بسم الله الرحمن الرحيم

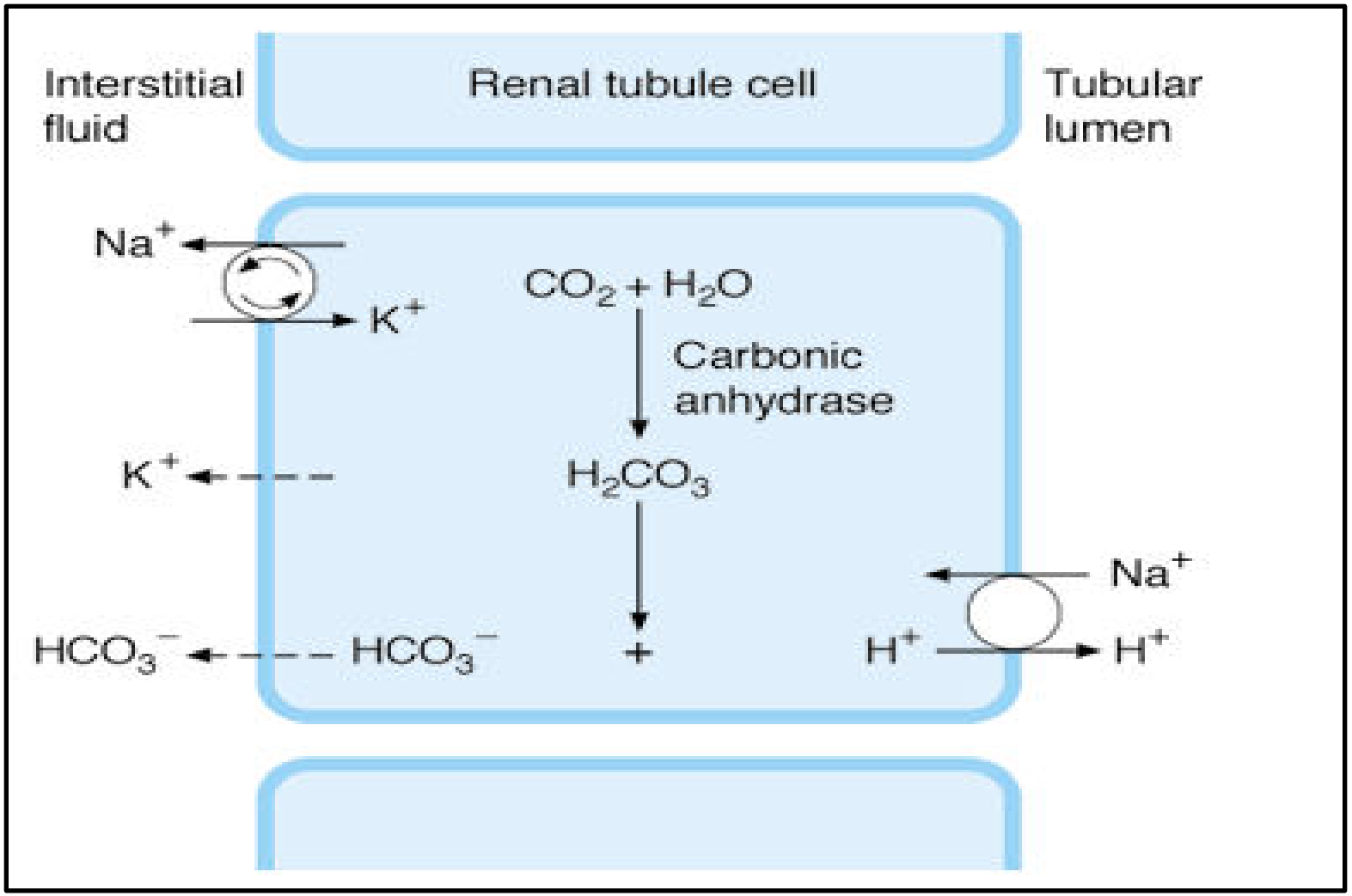
ACIDIFICATION OF THE URINE & BICARBONATE EXCRETION

H^+ Secretion

- The cells of the proximal and distal tubules, like the cells of the gastric glands, secrete hydrogen ions .
- Acidification also occurs in the collecting ducts.
- The reaction that is primarily responsible for H^+ secretion in the proximal tubules is ***Na^+-H^+ exchange*** .
- This is an example of secondary active transport; extrusion of Na^+ from the cells into the interstitium by ***Na^+-K^+ ATPase*** lowers intracellular Na^+, and this causes Na^+ to enter the cell from the tubular lumen, with coupled extrusion of H^+.

