Ions and buffer systems

Seminar No. 10

Average concentrations of plasma cations/anions

Cation	**Molarity of (mmol/l)**	
	Cation	**Charge**
Na^+	142	142
K^+	4	4
Ca^{2+}	2.5	5
Mg^{2+}	1.5	3

Total positive charge: 154

Anion	**Molarity of (mmol/l)**	
	Anion	**Charge**
Cl^-	103	103
HCO_3^-	25	25
$Protein^-$	2	18*
HPO_4^{2-}	1	2
SO_4^{2-}	0.5	1
OA**	4	5*

Total negative charge: 154

*Calculated by empirical formula. **Organic acid anions

Compare concentrations in mmol/l

Blood plasma	
Na$^+$	Cl$^-$
133-150	~ 100

Saline solution (0.9 %)	
Na$^+$	Cl$^-$
154	154

Saline solution of NaCl is **isotonic** with plasma but it has increased concentration of chloride ions compared to plasma!!

!

Q. 2

Commentary - Cations and anions in plasma

- every body fluid is **electroneutral system**
- in univalent ionic species (Na^+, K^+, Cl^-, HCO_3^-, lactate$^-$) ⇒

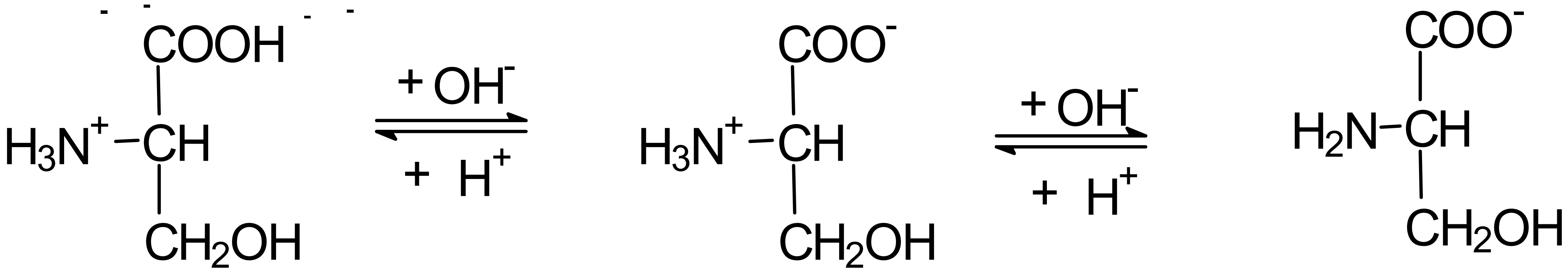
  molarity of charge = molarity of ion
- in polyvalent ionic species ⇒

  molarity of charge = charge × molarity of ion

  $Mg^{2+} \Rightarrow 2 \times [Mg^{2+}] = 2 \times 1 = 2$

  $SO_4^{2-} \Rightarrow 2 \times [SO_4^{2-}] = 2 \times 0.5 = 1$
- **plasma proteins have pI around 5 ⇒ at pH 7.40 they are polyanions**
- OA: lactate, free AA, oxalate, citrate, malate, ascorbate ...etc.
- charge molarity of proteins + org. ions is estimated by empirical formulas

Compare pH and pI



cation

$pH < pI$

amphion

$pH = pI$

anion

$pH > pI$

Compare ECF and ICF

Feature	**ECF**	**ICF**
Main cation	Na^+	K^+
Main anion	Cl^-	HPO_4^{2-}
Protein content	★	★ ★ ★
Main buffer base	HCO_3^-	HPO_4^{2-}

Give main dietary sources of magnesium

What is the main compound?

Q. 9

A. 9

approximate osmolality is calculated

according to empirical relationship:

$2\,[Na^+] + [urea] + [glucose] =$

$2 \times 146 + 4 + 5.6 =$ **301.6 mmol/kg H_2O**

Physiological range: 275 – 300 mmol/kg H_2O

Q. 10

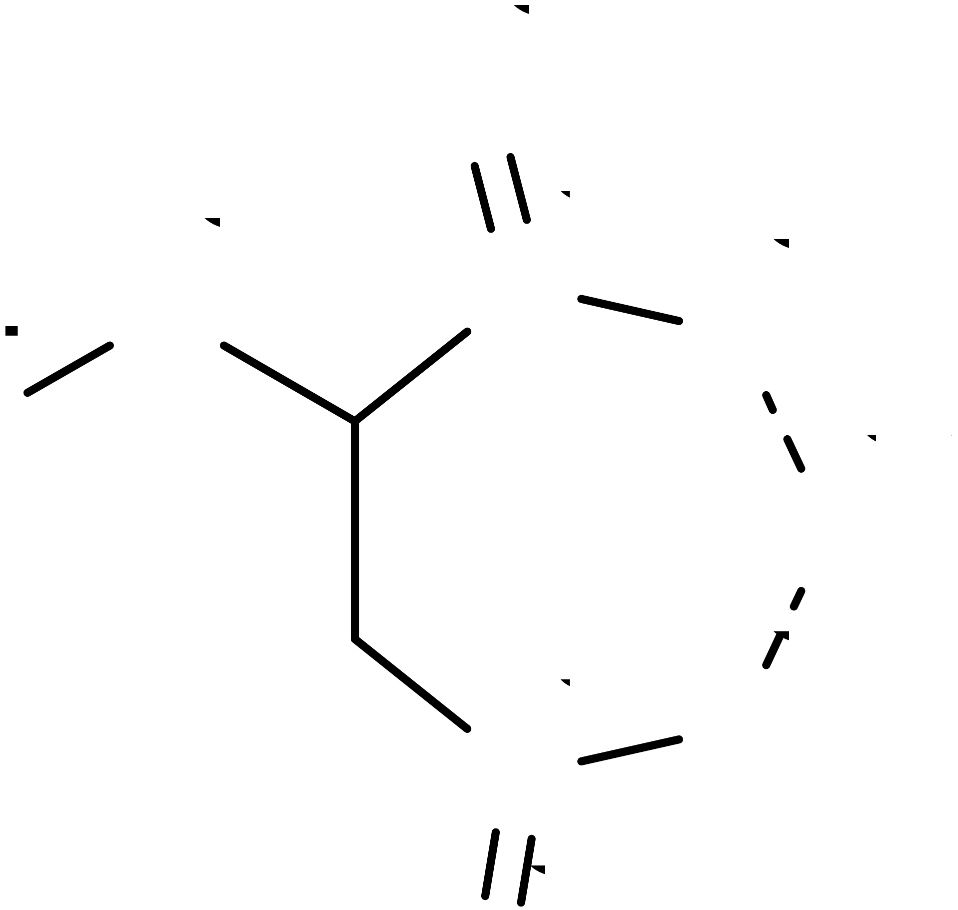
Hormones regulating calcium level

Hormone	**Blood Ca**	**Main actions**
Parathormone	↑	•Stimulates bone demineralization (osteoclasts) •Stimulates renal Ca resorption •Stimulates synthesis of calcitriol in kidneys
Calcitonine	↓	•Inhibits renal Ca resorption •Stimulates bone mineralization
Cacitriol	↑	•Stimulates intestinal Ca resorption •Stimulates the action of parathormone on kidney

What are the forms of calcium in blood?

