Acid-Base Regulation-1
Lecture 8 (9/4/2015)

Yanal A. Shafagoj MD. PhD

H^+ balance means (H^+ intake + H^+ production) = H^+ removal from the body.

Normally the [H^+] in the body fluids is kept at a low level, its concentration in the extracellular fluid is about 40nM/L (ranges from 4 times less (10 nM/L) to 4 times more than normal (160 nM/L).

The body can tolerate a greater increase of H+ from 40 to 160nM/L than decrease from 40 to 10nM/L in the [H^+] (i.e our body can tolerate a greater change in the acidic direction than the alkaline direction).

Compare H+ to Na+:   [Na+] = 145 mM/L

[H+] = 40 nM/L

[Na+]: 3.5 million times more [H+]

[H+] : the only ion that can vary widely from 10-160 nM (16X)

The Concept of the pH

- The pH = -log [H$^+$]. High [H+] means low pH
- $pCa^{++} = -log [Ca^{++}]$
- - "p" is the power of H with the sign reversed
- Low pH $\rightarrow$ high [H$^+$] $\rightarrow$ Acidosis
- High pH $\rightarrow$ low [H$^+$] $\rightarrow$ alkalosis
- So at normal extracellular H$^+$ concentration (40nM/L) our arterial blood pH is equal to 7.4 (range 7.35-7.45)
- Venous blood and interstitial fluid pH = 7.35 due to excess CO_2
- Intracellular pH ranges from 6.0 – 7.4 (In general 7.0 is the average).
- Urine pH ranges from 4.5 – 8 (5.5 is the average).
- (**Note : Hypoxia decreases intracellular pH due to acid accumulation**)

- An acid is a proton donor, while a base is a proton acceptor.
- Strong acids/bases dissociate (ionize) completely in solution such as HCl and NaOH.
- Weak acids partially ionize in solution such as H_2CO_3. It ionizes weakly to form H+.
- Weak bases also partially ionize such as NaHCO3- or HPO_4^{--} .

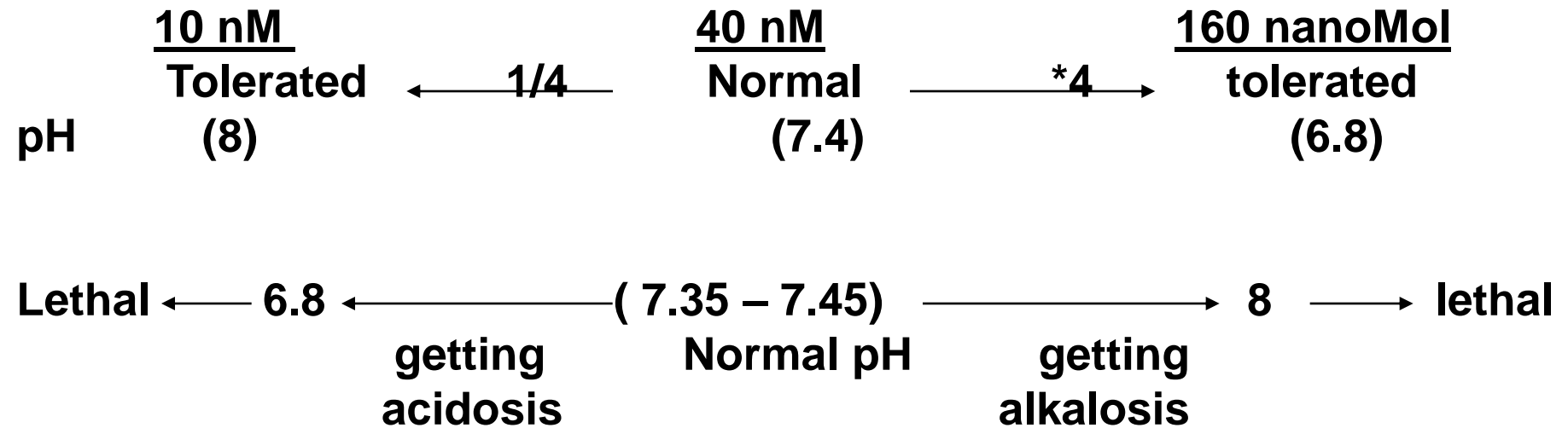(Note : Hemoglobin and other body proteins are of the most important body bases).

Most of our body acids and bases are weak acids and weak bases

Defense against changes in hydrogen ion concentration:

Our body is at a constant threat of becoming acidic, so how does it deal with these acids?

1. **First Line** of defense: Chemical acid-base buffer system (Very Fast)
2. **Second Line**: The respiratory center (removes or retain CO_2: intermediate speed, few minutes to start acting and few hours to give the full response)
3. **Third Line**: The kidneys (the most powerful regulatory system), a slow system that takes a few hours to start working and 3-5 days to reach full response.

10 nM
Tolerated   ←——— 1/4 ———   **40 nM**
Normal   ———— *4 ——→   **160 nanoMol**
tolerated

pH   (8)   (7.4)   (6.8)

Lethal ←——— 6.8 ←————————(7.35 – 7.45) ————————→ 8 ——→ lethal

getting   Normal pH   getting
acidosis   alkalosis

pH < 6.8   OR   > 8   is not compatible with life

$[H^+]$ range from 10 nM/L to 160 nM/L without causing death (16 times). Na^+ or K^+ can change only small percentages.

Acidosis OR Alkalosis, alter enzymatic activity leading to death

Acidosis: excess addition of H^+ to ECF$\rightarrow$ suppression of CNS $\rightarrow$ coma $\rightarrow$ death
Alkalosis: excess removal of H^+ form body fluid: convulsions of the respiratory muscle $\rightarrow$ death.

Acids can be volatile or Non-volatile.

Volatile Acids:

Is in the form of CO2 (300 L/D corresponds to 10 M/D) Produced in huge amounts, but usually will cause problems because it is engaged in this pathway:

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

CA: carbonic anhydrase enzyme fits here.

* If more H+ is produced in your body: reaction shift to left and CO_2 will be eliminated by the lungs and acidosis is corrected.

* If H+ is less, rxn shift to right; respiration is depressed More CO_2, is retained $\rightarrow$ forming H^+

Note: "CO_2 is masked H^+"

Non-volatile acids (Fixed Acids):

-Phosphoric acid from oxidation of phosphoproteins, phospholipids, nucleic acid:

-Sulphoric acid $\rightarrow$ oxidation of methionine and cysteine

-Others: lactic, pyrovic, beta-OH butyric acid, acetoacetic acids, and Krebs cycle acids. All these acids are not in the form of CO_2

The problem is that our body has tendency to form 50-80 mM/day fixed acids which cannot be taken care by the respiratory system (1 mM/kg). This is 25,000 times more than normal [H^+].

- 80 mM/D of fixed acids are produced.

80 mM if distributed in 14L of ECF, gets >5 mmol/L which correspond to pH less than 3: This, as mentioned before, not compatible with life.

- Why not secreting these 80 mMole of H+ in the urine in its free form???

Bcs: Minimum pH of urine = 4.5. this correspond to less than 0.1 mM.

- Other option: why not to buffer it: add 80 mM of HCO3- (**Buffer**)

80 mM H+ + 80 mM HCO3- $\rightarrow\rightarrow$ CO2 + H2O

The problem has been solved by converting these acids to CO2 and let the lung take care of it.

What is the Price? We lost 80 mM of HCO3-

How much HCO3- we have in ECF (HCO3- reserve!)?

24 mM/L * 14 l=336 mM which is enough for only 4-5 days. Still the problem has not been solved.

We must replace the lost 80 mM of HCO3- every day through the kidney.

Kidneys are Bicarbonate factory (continuous formation)

The Buffer System:
Buffer + H⁺ ↔ H:buffer

 If H^+ is added, the reaction moves to the right. If H^+ is reduced, reaction moves to the left.

Buffers don't eliminate H^+ or add it to our body, but keep them tied up until balance can be reestablished. It acts with a fraction of a second and is the first line of defense.

A buffer prevents a change in pH when H^+ is added or removed from a solution within certain limits

- Buffer is a substance that releases/binds H^+ reversibly to resist marked pH changes and keep it compatible with life. All chemicals can buffer up to 1000 mM H^+ before there is any significant shift in pH).