H^+ Secretion

- $^+$The H^+ comes from intracellular dissociation of H_2CO_3, and the HCO_3^- that is formed diffuses into the interstitial fluid.
- Thus, for each H^+ ion secreted, one Na^+ ion and one HCO_3^- ion enter the interstitial fluid.
- ***Carbonic anhydrase*** catalyzes the formation of H_2CO_3, and drugs that inhibit carbonic anhydrase depress both secretion of acid by the **proximal tubules** and the reactions which depend on it.



Secretion of acid by proximal tubular cells in the kidney. H^+ is transported into the tubular lumen by an antiport in exchange for Na^+. Active transport by Na^+-K^+ ATPase is indicated by arrows in the circle. Dashed arrows indicate diffusion.

H^+ Secretion

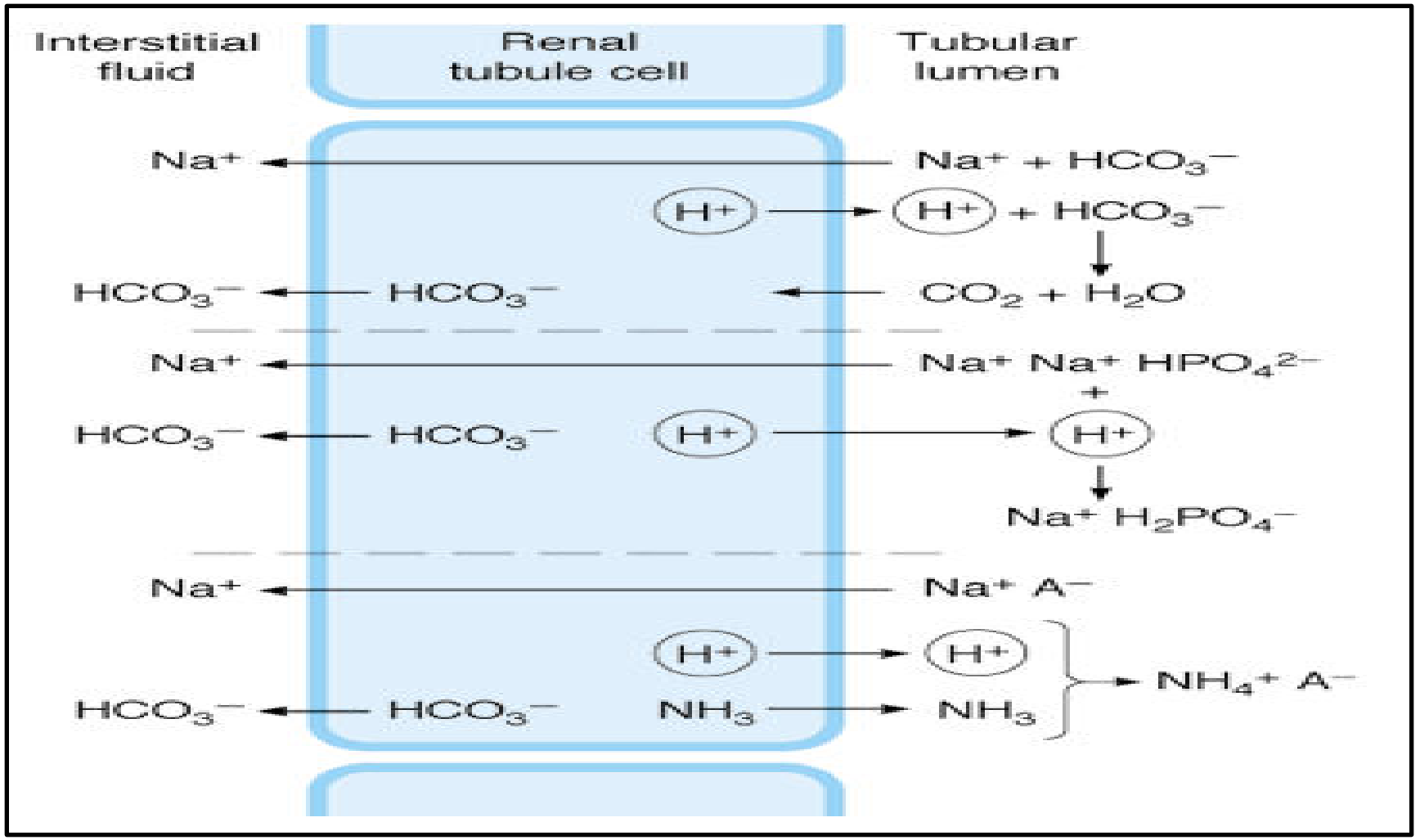
- This is in contrast to what occurs in the distal tubules and collecting ducts, where H^+ secretion is relatively independent of Na^+ in the tubular lumen.
- In this part of the tubule, most H^+ is secreted by an ATP-driven proton pump.