Give the structure of calcium chelated by malate

Calcium malate is chelate



Q. 13

Compare calcium ion concentrations

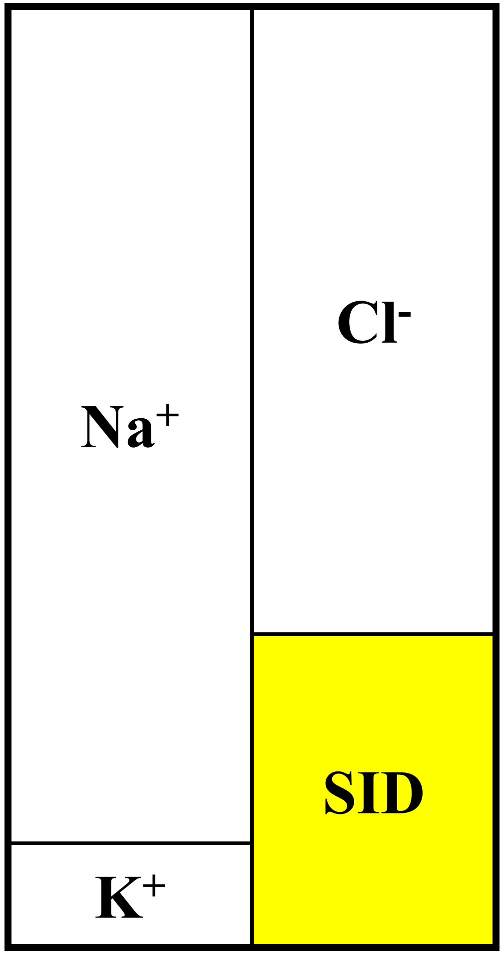
ECF	ICF
10^{-3} M	10^{-7} M

Difference by four orders !

SID (strong ion difference)

- strong ions **do not hydrolyze** in aqueous solution
- Na^+, K^+, Cl^-
- $SID = [Na^+] + [K^+] - [Cl^-] = 142 + 4 – 103 = 43$ mmol/l
- physiological range of SID = 38 – 46 mmol/l

SID ≈ buffer bases of serum/plasma

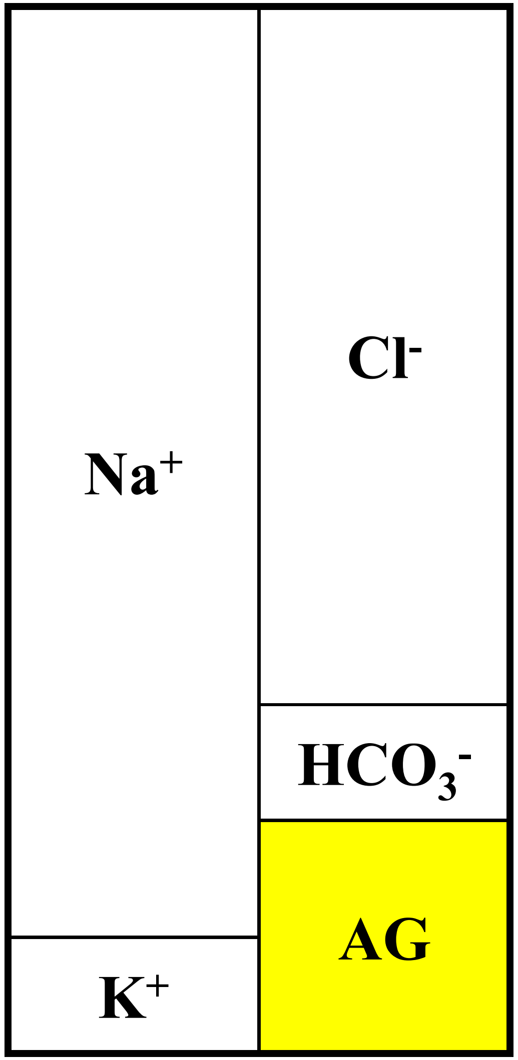


$$SID = HCO_3^- + HPO_4^{2-} + Prot^-$$

AG (anion gap)

- the approximate extent of unmeasured (unusual) anions
- AG = $[Na^+] + [K^+] - [Cl^-] - [HCO_3^-]$
- AG = 142 + 4 - 103 - 25 = 18 mmol/l
- physiological range of AG = 12 – 18 mmol/l

AG



Na^+

K^+

Cl^-

HCO_3^-

AG

$$AG = HPO_4^{2-} + Prot^- + SO_4^{2-} + OA$$

Elevated AG may be caused by various conditions

- kidney insufficiency (↑ HPO_4^{2-} + ↑ SO_4^{2-})
- diabetes, starvation (↑ OA: acetoacetate, β-hydroxybutyrate)
- poisoning by methanol (↑ OA: formate $HCOO^-$)
- lactoacidosis (↑ OA: lactate)
- severe dehydratation (↑ proteinates)

Q. 16

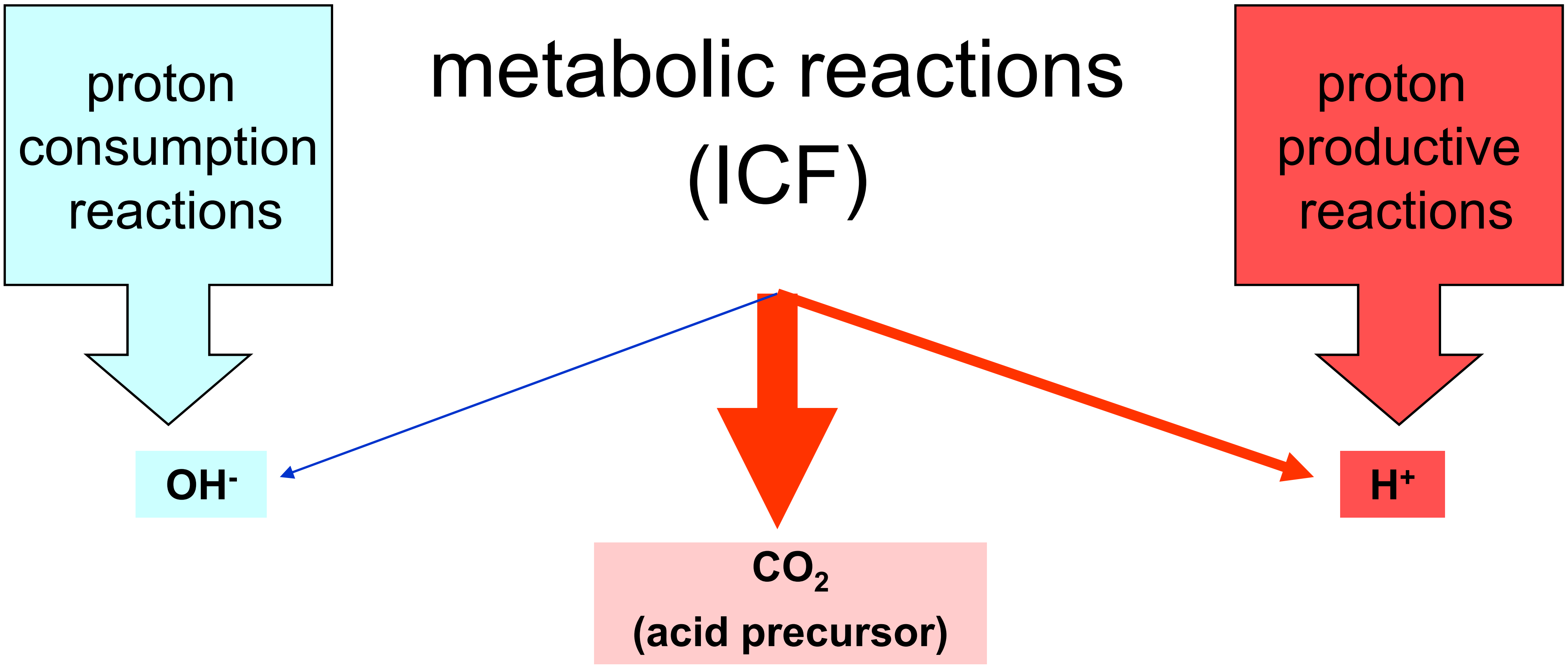
Condition	**Change in SID**
Increased concentration of chloride ions	
Increased KB	
Acute diarrhea (= loss of HCO_3^-)	
Hypoxia (lactate production in tissues)	
Ethylene glycol intoxication (oxalate)	
Long vomiting (loss of chloride)	