- **Principle body buffers:**

a) Bicarbonate/carbonic acid buffer system
   (most important system in the ECF)

b) Phosphate buffer system (HPO_4^{-2}, $H2PO_4^-$) Intratubular and intracellular

c) Proteins (important intracellular buffers, ex: Hemoglobin**)**

Buffer Systems in the Body

Bicarbonate : most important ECF buffer

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

Phosphate : important renal tubular buffer

$$HPO_4^{--} + H^+ \longleftrightarrow H_2PO_4^-$$

Ammonia : important renal tubular buffer

$$NH_3 + H^+ \longleftrightarrow NH_4^+$$

Proteins : important intracellular buffers

$$H^+ + Hb \longleftrightarrow HHb$$

(60-70% of buffering is in the cells)

Bicarbonate buffer system:

Consists of a weak acid (H_2CO_3) and a bicarbonate salt, predominantly $NaHCO_3$ which ionizes completely into Na^+ and HCO_3^-

Always remember this equation:

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

To calculate the pH of this buffer system, we use the *Henderson-Hesselbalch Equation*   pH = pK + log [Salt/Acid]

  pK is the pH of a solution when the salt form is equal to the acid form.

The pK for the bicarbonate/carbonic acid is pK= 6.1 $pH = Pk' + \log\left[\dfrac{Salt}{Acid}\right]$

The salt is the bicarbonate ion, and the acid is CO_2

CO_2 is measured by its partial pressure (PCO_2)

 To convert it to mMole :multiply by 0.03

Arterial PCO_2 = 40mm Hg correspond to 1.2 mMole (40 * 0.03)

Bicarbonate Buffer System

Is the most important buffer in extracellular fluid even though the concentration of the components are low and pK of the system is 6.1, which is not very close to normal extracellular fluid pH (7.4).

Reason: the components of the system (CO_2 and HCO_3^-) are closely regulated by the lungs and the kidneys

pH = 6.1 + log {HCO_3^-/[0.03 * PCO_2]}

Substituting the actual concentrations would give us:

pH = 6.1 + log [24/1.2] = 6.1 + log 20

    = 6.1 + 1.3

    = 7.4

We can calculate the pH of any buffer by using the above equation if we know the pK and the concentration of the buffer in its salt and acidic forms.

Ex: pK for phosphate buffer = 6.8

pH = 6.8 + log [1.0/ 0.25]=6.8 +log 4

    = 6.8 + 0.6

    = 7.4

Ex: ammonia/ammonium ion system (pK = 9.2):

(Note: its not one of the buffer systems mentioned above)

pH = 9.2 + log [NH_3 / NH_4^+]   the result is also 7.4

Isohydric principle: $$pH = pK_1 + \log\left[\frac{A_1}{HA_1}\right] = pK_2 + \log\left[\frac{A_2}{HA_2}\right] etc$$

States that all buffers in a common solution are in equilibrium with the same hydrogen ion concentration.

-Therefore, whenever there is a change in the ECF H^+ concentration, the balance of all other buffer systems changes at the same time.

- Changing the balance of one buffer system changes the others because the systems actually buffer each other.

•To see how this buffer system works, if we add HCl (strong acid) to the solution, the following reaction takes place to change the strong acid (HCl) into a weak acid (H_2CO_3):

•HCl + NaHCO$_3$ $\longrightarrow$ NaCl + H_2CO_3
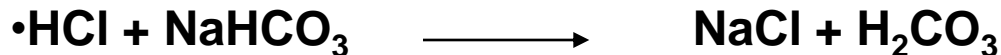
While if a strong base was added as NaOH, the buffer system changes it into a weak base (NaHCO$_3$) by the following reaction:

NaOH + H_2CO_3 $\longrightarrow$ NaHCO$_3$ + H_2O
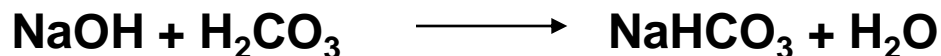
- The buffer is most effective within 1.0 pH unit of the pK of the buffer (i.e the linear portion of the curve in the following curve …next slide).

The bicarbonate buffer is most effective at pH range 5.1 – 7.1

- At normal body pH (7.4), the ratio of the basic form is 20 times more than the acid form.

We worry about acids in our body, and increasing H^+ will shift the curve closer to the linear portion (5.1 – 7.1), so it can work effectively.

- <u>Criteria to determine the buffering power and capacity of a system:</u>

1. The absolute / total concentration.
2. The relative concentration (pK of the system relative to pH of the surrounding).
3. The renewal tendency of the buffer.

Titration curve for bicarbonate buffer system.
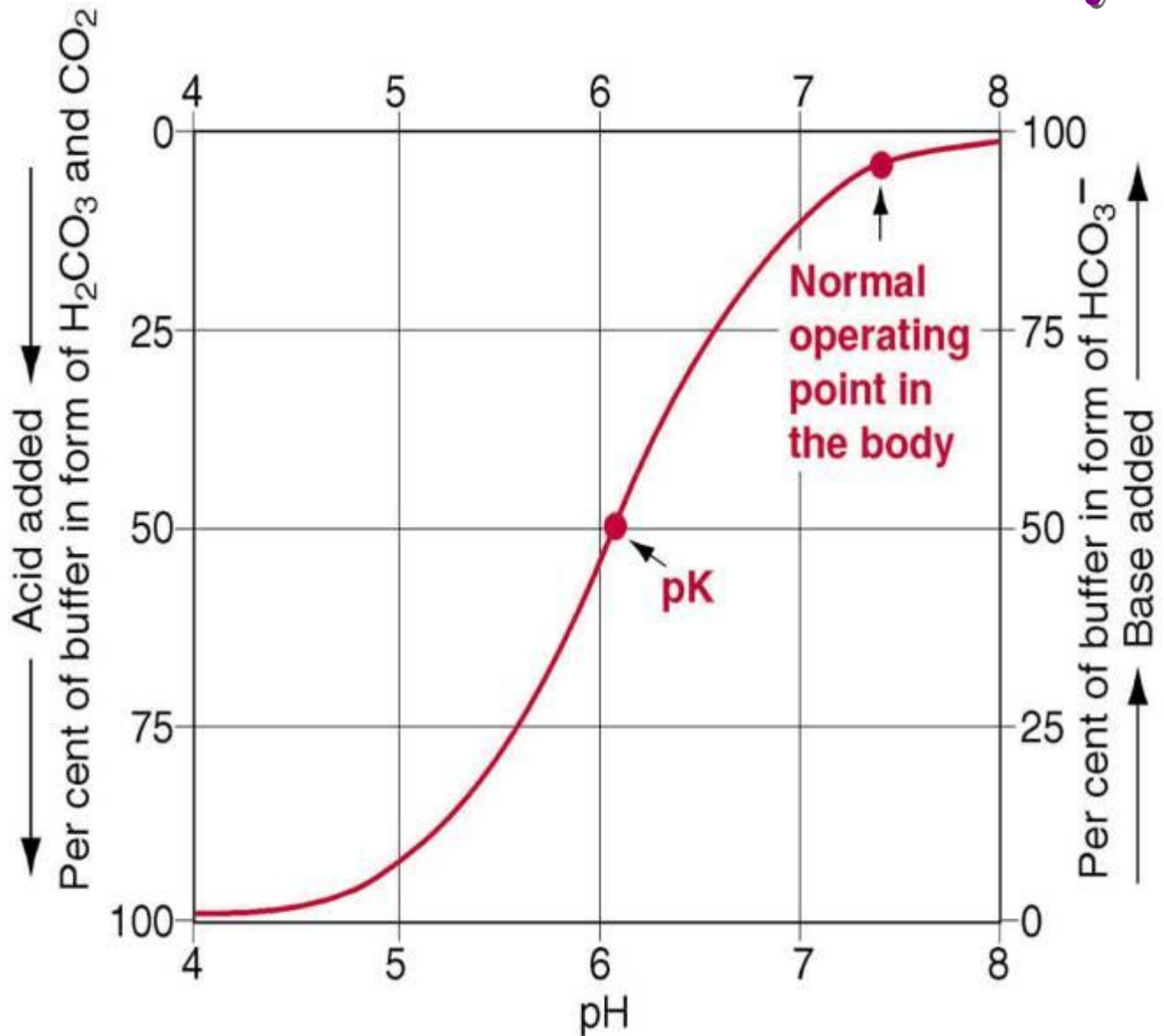
The Phosphate Buffer System:

Its concentration in plasma is low = 1mmol/L but its pK is equal to 6.8, which is closer to intracellular pH (7.0).

• We get the phosphate mostly from food and its plasma concentration is under the control of the kidney.

•Phosphate is 90% reabsorbed and 10% excreted.

• Its filtration load is equal to 180L/day * (1-1.5mmol/L) = 200-250 mmol/day

• The phosphate is more concentrated inside the cells and in TF. Where the local pH is closer to its pK.

•Hence, phosphate is a good buffer intracellular and intratubular, but not important as extracellular buffer.

•

Note: PTH inhibits phosphate reabsorption by affecting its T max.