H^+ Secretion

- Aldosterone acts on this pump to increase distal H^+ secretion.
- The I cells in this part of the renal tubule secrete acid and, like the parietal cells in the stomach, contain abundant carbonic anhydrase and numerous tubulovesicular structures.
- Some of the H^+ is also secreted by H^+-K^+ ATPase.

Fate of H^+ in the Urine

- The amount of acid secreted depends upon the subsequent events in the tubular urine.
- The maximal H^+ gradient against which the transport mechanisms can secrete in humans corresponds to a urine pH of about 4.5, ie, an H^+ concentration in the urine that is 1000 times the concentration in plasma.
- pH 4.5 is thus the **limiting pH.**
- If there were no buffers that "tied up" H^+ in the urine, this pH would be reached rapidly, and H^+ secretion would stop.
- However, three important reactions in the tubular fluid remove free H^+, permitting more acid to be secreted.
- These are the reactions with HCO_3^- to form CO_2 and H_2O, with HPO_4^{2-} to form $H_2PO_4^-$, and with NH_3 to form NH_4^+.



- Fate of H^+ secreted into a tubule in exchange for Na^+. **Top:** Reabsorption of filtered bicarbonate via CO_2. **Middle:** Formation of monobasic phosphate. **Bottom:** Ammonium formation. Note that in each instance one Na^+ ion and one HCO_3^- ion enter the bloodstream for each H^+ ion secreted. A^-, anion.

Reaction With Buffers

- In the proximal tubule, most of the secreted H^+ reacts with HCO_3^- to form H_2CO_3 .
- The H_2CO_3 breaks down to form CO_2 and H_2O.
- In the proximal (but not in the distal) tubule, there is carbonic anhydrase in the brush border of the cells; this facilitates the formation of CO_2 and H_2O in the tubular fluid.
- The CO_2, which diffuses readily across all biological membranes, enters the tubular cells, where it adds to the pool of CO_2 available to form H_2CO_3.
- Since most of the H^+ is removed from the tubule, the pH of the fluid is changed very little.
- This is the mechanism by which HCO_3^- is reabsorbed; for each mole of HCO_3^- removed from the tubular fluid, 1 mole of HCO_3^- diffuses from the tubular cells into the blood,

Reaction With Buffers

- Secreted H^+ also reacts with dibasic phosphate (HPO_4^{2-}) to form monobasic phosphate ($H_2PO_4^-$).
- This happens to the greatest extent in the distal tubules and collecting ducts,
- The reaction with NH_3 occurs in the proximal and distal tubules.
- H^+ also combines to a minor degree with other buffer anions.

Ammonia Secretion

- Reactions in the renal tubular cells produce NH_4^+ and HCO_3^-.
- NH_4^+ is in equilibrium with $NH_3 + H^+$ in the cells.
- Since the pK´ of this reaction is 9.0, the ratio of NH_3 to NH_4^+ at pH 7.0 is 1:100 .
- However, NH_3 is lipid-soluble and diffuses across the cell membranes down its concentration gradient into the interstitial fluid and tubular urine.
- In the urine it reacts with H^+ to form NH_4^+, and the NH_4^+ remains in the urine.

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

$$pH = pK' + \log \frac{[NH_3]}{[NH_4^+]}$$

$$\text{Glutamine} \xrightarrow{\text{Glutaminase}} \text{Glutamate} + NH_4^+$$

$$\text{Glutamate} \xrightarrow[\text{dehydrogenase}]{\text{Glutamic}} \alpha\text{–Ketoglutarate} + NH_4^+$$

Subsequent metabolism of α-ketoglutarate utilizes $2H^+$, freeing $2HCO_3^-$.

Major reactions involved in ammonia production in the kidneys.