Formula

A. 16

Condition	**Change in SID**
Increased concentration of chloride ions	↓
Increased KB	↓
Acute diarrhea (= loss of HCO_3^-)	↓
Hypoxia (lactate production in tissues)	↓
Ethylene glycol intoxication (oxalate)	↓
Long vomiting (loss of chloride)	↑

Metabolism from acid-base point of view



proton consumption reactions

metabolic reactions (ICF)

proton productive reactions

OH^-

H^+

CO_2
(acid precursor)

acid-base reactions in ECF with buffers systems

Which compounds are responsible for the pH of:

- Lemon juice (2.3)
- Pepsi-Cola (2.5)

- Gastric juice (1-2)
- Bile (6.2 - 8.5)

Proton-consumption reaction:

$\text{anion}^- + H^+ \rightarrow$ non-electrolyte

Gluconeogenesis from lactate:

$2\ \text{lactate}^- + 2\ H^+ \rightarrow 1\ \text{glucose}$

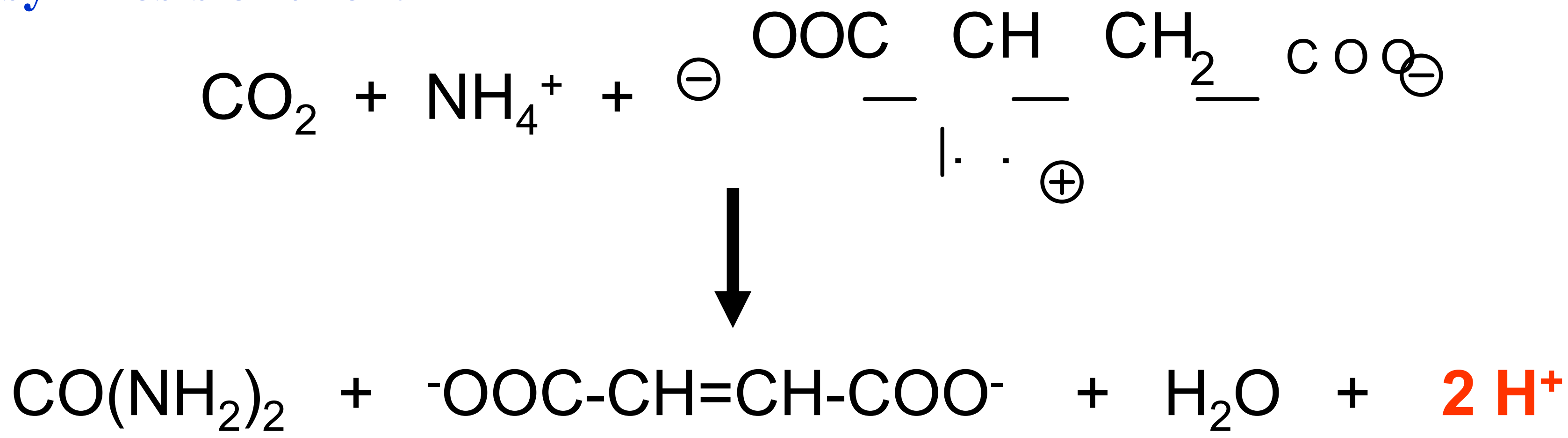
- protons are consumed in the synthesis of non-electrolyte from anion
- proton-consumption is equivalent to OH^- production

Proton-productive reactions

- **non-electrolyte → acid → anion$^-$ + H$^+$**

  e.g. anaerobic glycolysis, glucose → 2 lactate$^-$ + **2 H$^+$**

- **synthesis of urea:**



$$CO_2 + NH_4^+ + {}^{\ominus}OOC - \underset{|.\ .\ \oplus}{CH} - CH_2 - COO^{\ominus}$$

$$\downarrow$$

$$CO(NH_2)_2 + {}^-OOC\text{-}CH{=}CH\text{-}COO^- + H_2O + 2\,H^+$$

Q. 17

The main acidic catabolite is CO_2

compare daily production of acid equivalents:

CO_2 .... up to **25 000** mmol/day

H^+ as NH_4^+ and $H_2PO_4^-$ .... up to **80** mmol/day

How is CO_2 made in the body?

Production of CO_2 in the body

- CO_2 is produced in **decarboxylation** reactions
- oxidative decarboxylation of pyruvate → acetyl-CoA
- two decarboxylations in CAC (isocitrate, 2-oxoglutarate)

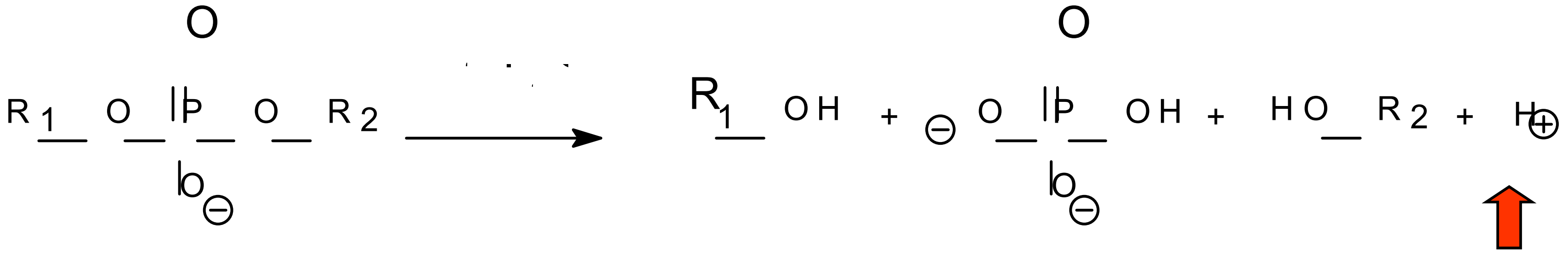
main sources of CO_2

- decarboxylation of aminoacids → biogenous amines
- non-enzymatic decarboxylation of acetoacetate → acetone
- catabolism of pyrimidine bases

  (cytosine, uracil $\rightarrow CO_2 + NH_3 + \beta$-alanine)
- catabolism of glycine $\rightarrow CO_2 + NH_3 +$ methylen-THF

Overview of acidic catabolites

- aerobic metabolism of nutrients → **$CO_2 \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$**
- anaerobic glycolysis → **lactic acid → lactate + H^+**
- KB production (starvation) → **acetoacetic/β-hydroxybutyric acid**
- catabolism of cystein (-SH) → **sulfuric acid → SO_4^{2-} + 2 H^+**
- catabolism of purine bases → **uric acid → urate + H^+**
- catabolism of phospholipids, DNA, RNA → **HPO_4^{2-} + H^+**