- **Since 10% of the phosphate is excreted and less than 1% of water is excreted, phosphate becomes 10 times more concentrated. Again the proximal tubular pH is around 6.5 which is also close to its pK (6.8).**

$HPO_4^=$ (dibasic phosphate) **and $H_2PO_4^-$** (monobasic phosphate).

$HPO_4^=$ = 1 mM/L **and $H_2PO_4^-$ =** 0.25 mM/L.

$$pH = 6.8 + \log(4) \cong 6.8 + 0.6 = 7.4$$

We care about **$HPO_4^=$** (1mM/L) = Amount filtered per day ≈200 mM. Normally, most of the filtered (90%) is reabsorbed.
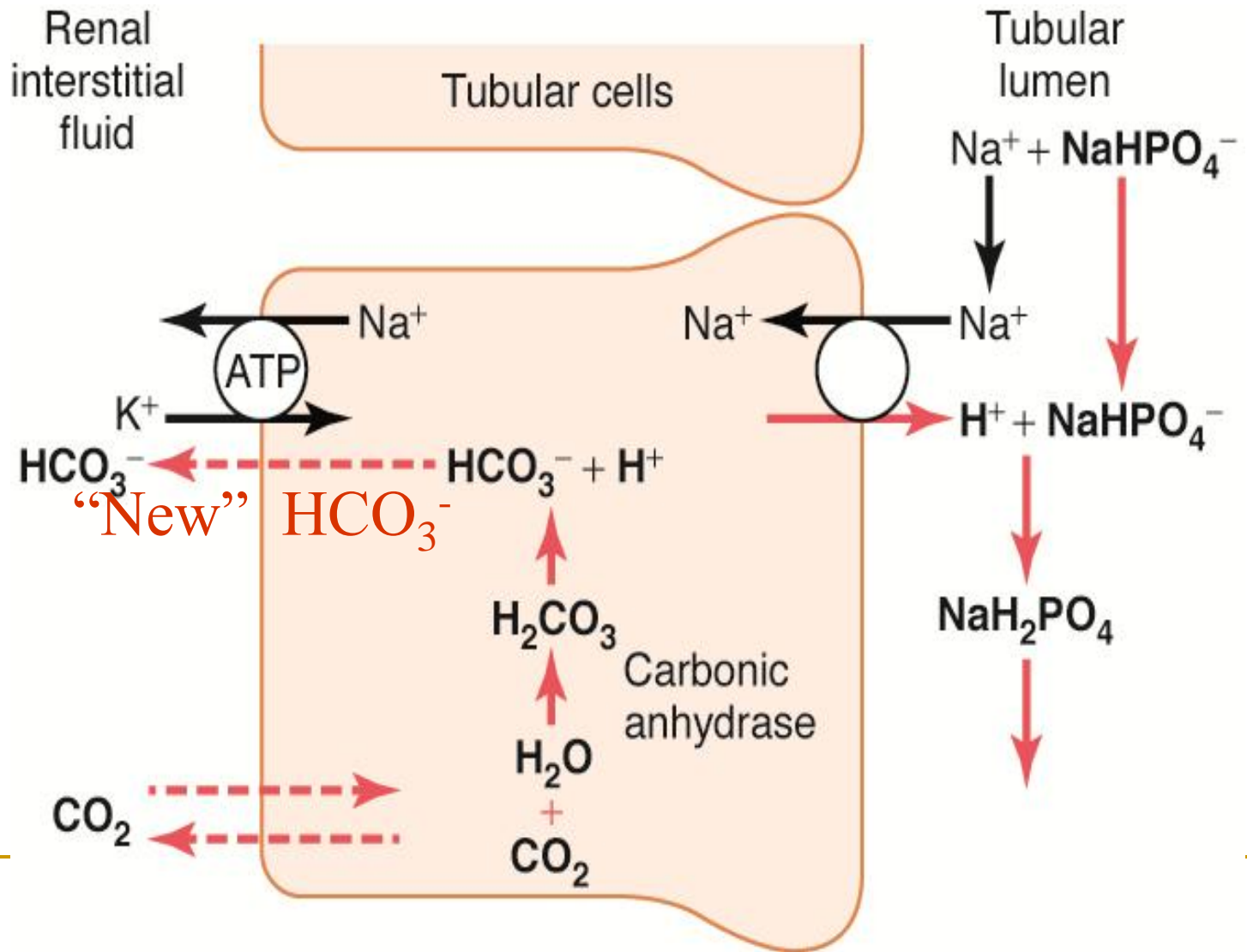
Phosphate as a Tubular Fluid Buffer

There is a high concentration of phosphate in the tubular fluid.

Phosphate buffering capacity does not change much with acid-base disturbances (phosphate is not the major tubular buffer in chronic acidosis

$$NaHPO_4^- + H^+ \longrightarrow NaH_2PO_4$$

Buffering of secreted H^+ by filtered phosphate $(NaHPO_4^-)$ and generation of "new" HCO_3^-

The protein Buffer System:

- An important intracellular buffer
- Its plasma concentration is negligible.
- Proteins have an imidazole group that binds to H^+ reversibly. The pK of most proteins is around 7.0, almost the same as intracellular pH.
- Intracellular proteins as hemoglobin have other functions but they work secondarily as buffers.
- Their concentrations cannot be controlled and they are not renewed.

All chemical buffers can buffer up to 1000 mmol of H^+.

$\rightarrow$ 70% of the buffering is due to proteins.

Since H^+ ion can't penetrate the cell membrane easily, the proteins can't really work acutely, but in chronic conditions they help.

Mechanisms of Hydrogen Ion Regulation

(pH range 7.35 -7.45)

1. Body fluid chemical buffers (rapid but temporary)
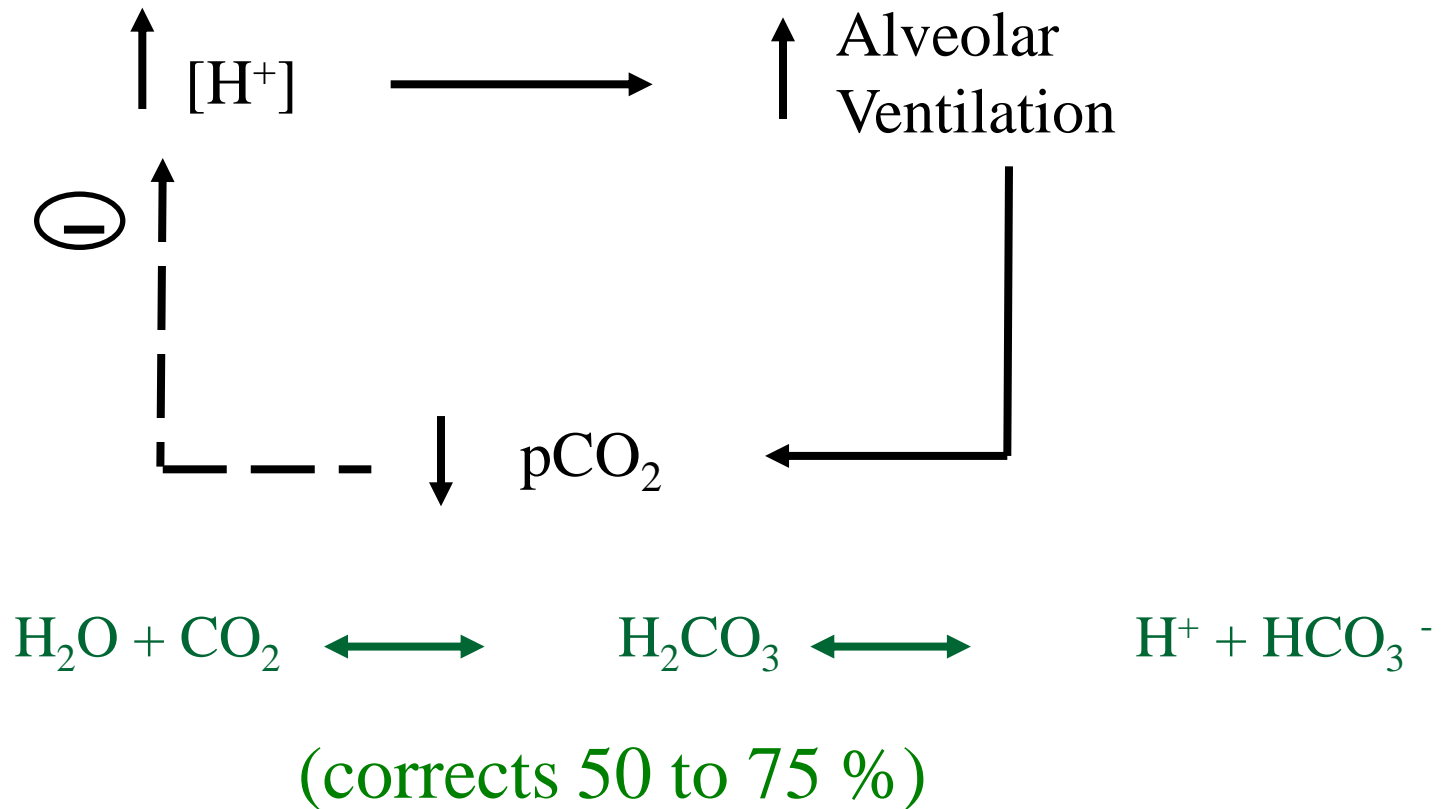- bicarbonate   - proteins   - phosphate