Ammonia Secretion

- The principal reaction producing NH_4^+ in cells is conversion of glutamine to glutamate.
- This reaction is catalyzed by the enzyme **glutaminase,** which is abundant in renal tubular cells .
- **Glutamic dehydrogenase** catalyzes the conversion of glutamate to α-ketoglutarate, with the production of more NH_4^+.
- Subsequent metabolism of α-ketoglutarate utilizes $2H^+$, freeing $2HCO_3^-$.

Factors Affecting Acid Secretion

- Renal acid secretion is altered by changes in the intracellular PCO_2, K^+ concentration, carbonic anhydrase level, and adrenocortical hormone concentration.
- When the PCO_2 is high (respiratory acidosis), more intracellular H_2CO_3 is available to buffer the hydroxyl ions and acid secretion is enhanced, whereas the reverse is true when the PCO_2 falls.
- K^+ depletion enhances acid secretion, apparently because the loss of K^+ causes intracellular acidosis even though the plasma pH may be elevated.
- Conversely, K^+ excess in the cells inhibits acid secretion.
- When carbonic anhydrase is inhibited, acid secretion is inhibited, because the formation of H_2CO_3 is decreased.
- Aldosterone and the other adrenocortical steroids that enhance tubular reabsorption of Na^+ also increase the secretion of H^+ and K^+.

Bicarbonate Excretion

- Although the process of HCO_3^- reabsorption does not actually involve transport of this ion into the tubular cells, ***HCO_3^- reabsorption is proportionate to the amount filtered over a relatively wide range.***

Bicarbonate Excretion

- When the plasma HCO_3^- concentration is low, all the filtered HCO_3^- is reabsorbed; but when the plasma HCO_3^- concentration is high, ie, above 26-28 meq/L (***the renal threshold for HCO_3^-***), HCO_3^- appears in the urine and the urine becomes alkaline.
- Conversely, when the plasma HCO_3^- falls below about 26 meq/L, the value at which all the secreted H^+ is being used to reabsorb HCO_3^-, more H^+ becomes available to combine with other buffer anions.
- Therefore, the lower the plasma HCO_3^- concentration drops, the more acidic the urine becomes and the greater its NH_4^+ content.

Implications of Urinary pH Changes

- Depending upon the rates of the interrelated processes of acid secretion, NH_4^+ production, and HCO_3^- excretion, the pH of the urine in humans varies from 4.5 to 8.0.
- Acids are buffered in the plasma and cells, the overall reaction being $HA + NaHCO_3 \rightarrow NaA + H_2CO_3$.
- The H_2CO_3 forms CO_2 and H_2O, and the CO_2 is expired, while the NaA appears in the glomerular filtrate.
- To the extent that the Na^+ is replaced by H^+ in the urine, Na^+ is conserved in the body.
- Furthermore, for each H^+ ion excreted with phosphate or as NH_4^+, there is a net gain of one HCO_3^- ion in the blood, replenishing the supply of this important buffer anion.

Implications of Urinary pH Changes

- Conversely, when base is added to the body fluids, the OH^- ions are buffered, raising the plasma HCO_3^-.
- When the plasma level exceeds 28 meq/L, the urine becomes alkaline and the extra HCO_3^- is excreted in the urine.
- Because the rate of maximal H^+ secretion by the tubules varies directly with the arterial PCO_2, HCO_3^- reabsorption also is affected by the PCO_2.

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Regulation of Extracellular Fluid Composition & Volume