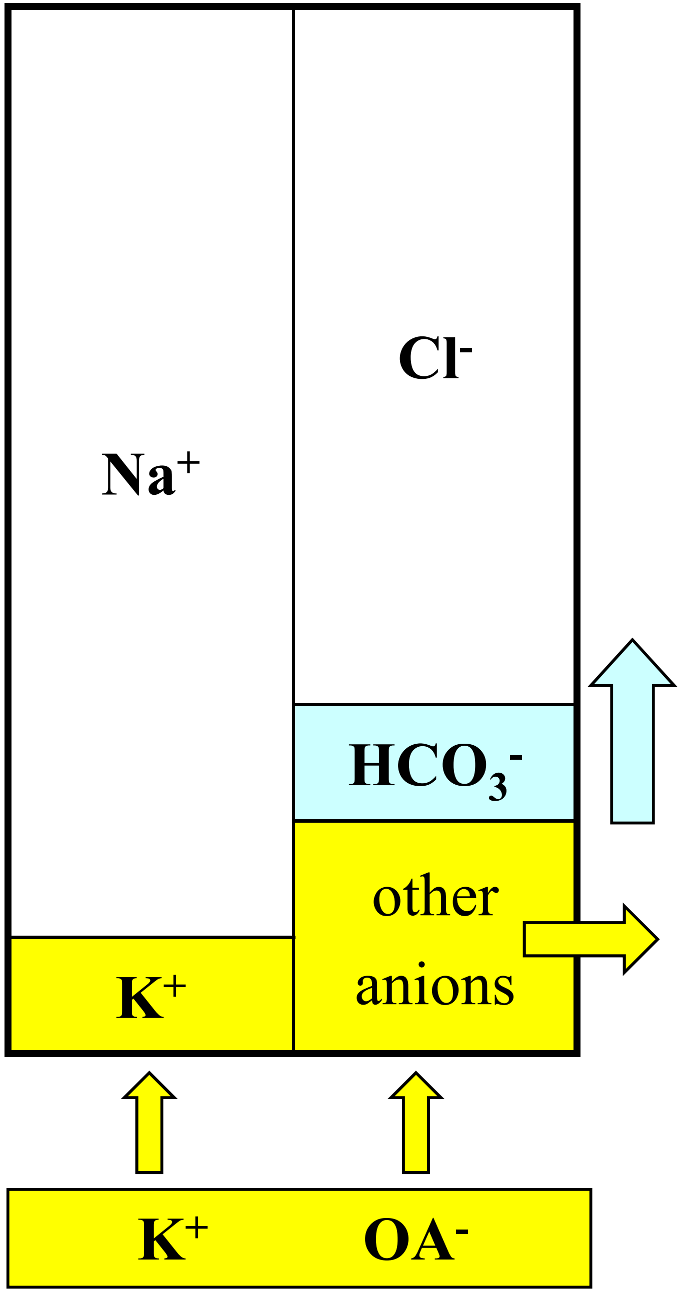


$$R_1-O-\overset{O}{\underset{O^-}{P}}-O-R_2 \longrightarrow R_1-OH + {}^-O-\overset{O}{\underset{O^-}{P}}-OH + HO-R_2 + H^+ \uparrow$$

phosphodiesteric linkage

Q. 18

A. 18



- **strictly vegetarian diet**
- contains a lot of potassium citrate/malate
- potassium salts get into blood plasma
- organic anions (OA) enter cells and are metabolized (CAC, malic enzyme etc.)
- K^+ cations remain in plasma
- to keep electroneutrality of plasma ⇒ HCO_3^- concentration increases
- **result: mild physiological alkalosis**

What is the conversion of malate in:

a) CAC

b) malic enzyme reaction

Buffer systems in blood

Buffer system	**Abundance**	**Buffer base**	**Buffer acid**	**pK_A**
Hydrogen carbonate	50 %	HCO_3^-	H_2CO_3, CO_2	6.1
Proteins[a]	45 %	Prot-His	Prot-His-H^+	6.0-8.0[b]
Hydrogen phosphate	5 %	HPO_4^{2-}	$H_2PO_4^-$	6.8

[a] In plasma mainly albumin, in erythrocytes hemoglobin

[b] The pK_A value depends on the type of protein

Buffer bases in (arterial) plasma

Buffer base	**mmol/l**
HCO_3^-	24
Protein-His	17*
HPO_4^{2-}	1
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Total	**42**

* Molarity of negative charge = binding sites for H^+

Q. 19

A. 19

$$\mathrm{pH} = \mathrm{p}K_\mathrm{A} + \log \frac{[\text{buffer base}]}{[\text{buffer acid}]}$$

Q. 20

A. 20

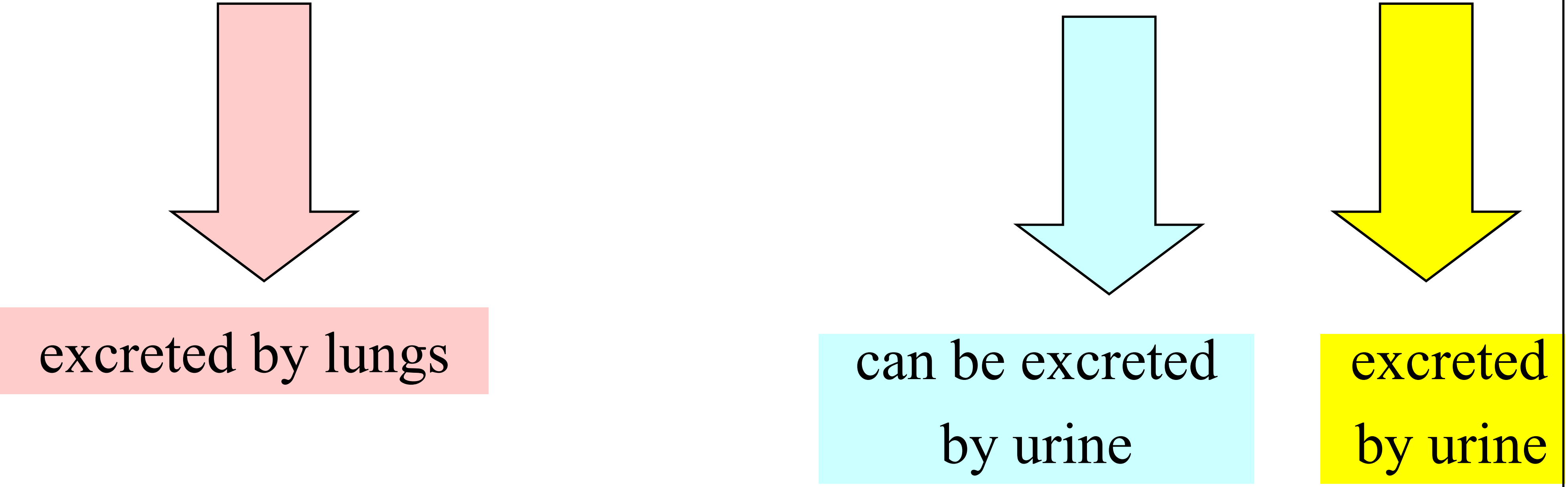
- concentration of both components
- the ratio of both components

- the best capacity if: [buffer base] = [buffer acid]

Hydrogen carbonate buffer is the only system which communicates with external environment