2. Lungs (rapid, eliminates CO_2)

↑ $[H^+]$ ⟶ ↑ventilation ⟶ ↑ CO_2 loss

3. Kidneys (slow, powerful); eliminates non-volatile acids
- secretes H^+
- reabsorbs HCO_3^-
- generates new HCO_3^-

Respiratory Regulation of Acid-Base Balance

$$\uparrow [H^+] \longrightarrow \uparrow \text{Alveolar Ventilation}$$

$$\downarrow pCO_2$$

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

(corrects 50 to 75 %)

Renal Regulation of Acid-Base Balance

- **Kidneys must not lose HCO3- in the urine, a task which is more important than secreting the nonvolatile acids**
- Still kidneys must eliminate the non-volatileacids (H_2SO_4, H_3PO_4) (~ 80 mmol/day)
- Filtered load of HCO_3^- = **180L/day * 24mEq/L = 4320 mEq/day**(~ 4320 mmol/day)
- Secretion of H^+ (~ 4400 mmol/day). The 4400 mEq of H^+ does not mean 4400 H+, because one H^+ molecule if it recycle 4400 times it is enough, we should keep in mind that the H^+ secreted is return back to the cell in form of CO_2.
- Net H^+ secretion is only 80 mM/D
- Reabsorption of HCO_3^- (~ 4319 mmol/day)
- Production of new HCO_3^- (~ 80 mmol/day)
- Excretion of HCO_3^- (1 mmol/day)

Kidneys conserve HCO_3^- and excrete acidic
or basic urine depending on body needs

The Renal control of the Acid-Base Balance:
1. Reabsorption of filtered HCO_3^-.
2. Secretion of H+.
3. Making new HCO_3^-.

Reabsorption of filtered HCO_3^-.

•HCO3- is very precious: we can't really afford losing any in urine.

(Full reabsorption, primarily in the proximal tubules)
80-90% of the HCO_3^- reabsorption and thus H+ secretion occurs at proximal tubule, 10% in thick ascending, 4.9 % in collecting duct and distal tubule, and less than 0.1% is excreted. With the exception of descending and thin ascending loop of Henle, the kidney tubules secrete H+ and reabsorb HCO3- at all other segments.

• The clearance is of HCO3- is negative→

•Quantity aspect: The reabsorption is more important than the production since its amount is (4320): it is greater.

• Again: 4320 molecules of bicarbonate can be reabsorbed by only on proton (H^+), there is no net secretion of hydrogen ions
(H+ recycle again and again)
→ No HCO_3^- gain sofar, but only reabsorption of filtered HCO_3^-
- After complete HCO_3^- reabsorption: any further H^+ secretion is net secretion resulting in: "HCO_3^- gain"

• The majority of H^+ secretion occurs in the proximal tubule by Na^+ countertransport mechanism and can cause a concentration difference of H+ across the luminal membrane up to 5-6 times only: but a tremendous amount of H+ is secreted (80-90%)….This makes pH of TF around 6.5 only.

•Primary Active Secretion Of H+ in Intercalated Cells (brown cells) of Late Distal Tubules & Collecting Ducts
• In the collecting ducts, we have H^+ pump which secrete H^+ actively and can increase the concentration difference up to 900 times leading to a very high concentration gradient. pH =4.5

|	Reabsorption of HCO3-	Gaining of HCO3-
Proximal parts	**80%**	**55 mM**
Distal parts	**20%**	**15 mM**

HCO3- Gain

→ As mentioned earlier, we also need additional amounts (80mmol/day) to be supplied by the kidney to neutralize the 80mmol of non-volatile (fixed) acids.

The presence of TF buffers allow us to secrete H+ and make new HCO3-: Main TF buffers are phosphate $HPO_4^=$ which can blind H+ to form H2PO4

If we excrete 80 mMole/D of phosphate then we gain 80 mMole/D of HCO3- → → → Thus we solved the problem

Unfortunately, we excrete only 20-30 mMole of phosphate
We still need additional 50 mMole of new HCO3- through other source→ This achieved by by ammonium production
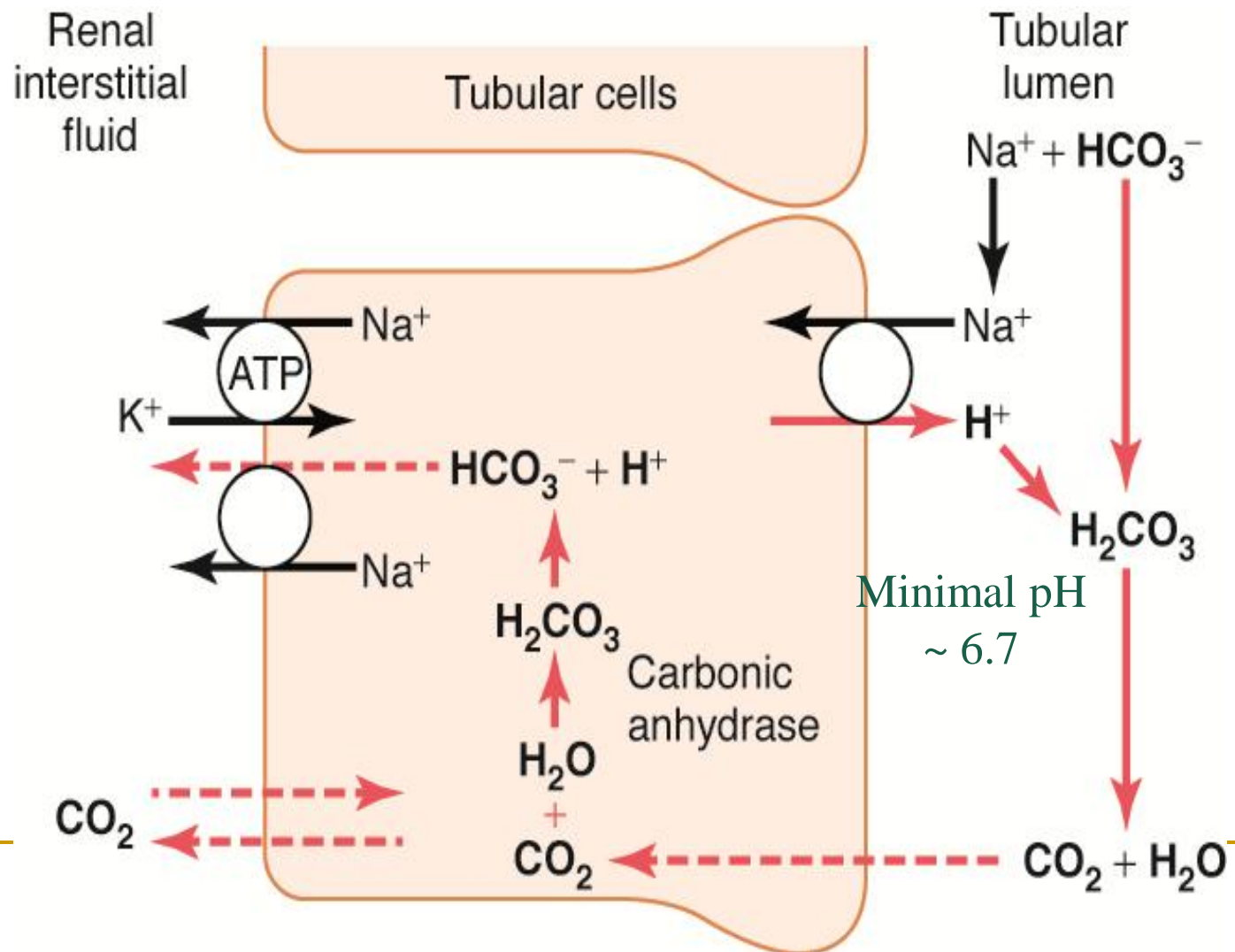
Reabsorption of bicarbonate (and H$^+$ secretion) in different segments of renal tubule.
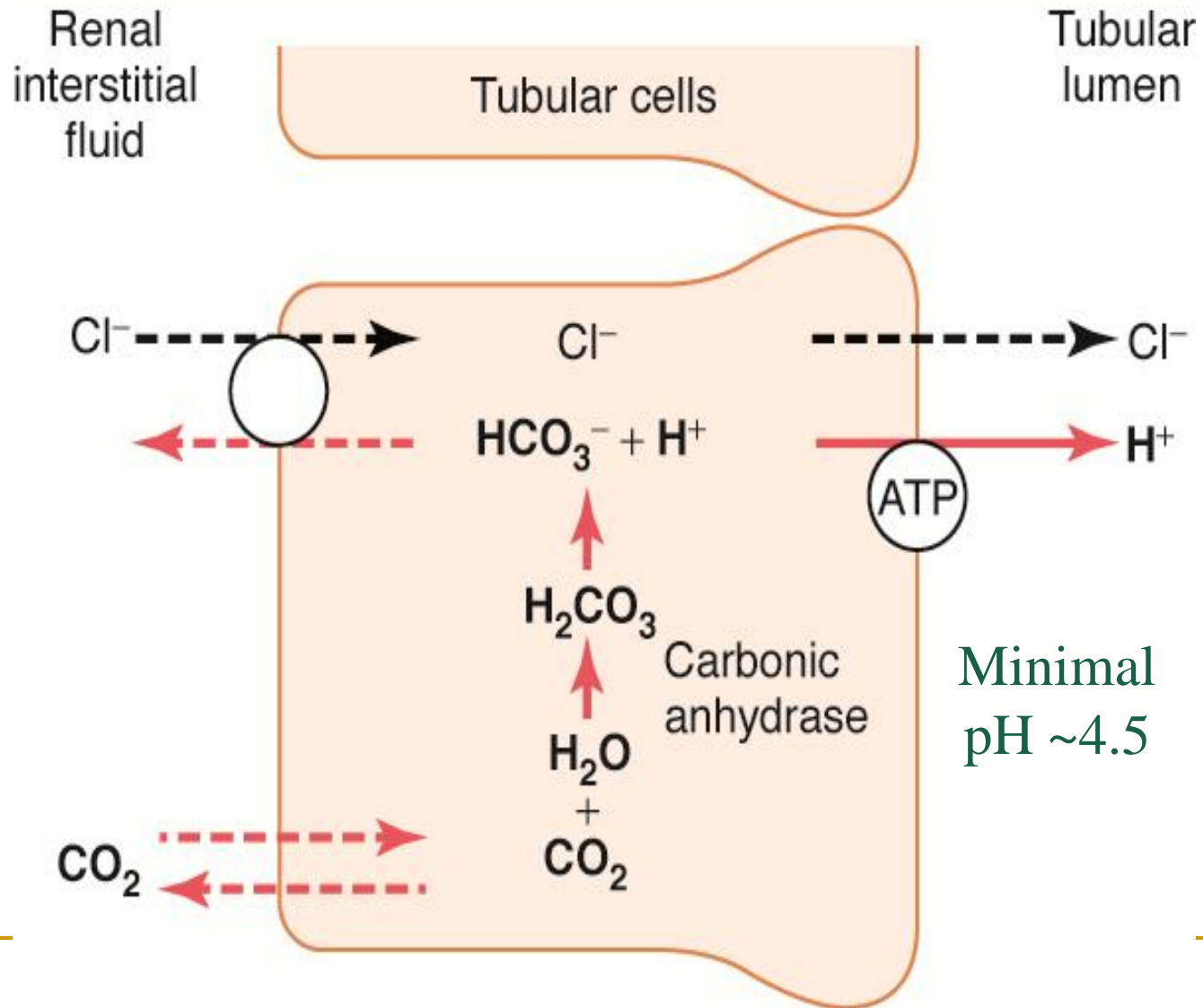
85%
(3672 mEq/day)