DEFENSE OF H^+ CONCENTRATION

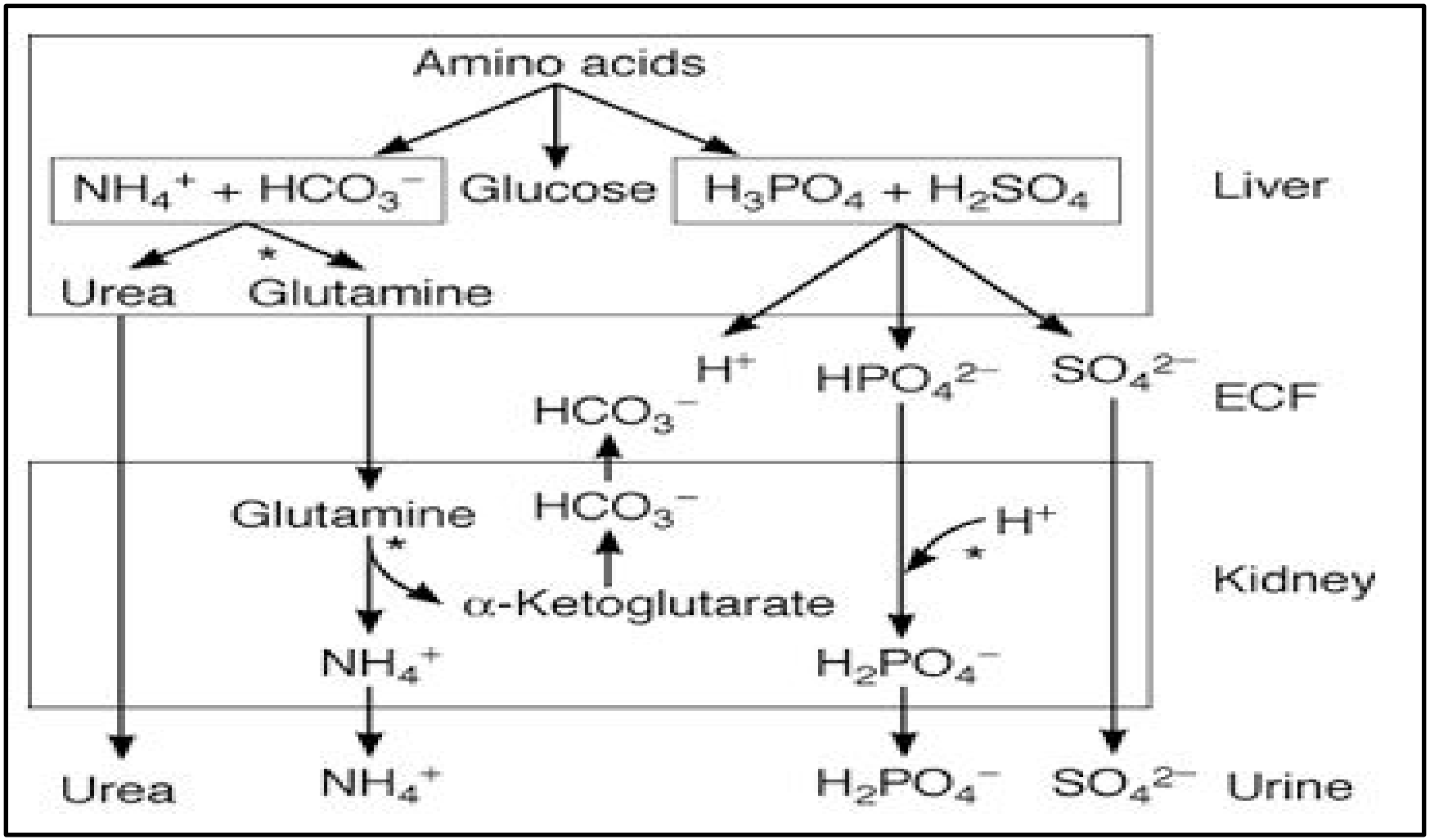
- The pH notation is a useful means of expressing H^+ concentrations in the body, because the H^+ concentrations happen to be low relative to those of other cations.
- Thus, the normal Na^+ concentration of arterial plasma that has been equilibrated with red blood cells is about 140 meq/L, whereas the H^+ concentration is 0.00004 meq/L .
- ***The pH, the negative logarithm of 0.00004, is therefore 7.4.***
- Of course, a decrease in pH of 1 unit, eg, from 7.0 to 6.0, represents a tenfold increase in H^+ concentration.
- It is important to remember that the pH of blood is the pH of **true plasma**—plasma that has been in equilibrium with red cells—because the red cells contain hemoglobin, which is quantitatively one of the most important blood buffers .

H^+ Balance

- The pH of the arterial plasma is normally 7.40 and that of venous plasma slightly lower.
- Technically, **acidosis** is present whenever the arterial pH is below 7.40, and **alkalosis** is present whenever it is above 7.40, although variations of up to 0.05 pH unit occur without untoward effects.

H^+ Balance

- Amino acids are utilized in the liver for gluconeogenesis, leaving as products NH_4^+ and HCO_3^- from their amino and carboxyl groups .
- The NH_4^+ is incorporated into urea and the protons that are formed are buffered intracellularly by HCO_3^-, so little NH_4^+ and HCO_3^- escape into the circulation.
- However, metabolism of sulfur-containing amino acids produces H_2SO_4, and metabolism of phosphorylated amino acids such as phosphoserine produces H_3PO_4.
- These strong acids enter the circulation and present a major H^+ load to the buffers in the ECF.