Describe double-equilibrium

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



excreted by lungs

can be excreted by urine

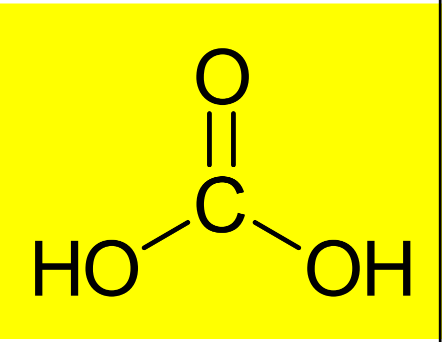
excreted by urine

Q. 23

Carbonic acid double equilibrium *in vitro* = carbonated water

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

$$800 \quad : \quad 1 \quad : \quad 0.03$$



- weak diprotic acid (p$K_{A1} = 6.37$; p$K_{A2} = 10.33$)
- does exist only in aq. solution, easily decomposes to CO_2 and water
- CO_2 predominates 800 × in sol. ⇒ therefore CO_2 is included into K_A

$$K_{A\,\mathrm{eff}} = \frac{[H^+][HCO_3^-]}{[CO_2 + H_2CO_3]}$$

$K_{A\,\mathrm{eff}}$ = effective dissociation constant

Carbonic acid double equilibrium in blood plasma

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

1 : traces : 20

- CO_2 hydration is catalyzed by carbonic anhydrase
- under physiological conditions: $pK_{A1} = 6.10$
- CO_2 is continually eliminated from body by lungs
- the overall concentration of carbonic acid:

$$\mathbf{[CO_2 + H_2CO_3] = pCO_2 \times \mathit{s} = 0.23\ pCO_2\ (kPa)}$$

directly measurable quantity

Compare: CO_2 in water and blood !

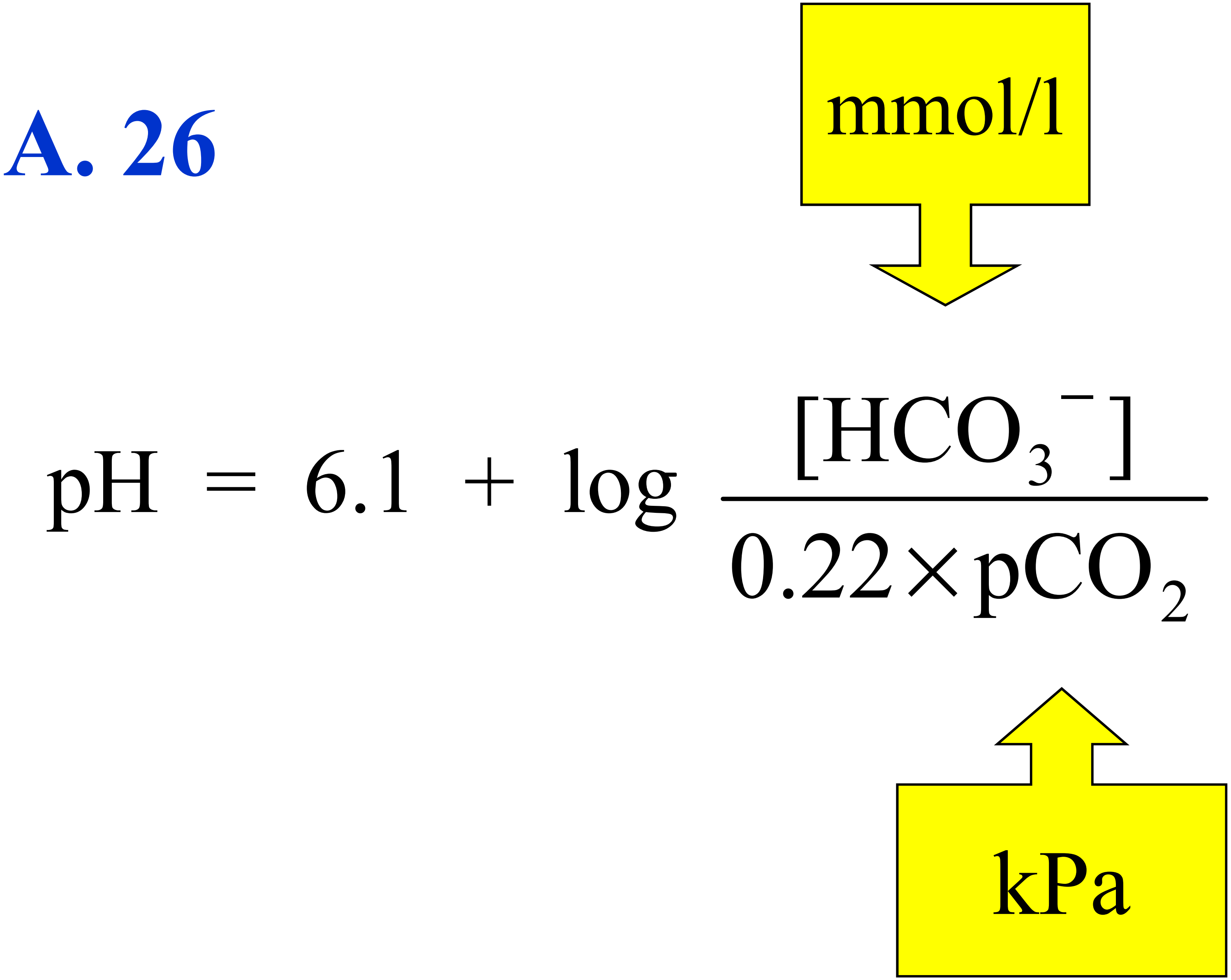
Liquid	**pH**	**$[CO_2] : [HCO_3^-]$**
Carbonated water[a]	3.50 – 5.00	800 : 0.03
Blood[b]	7.36 – 7.44	1 : 20

[a] **Closed system** (PET bottle), 25 °C, $pK_{A1} = 6.37$
pH ~ pCO_2 ~ the pressure of CO_2 applied in saturation process

[b] **Open system**, 37 °C, $pK_{A1} = 6.10$
CO_2 continually eliminated, pCO_2 in lung alveoli ~ 5.3 kPa,
acid component of hydrogen carbonate buffer

Q. 26

A. 26



mmol/l

$$\mathrm{pH} = 6.1 + \log \frac{[\mathrm{HCO_3}^-]}{0.22 \times p\mathrm{CO_2}}$$

kPa

Q. 27

A. 27

protons are eliminated in the reaction with buffer base

$HCO_3^- + \mathbf{H^+} \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$

hydroxide ions are eliminated in the reaction with buffer acid

$H_2CO_3 + \mathbf{OH^-} \rightarrow H_2O + HCO_3^-$

Q. 28

	Initial status	**Closed system**	**Open system**
$[HCO_3^-]$	24 mmol/l	**22**	
$[CO_2+H_2CO_3]$	1.2 mmol/l	**3.2**	
pH	7.40	**6.94**	

2 H^+ react with buffer base ⇒ 24 – 2 = **22 HCO_3^-** + 2 CO_2

newly formed CO_2 remain in the system ⇒ 1.2 + 2 = **3.2 CO_2**

	Initial status	**Closed system**	**Open system**
$[HCO_3^-]$	24 mmol/l	22	**22**
$[CO_2+H_2CO_3]$	1.2 mmol/l	3.2	**1.2**
pH	7.40	6.94	**7.36**

2 H^+ react with buffer base ⇒ 24 - 2 = **22 HCO_3^-** + 2 CO_2

newly formed CO_2 is eliminated by lungs ⇒ 3.2 - 2 = **1.2 CO_2**

Q. 29

A. 29

$7.40 = 6.1 + \log x$

$\log x = 1.3$

$x = 10^{1.3} = 20 = \boxed{20 : 1 = [HCO_3^-] : [CO_2 + H_2CO_3]}$

Calculate pCO_2 if pH = 7.30, $[HCO_3^-]$ = 20 mmol/l

$$7.30 = 6.1 + \log (20 / 0.22pCO_2)$$

$$1.2 = \log x$$

$$x = 10^{1.2} = 15.85 = 20 / 0.22pCO_2$$

$$pCO_2 = 20 / 0.22 \times 15.85 = 5.74 \text{ kPa}$$

Q. 30

A. 30

- $[HCO_3^-] : [CO_2 + H_2CO_3] = 20 : 1$
- the concentration of buffer base is **20 × higher**

  than the concentration of buffer acid
- hydrogen carbonate buffer is 20 × more resistant to acids