4320 mEq/day

10%
(432 mEq/day)

>4.9%
(215 mEq/day)

(1 mEq/day)
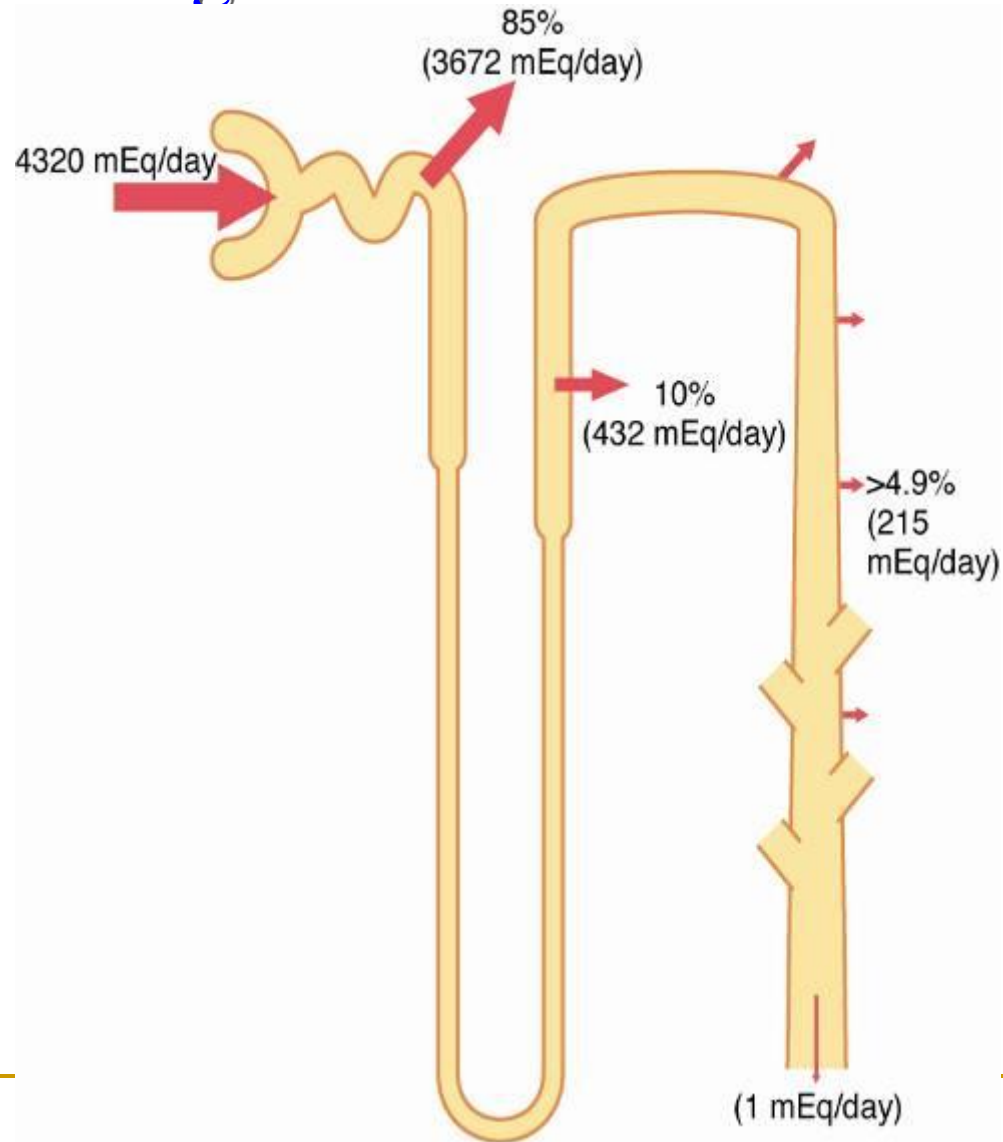
Key point:
For each HCO$_3^-$
reabsorbed, there
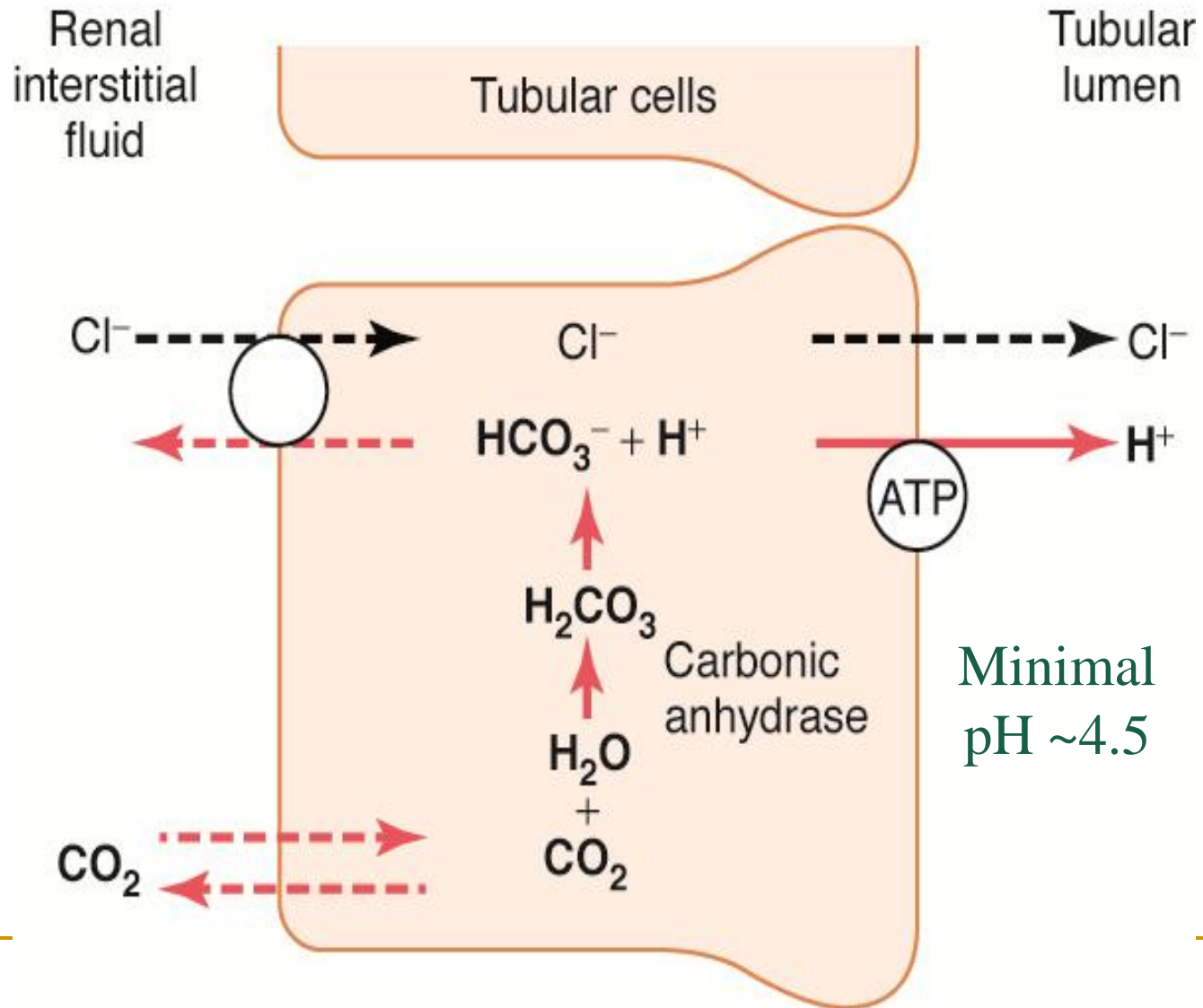must be a H$^+$
Secretion (but, no net
 H+ secretion)

