changes in arterial pH
- These two electrodes are connected via blood to create an electrical circuit → electrical potential difference produced is proportional to pH (and $[H^+]$) gradient → 61.5 mV/pH unit
- It has an accuracy of +/- 0.005 pH units
- Issues:
  - (i) Both electrodes must be:
    - Kept at 37°C → due to temperature-dependent changes in pH (acid/bases dissociate at ↑ temperatures → so pH ↓ 0.015 unit per ↑ 1°C → Rosenthal factor) and solubility of gases (Ie. CO_2)
    - Kept clean (esp free from protein and cellular deposits)
    - Calibrated with 2x PO_4^{3-} buffer solutions of known pH
  - (ii) Delicate plastic membrane → damage leads to inaccurate results (due to protein or microorganism contamination of electrodes)

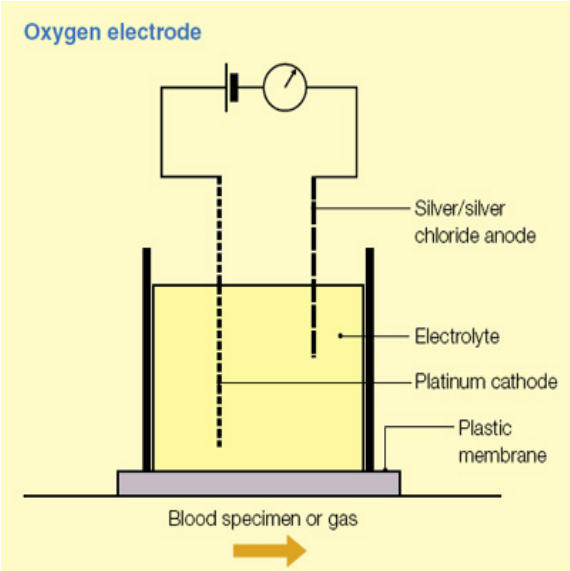
Measurement of PCO_2: CO_2 electrode (Severinghaus electrode)

- Modified pH electrode → two ion-selective electrodes contained in a CO_2-permeable plastic membrane (not permeable to liquids and solids, such as proteins) that separates them from blood sample:
  - (i) H^+-sensitive glass electrode → covered in nylon mesh and coated in thin film of $NaHCO_3$ solution
  - (ii) Ag/AgCl reference electrode



- CO_2 diffuses from blood sample across plastic membrane into the $NaHCO_3$-coated nylon mesh → CO_2 hydrated to form H^+ and HCO_3^- → glass electrode measures change in H^+ (and pH) in $NaHCO_3$ solution, which is proportional to changes in CO_2 tension
- It has an accuracy of +/- 1 mmHg
- Issues:
    - (i) Slow response time (2-3 mins) → because CO_2 needs to diffuse across membrane and react with H_2O to form H^+ and HCO_3^- (Nb. Can be accelerated by addition of CA)
    - (ii) Delicate plastic membrane → damage leads to inaccurate results
    - (iii) Both electrodes must be kept at 37°C, clean (Ie. free from protein deposits) and calibrated with buffer solutions of known PCO_2

Measurement of PO_2: O_2 electrode (Clark electrode)



- Cathode (Pt wire in glass rod) and anode (Ag wire in AgCl gel) placed in KCl solution → this is wrapped in an O_2-permeable plastic membrane (not permeable to liquids and solids, such as proteins) that separates electrodes from blood sample
- O_2 diffuses across membrane from blood sample to equilibrate with electrolyte solution → potential difference of 600 mV is then applied to electrodes → causes an electron flow from anode to cathode as per the following reactions:

At cathode: $O_2 + 2\ H_2O + 4e^- \rightarrow 4\ OH^-$
At anode: $Ag + Cl^- \rightarrow AgCl + e^-$
In solution: $KCl + OH^- \rightarrow KOH + Cl^-$

- Only at an applied voltage of 600 mV → current generated in circuit is directly proportional to PO_2 (Ie. linear relationship)
- It has an accuracy of +/- 2 mmHg
- Issues:
    - (i) Electrodes → must be kept at 37°C, clean and contaminant-free (Ie. free from protein deposits and O_2-consuming cells/microorganisms), and calibrated with solutions of known PO_2 (with N_2 as zero-point)
    - (ii) Delicate plastic membrane → damage leads to inaccurate results (due to protein or microorganism contamination of electrodes)
    - (iii) Halothane can produce falsely ↑ PO_2 readings → avoid this issue by using membrane impermeable to it
    - (iv) Avoid delay in sample analysis → cells consume O_2 which causes falsely lower PO_2 (Nb. store sample in ice to retard cell O_2 consumption)
    - (v) Applied voltage must be 600 mV → this is b/c:
        - (a) Calibration curve of current vs. PO_2 is linear
        - (b) Relationship of current and voltage is non-linear at a given PO_2, EXCEPT within a plateau region (400-800 mV) → in this range, current flow for a given PO_2 is not altered by small changes in applied voltage (Ie. less likely that fluctuations in applied voltage will bias PO_2 readings)