Hydrogen phosphate buffer

- buffer base: HPO_4^{2-}
- buffer acid: $H_2PO_4^-$

- occurs mainly in ICF, bones, urine

Q. 31 a)

A. 31 a)

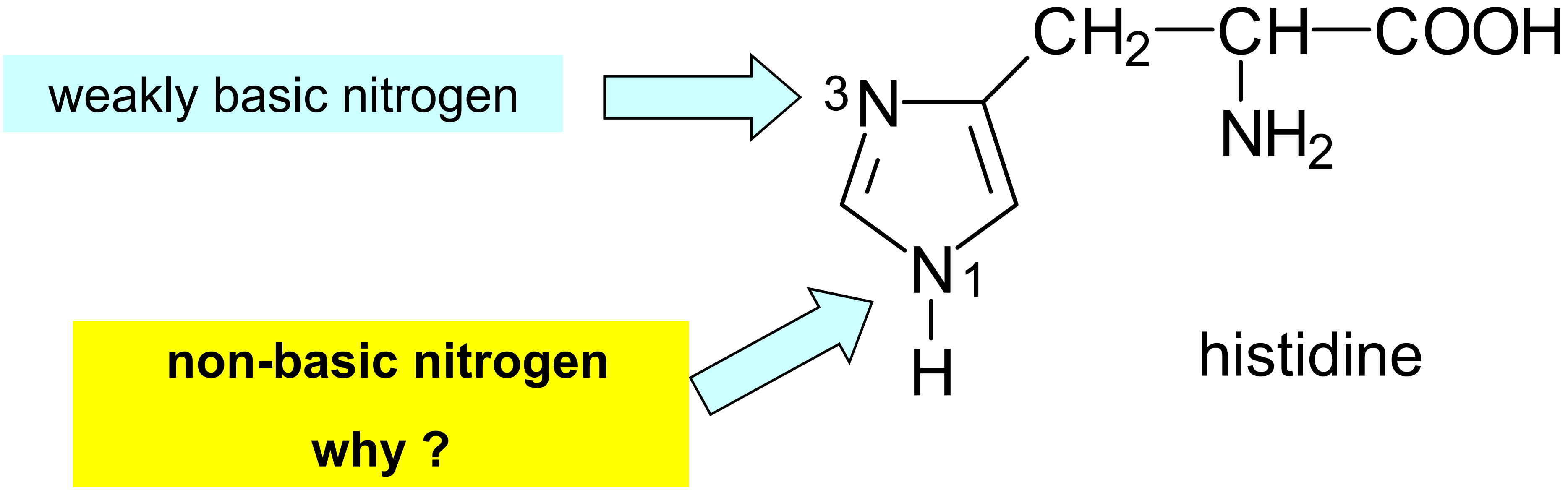
$7.40 = 6.80 + \log x$

$\log x = 0.6$

$x = 10^{0.6} = 4 \Rightarrow \boxed{[HPO_4^{2-}] : [H_2PO_4^{-}] = 4 : 1}$

Protein and hemoglobin buffers

- hemoglobin (Hb) contains a lot of histidine (His)



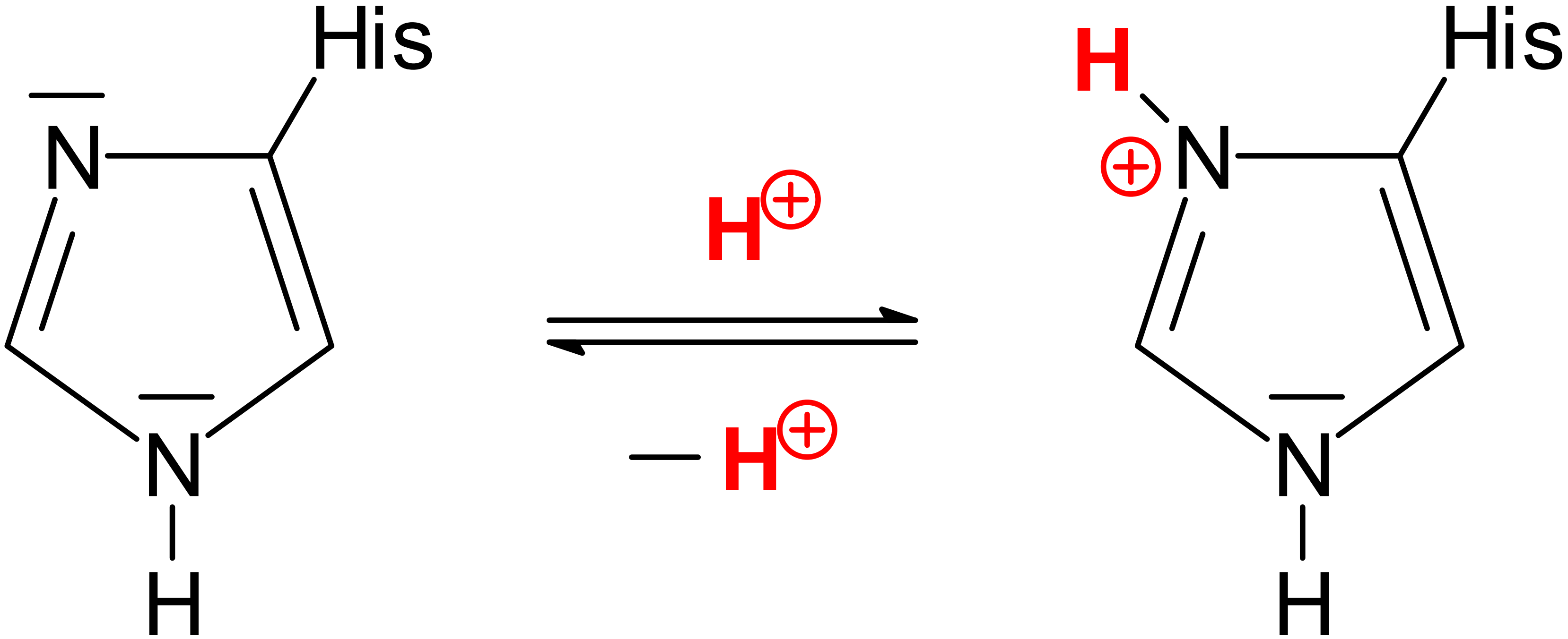
weakly basic nitrogen

non-basic nitrogen

why ?