Mechanisms for HCO_3^- reabsorption and Na^+ - H^+ exchange in proximal tubule and thick loop of Henle

HCO$_3^-$ reabsorption and H$^+$ secretion in intercalated cells of late distal and collecting tubules

HCO$_3^-$ reabsorption and H$^+$ secretion in intercalated cells of late distal and collecting tubules

Ammonium productionNH_4^+

(Ammonium NH_4^+ (ion), ammonia NH_3 is not).

Glutamine from blood enter the proximal cells where it is converted to glutamate then to alpha keto-glutamate which forms $2 NH_4^+ + 2HCO3-$

• Ammonium is secreted into the lumen by counter-transport mechanism in exchange of sodium in proximal tubules, thick ascending loop of Henley and distal tubules.
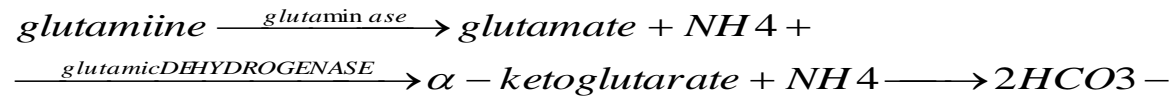
• In collecting tubules:

H+ is secreted into the lumen where it combines with NH3(ammonia) to form NH4+ (ammonium).

→ Collecting tubules membrane is much less permeable for ammonium than ammonia, thus it is trapped in the lumen in from of ammonium,

→ called ammonia trapping.

• Ammonium production can be induced unlike phosphate buffer system which is fixed.

• Whenever a hydrogen ion secreted into the tubular lumen combines with a buffer other than bicarbonate, the net effect is the addition of new bicarbonate ion to the blood.

$$glutamiine \xrightarrow{glutaminase} glutamate + NH4+$$

$$\xrightarrow{glutamicDEHYDROGENASE} \alpha - ketoglutarate + NH4 \longrightarrow 2HCO3-$$

Glutamine is actively transport to the proximal, thick ascending and distal from lumen and interstitium to inside the cell. Glutamine $\rightarrow$ Glutamate + NH4$^+$ Thus for every glutamine molecule there are two HCO_3^- are being gained to the blood and two NH4$^+$ are being secreted as counter-transport with Na$^+$ and excreted in the urine as NH4Cl. Then $NaHCO_3$ goes back to blood.

NH$_4^+$ excretion might exceed 500 mEq/day in chronic acidosis, thus NH$_4^+$ is the most important mechanism by which kidneys handle chronic acidosis. The same amount of new HCO_3^- are being formed too.

• Low blood pH induces glutaminase enzyme. It ends up in formation of HCO3- and NH_4^+, so the urine will be full of ammonium which is secreted in the from of NH_4Cl

If the kidney cannot absorb HCO3- or cannot secrete H+ then there is acidosis (<u>renal tubular acidosis)</u>

• <u>This is urine test to measure how much HCO3 has been added to the blood</u>

• HCO3- added /day = NH_4Cl excretion + titratable acids - HCO3-

Phosphate and Ammonium Buffering In Chronic Acidosis

Acid Excretion (mmoles/day)

$H_2PO_4^- + HSO_4^-$

NH_4^+

Normal

Acidosis for 4 Days
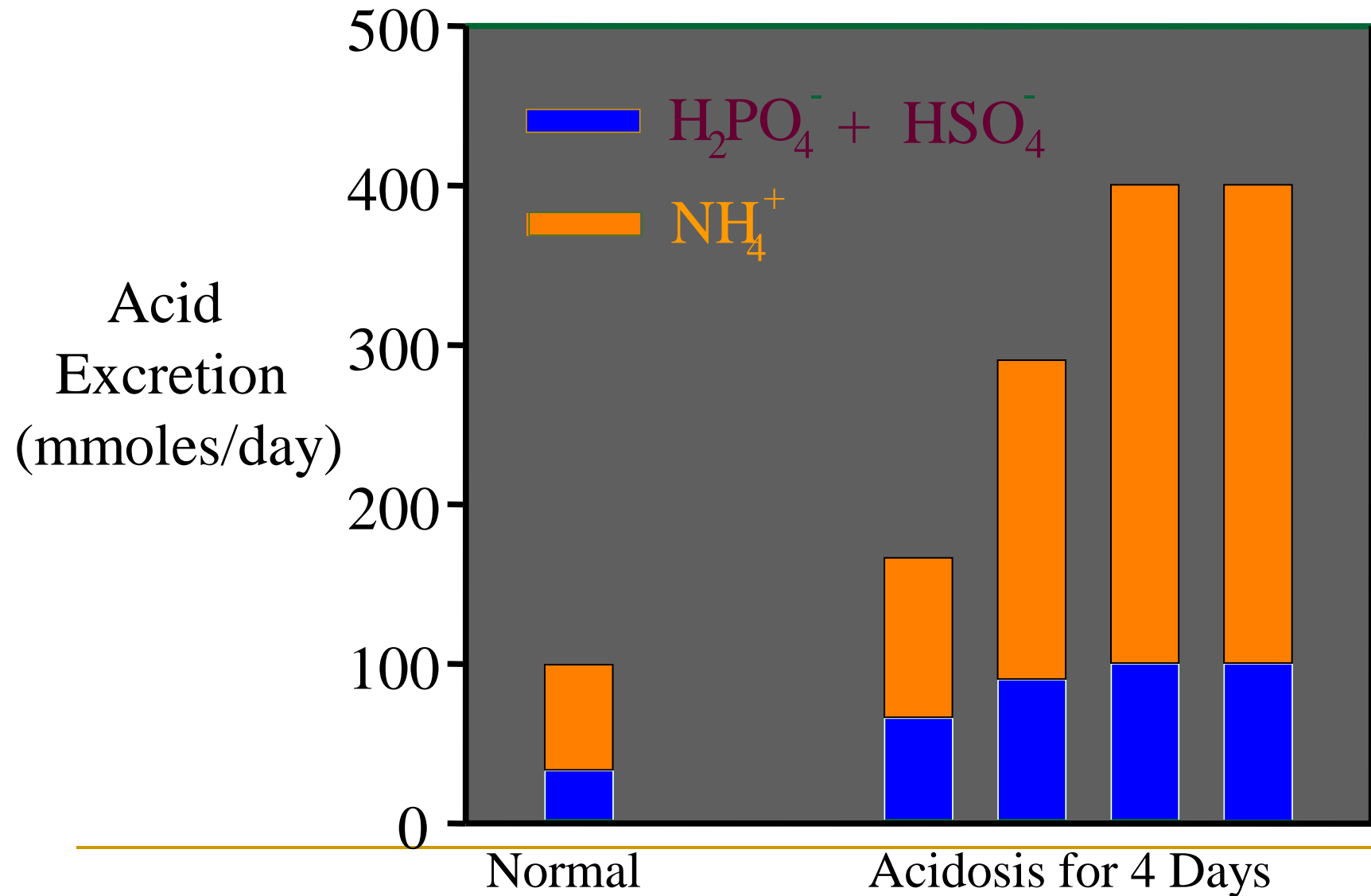
Regulation of H$^+$ secretion