Q. 35

Buffering action of proteins is performed by the side chain of histidine



imidazol

$\mathrm{p}K_B$ (His) = 8

imidazolium

$\mathrm{p}K_A$ (His) = 14 - 8 = 6

$\mathrm{p}K_A$ (His in proteins) = 6 - 8

Describe the transport of:

1) CO_2 from tissues to air

2) O_2 from air to tissues

Three ways of CO_2 transport in blood

1. **cca 85 % in the form of HCO_3^-**

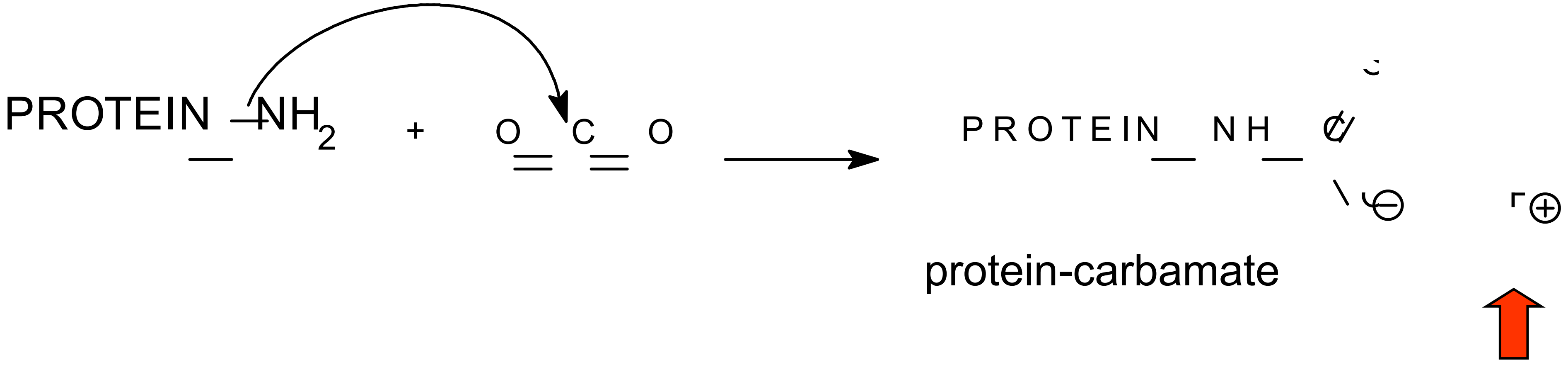
   it is formed in ery by the action of carbonic anhydrase, then is transported to plasma, exchange for chloride is needed to maintain **electroneutrality** in ery

2. **cca 10 % in the form of unstable carbamates**

3. **cca 5 % of physically dissolved CO_2**

Q. 44

A. 44



protein-carbamate

- the nitrogen atom of N-terminal adds to carbon atom of CO_2
- released proton is buffered by the protein itself
- in lungs, protein-carbamates are non-enzymatically hydrolyzed to Prot-NH_2 and CO_2 which is exhaled

Kidney functions in acid-base balance

- **kidneys excrete acid species:**

ammonium cation NH_4^+

dihydrogen phosphate anion $H_2PO_4^-$

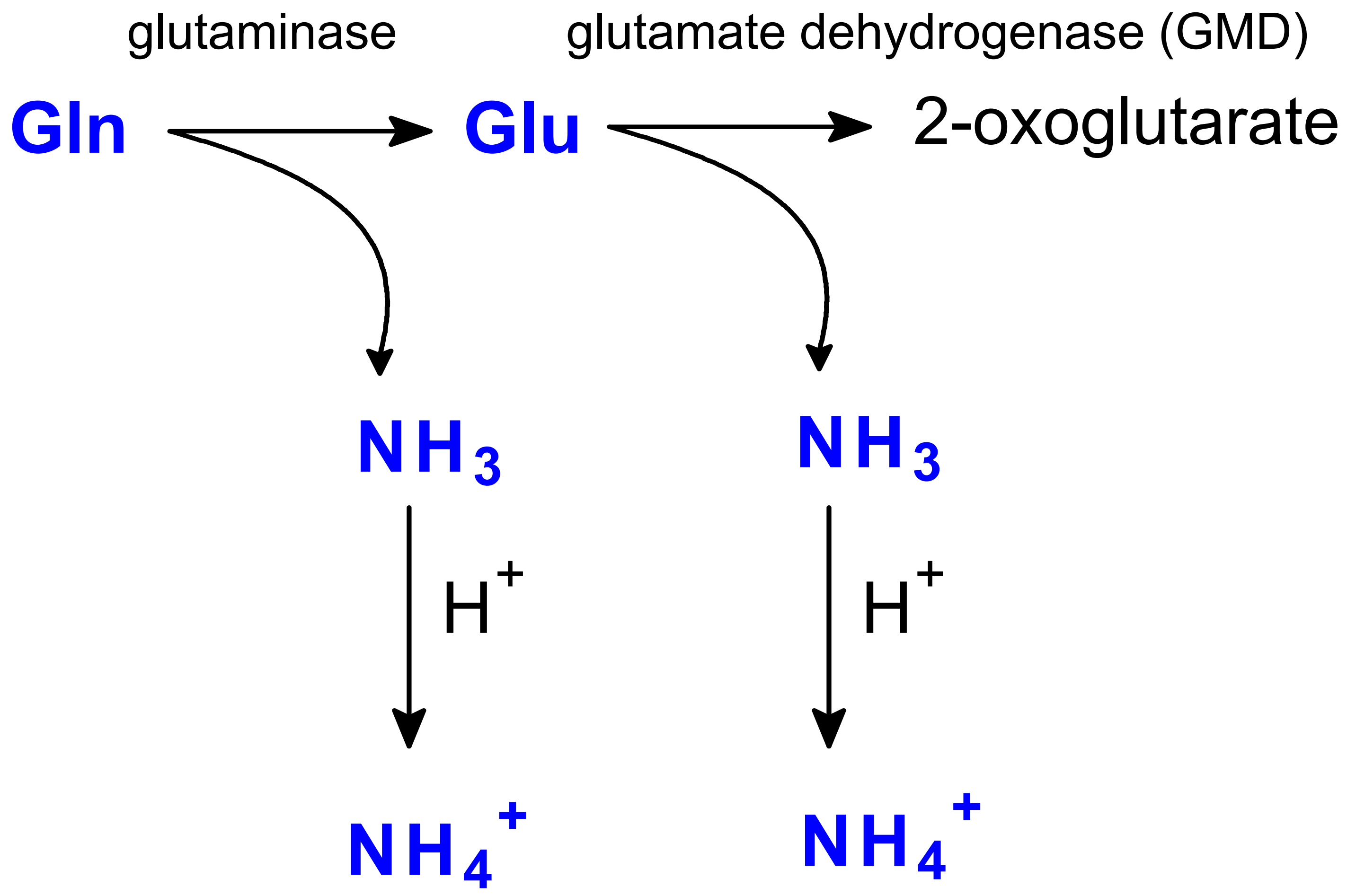
(uric acid and some other ...)

- **kidneys resorb basic species:**

the main buffer base = hydrogen carbonate anion HCO_3^-

Q. 45

Glutaminase and GMD reactions produce NH_4^+ in tubular cells



glutaminase

glutamate dehydrogenase (GMD)

Gln → **Glu** → 2-oxoglutarate

NH_3 $\xrightarrow{H^+}$ NH_4^+

NH_3 $\xrightarrow{H^+}$ NH_4^+

ammonium gets into urine by K^+-channel
or by K^+/Na^+-antiport transporter

Q. 47

A. 47

Acid	**Type**	**$\mathrm{p}K_\mathrm{A}$**	**Daily excretion**
NH_4^+	cation	9.25	~ 50 mmol/d
$H_2PO_4^-$	anion	6.80	~ 30 mmol/d
Uric acid	neutral	5.40	~ 2 mmol/d

Q. 48

$4.8 = 6.8 + \log x$

$\log x = -2$

$x = 10^{-2} = 0.01 = 1/100 \Rightarrow \boxed{[HPO_4^{2-}] : [H_2PO_4^-] = 1 : 100}$

under normal conditions (in mildly acidic urine)

the essentially prevailing species is dihydrogen phosphate

What is the consequence of reversed ratio in urine?

$HPO_4^{2-} / H_2PO_4^- > 1$

Formation of $CaHPO_4$ concrements

$$Ca_3(PO_4)_2 \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} CaHPO_4 \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} Ca(H_2PO_4)_2$$

insoluble	**insoluble**	**soluble**
pH > 8	**pH 6-8**	**pH 4-6**

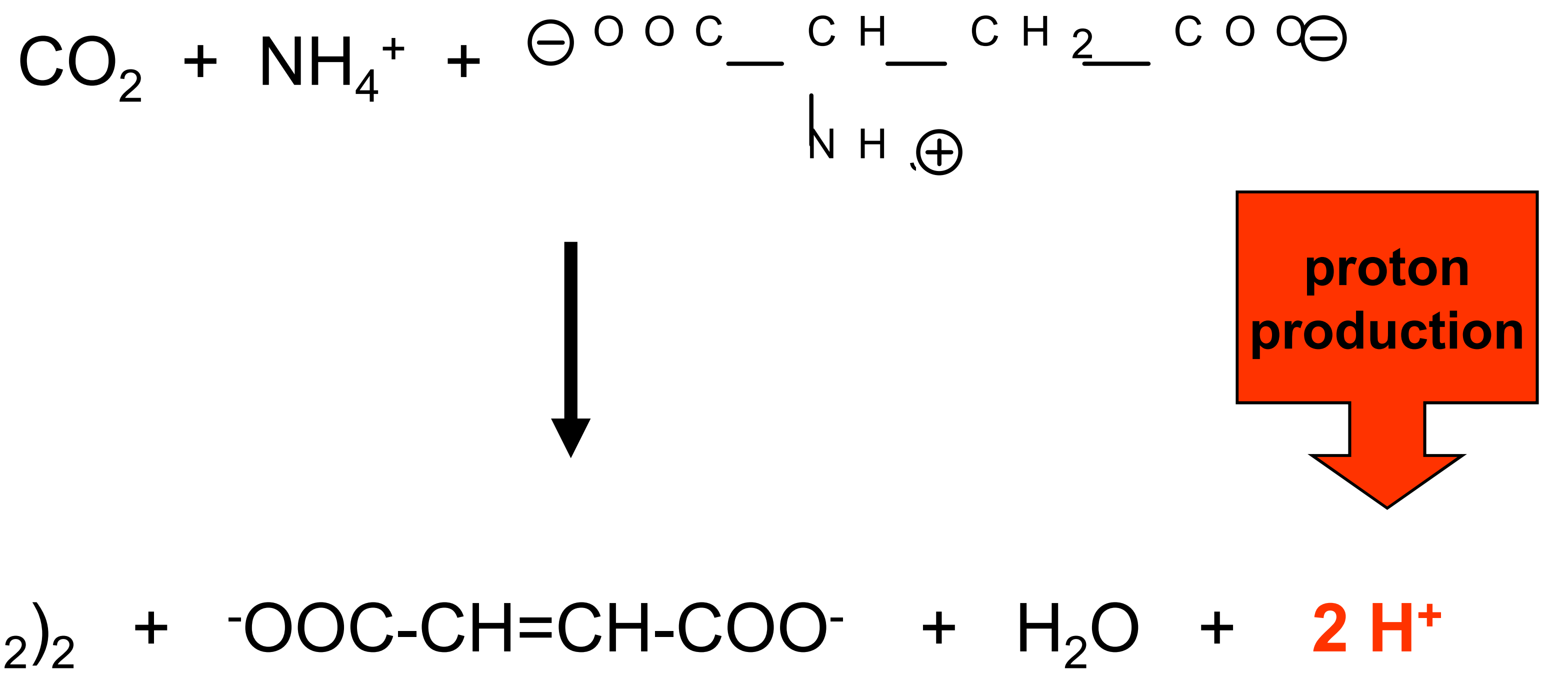
Compare and remember

Liquid	**pH**	**Prevailing phosphate species**
ECF, ICF	~ 7.4	HPO_4^{2-}
Urine	~ 5.5	$H_2PO_4^-$
Coca-Cola	~ 2.5	H_3PO_4

!

Q. 49

Synthesis of urea is proton-productive



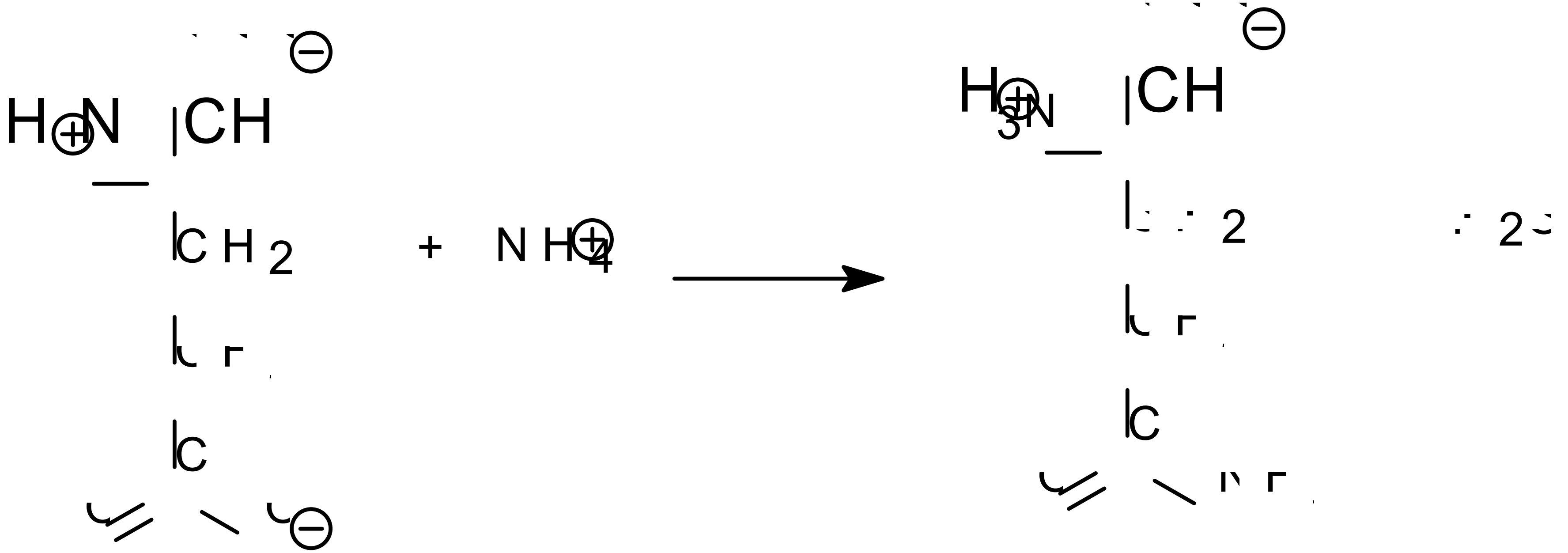
$$CO_2 + NH_4^+ + {}^{\ominus}OOC\text{—}\underset{|\atop NH_3^{\oplus}}{CH}\text{—}CH_2\text{—}COO^{\ominus}$$

$$\downarrow$$

$$CO(NH_2)_2 + {}^{-}OOC\text{-}CH\text{=}CH\text{-}COO^{-} + H_2O + 2\,H^+$$

proton production

Synthesis of glutamine is proton-neutral



Liver functions maintaining acid-base balance

- in acidosis, liver preferably makes glutamine instead of urea
- glutamine is transported by blood to kidneys, where it is hydrolyzed (glutaminase) - NH_4^+ cation is released into urine
- glutamate can be further deaminated and NH_4^+ cation is again released into urine
- urine pH is rather acidic