- Increased pCO_2 increases H$^+$ secretion

  i.e. respiratory acidosis

- Increased extracellular H$^+$ increases H$^+$ secretion

  i.e. metabolic or respiratory acidosis

- Increased tubular fluid buffers increases H$^+$ secretion

  i.e. metabolic or respiratory acidosis

Renal Compensations for Acid-Base Disorders
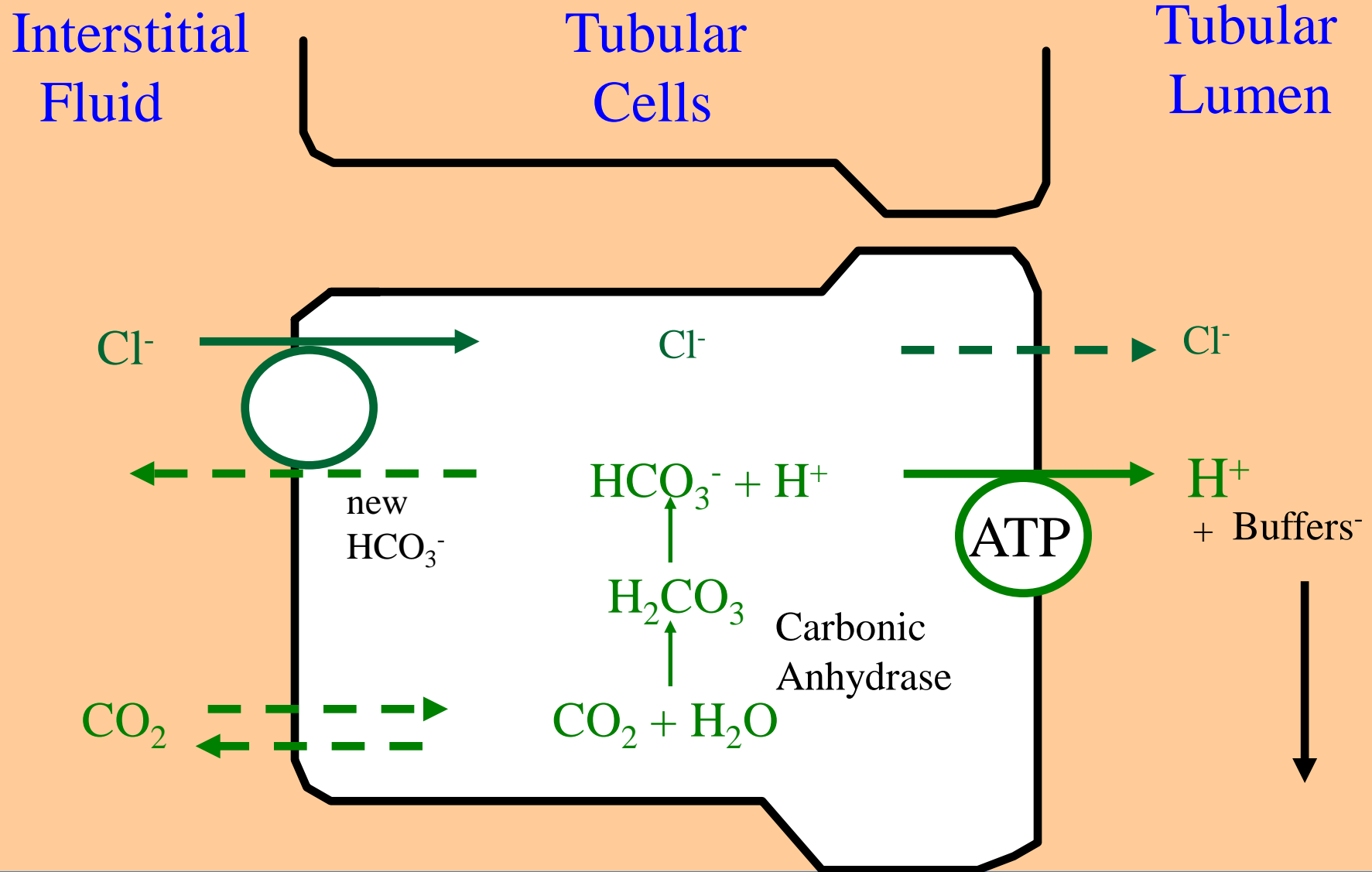
- Acidosis:

    - increased H^+ secretion

    - increased HCO_3^- reabsorption

    - production of new HCO_3^-

- Alkalosis:

    - decreased H^+ secretion

    - decreased HCO_3^- reabsorption

    - loss of HCO_3^- in urine

In acidosis all HCO_3^- is reabsorbed and excess H^+ in tubule is buffered

Interstitial Fluid

Tubular Cells

Tubular Lumen

Cl^- $\longrightarrow$ Cl^- $\dashrightarrow$ Cl^-

new HCO_3^- $\dashleftarrow$ $HCO_3^- + H^+$ $\longrightarrow$ H^+
+ Buffers$^-$

ATP

H_2CO_3 — Carbonic Anhydrase

CO_2 $\dashrightarrow$ $CO_2 + H_2O$

Importance of Renal Tubular Buffers

Minimum urine pH = 4.5
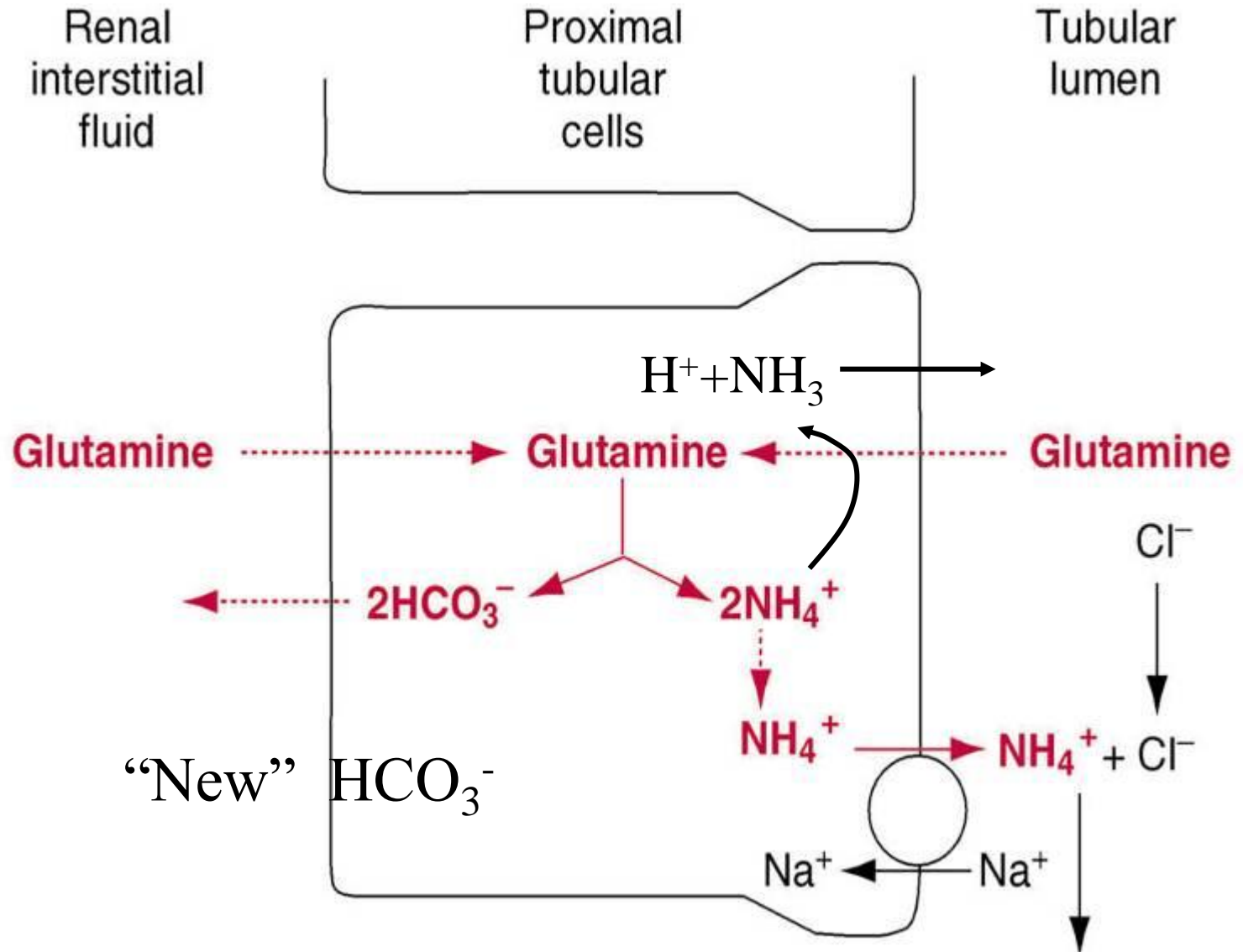
$$= 10^{-4.5}$$

$$= 3 \times 10^{-5} \text{ moles/L}$$

i.e. the maximal $[H^+]$ of urine is 0.03 mmol/L
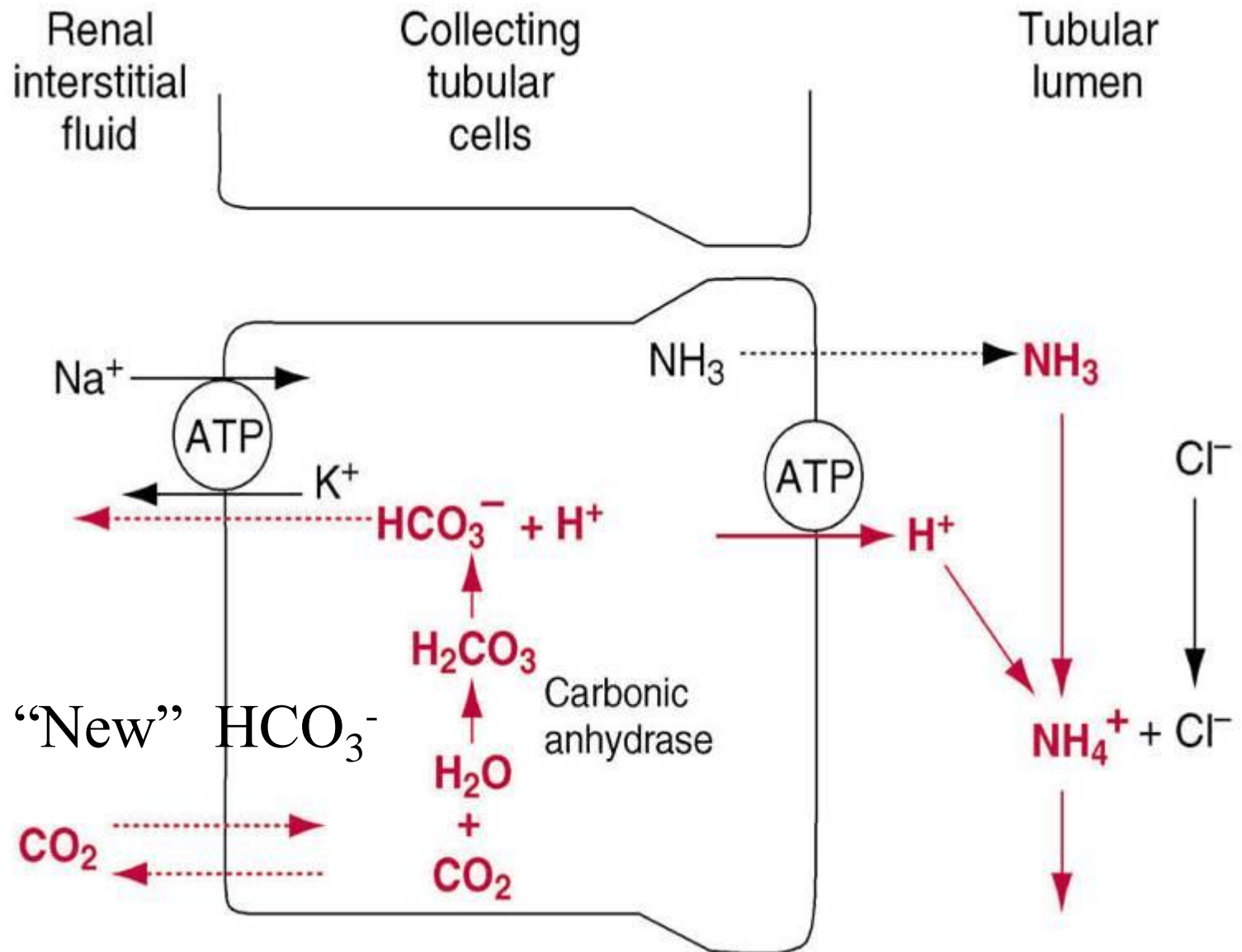
Yet, the kidneys must excrete, under normal conditions, at least 80 mmol non-volatile acids each day. To excrete this as free H^+ would require :

$$\frac{80 \text{ mmol}}{.03 \text{mmol/L}} \qquad > 2000 \text{ L per day !!!}$$

Production and secretion of NH_4^+ and HCO_3^- by proximal, thick loop of Henle, and distal tubules

Buffering of hydrogen ion secretion by ammonia (NH_3) in the collecting tubules.

Quantification of Normal Renal Acid-Base Regulation

Total H^+ secretion = 4380 mmol/day

$= HCO_3^-$ reabsorption (4320 mmol/d)

$+$ titratable acid ($NaHPO_4^-$) (30 mmol/d)

$+ NH_4^+$ excretion (30 mmol/d)

Titratable Acids: The # of mEq of NaOH needed to be added to the urine to bring its pH of the urine back to 7.4. This amount indicates how much H^+ is secreted in form of phosphate, citrate, urate. Titratable acids don't include the H^+ secreted as NH_4^+ because its pK is 9.2 and thus titration with NaOH does not remove H^+ combines with NH_4^+. Very little OH can bring pH to 7.4 without affecting NH_4^+ concentration

Net H^+ excretion = 59 mmol/day

$=$ titratable acid (30 mmol/d)

$+ NH_4^+$ excretion (30 mmol/d)

$- HCO_3^-$ excretion (1 mmol/d)

Normal Renal Acid-Base Regulation

Net addition of HCO_3^- to body
(i.e. net loss of H^+)

Titratable acid $\quad\quad\quad$ = 30 mmol/day
+ NH_4^+ excretion $\quad$ = 30 mmol/day
- HCO_3^- excretion $\quad$ = 1 mmol/day
Total $\quad\quad\quad\quad\quad\quad$ = 59 mmol/day

Renal Compensation for Acidosis

Increased addition of HCO_3^- to body by kidneys
(increased H^+ loss by kidneys)

Titratable acid   = 35 mmol/day (small increase)
NH_4^+ excretion   = 165 mmol/day (increased)
HCO_3^- excretion   = 0 mmol/day (decreased)
      Total   = 200 mmol/day

This can increase to as high as 500 mmol/day

Renal Compensation for Alkalosis

Net loss of HCO_3^- from body
(i.e. decreased H^+ loss by kidneys)

Titratable acid	=	0 mmol/day (decreased)
NH_4^+ excretion	=	0 mmol/day (decreased)
HCO_3^- excretion	=	80 mmol/day (increased)
Total	=	80 mmol/day

HCO_3^- excretion can increase markedly in alkalosis

Renal Responses to Respiratory Acidosis

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

Respiratory acidosis : $\downarrow$ pH $\uparrow$ pCO$_2$ $\uparrow$ HCO$_3^-$

$\uparrow$ PCO$_2$ $\longrightarrow$ $\uparrow$ H$^+$ secretion $\longrightarrow$ complete HCO$_3^-$ reabs.

$+$

excess tubular H$^+$

$\downarrow$ pH   Buffers (NH$_4^+$, NaHPO$_4^-$) $\longrightarrow$ $\downarrow$

$\ominus$

$\uparrow$ H$^+$ Buffers$^-$

$+$

$\uparrow$ new

HCO$_3^-$