- Role of the liver and kidneys in the handling of metabolically produced acid loads. Sites where regulation occurs are indicated by asterisks.

H^+ Balance

- The H^+ load from amino acid metabolism is normally about 50 meq/d.
- The CO_2 formed by metabolism in the tissues is in large part hydrated to H_2CO_3 , and the total H^+ load from this source is over 12,500 meq/d.
- However, most of the CO_2 is excreted in the lungs, and only small quantities of the H^+ remain to be excreted by the kidneys.

H^+ Balance

- Common sources of extra acid loads are strenuous exercise (lactic acid), diabetic ketosis (***acetoacetic acid and β-hydroxybutyric acid***), and ingestion of acidifying salts such as NH_4Cl and $CaCl_2$, which in effect add HCl to the body.
- Failure of diseased kidneys to excrete normal amounts of acid is also a cause of acidosis.
- Fruits are the main dietary source of alkali.
- but a more common cause of alkalosis is loss of acid from the body as a result of vomiting of gastric juice rich in HCl. This is, of course, equivalent to adding alkali to the body.

Buffering

- Intracellular and extracellular pH are generally maintained at very constant levels.
- For example, the pH of the ECF is 7.40, and in health, this value usually varies less than ±0.05 pH unit.
- Body pH is stabilized by the **buffering capacity** of the body fluids.
- A buffer is a substance that has the ability to bind or release H^+ in solution, thus keeping the pH of the solution relatively constant despite the addition of considerable quantities of acid or base.

Buffering

- One buffer in the body is carbonic acid. This acid is only partly dissociated into H^+ and bicarbonate:

  $H_2CO_3 \leftrightarrow\rightarrow H^+ + HCO_3^-$.
- If H^+ is added to a solution of carbonic acid, the equilibrium shifts to the left and most of the added H^+ is removed from solution.
- If OH^- is added, H^+ and OH^- combine, taking H^+ out of solution. However, the decrease is countered by more dissociation of H_2CO_3, and the decline in H^+ concentration is minimized.
- Other buffers include the blood proteins and the proteins in cells .

The Henderson-Hasselbalch Equation

- The general equation for a buffer system is
- $HA \leftrightarrow H^+ + A^-$

  A^- represents any anion and HA the undissociated acid.
- If an acid stronger than HA is added to a solution containing this system, the equilibrium is shifted to the left.
- Hydrogen ions are "tied up" in the formation of more undissociated HA, so the increase in H^+ concentration is much less than it would otherwise be.
- Conversely, if a base is added to the solution, H^+ and OH^- react to form H_2O; but more HA dissociates, limiting the decrease in H^+ concentration.

The Henderson-Hasselbalch Equation

- By the law of ***mass action***, the product of the concentrations of the products in a chemical reaction divided by the product of the concentration of the reactants at equilibrium is a constant:

$$[H]\,[A^-]/[HA] = K$$

The Henderson-Hasselbalch Equation

- If this equation is solved for H^+ and put in pH notation (pH is the negative log of $[H^+]$), the resulting equation is that originally derived by Henderson and Hasselbalch to describe the pH changes resulting from addition of H^+ or OH^- to any buffer system **(Henderson-Hasselbalch equation):**

  PH = PK + log [A-] / [AH]

The Henderson-Hasselbalch Equation

- It is apparent from these equations that the buffering capacity of a system is greatest when the amount of free anion is equal to the amount of undissociated HA, ie, when $[A^-]/[HA] = 1$, so that log $[A^-]/[HA] = 0$ and pH = pK.
- This is why the most effective buffers in the body would be expected to be those with pKs close to the pH in which they operate.

DEFENSE OF TONICITY

- The defense of the tonicity of the ECF is primarily the function of the vasopressin-secreting and thirst mechanisms.
- The total body osmolality is directly proportionate to the ***total body sodium plus the total body potassium divided by the total body water***, so that changes in the osmolality of the body fluids occur when there is a disproportion between the amount of these electrolytes and the amount of water ingested or lost from the body .
- When the effective osmotic pressure of the plasma rises, vasopressin secretion is increased and the thirst mechanism is stimulated.

DEFENSE OF TONICITY

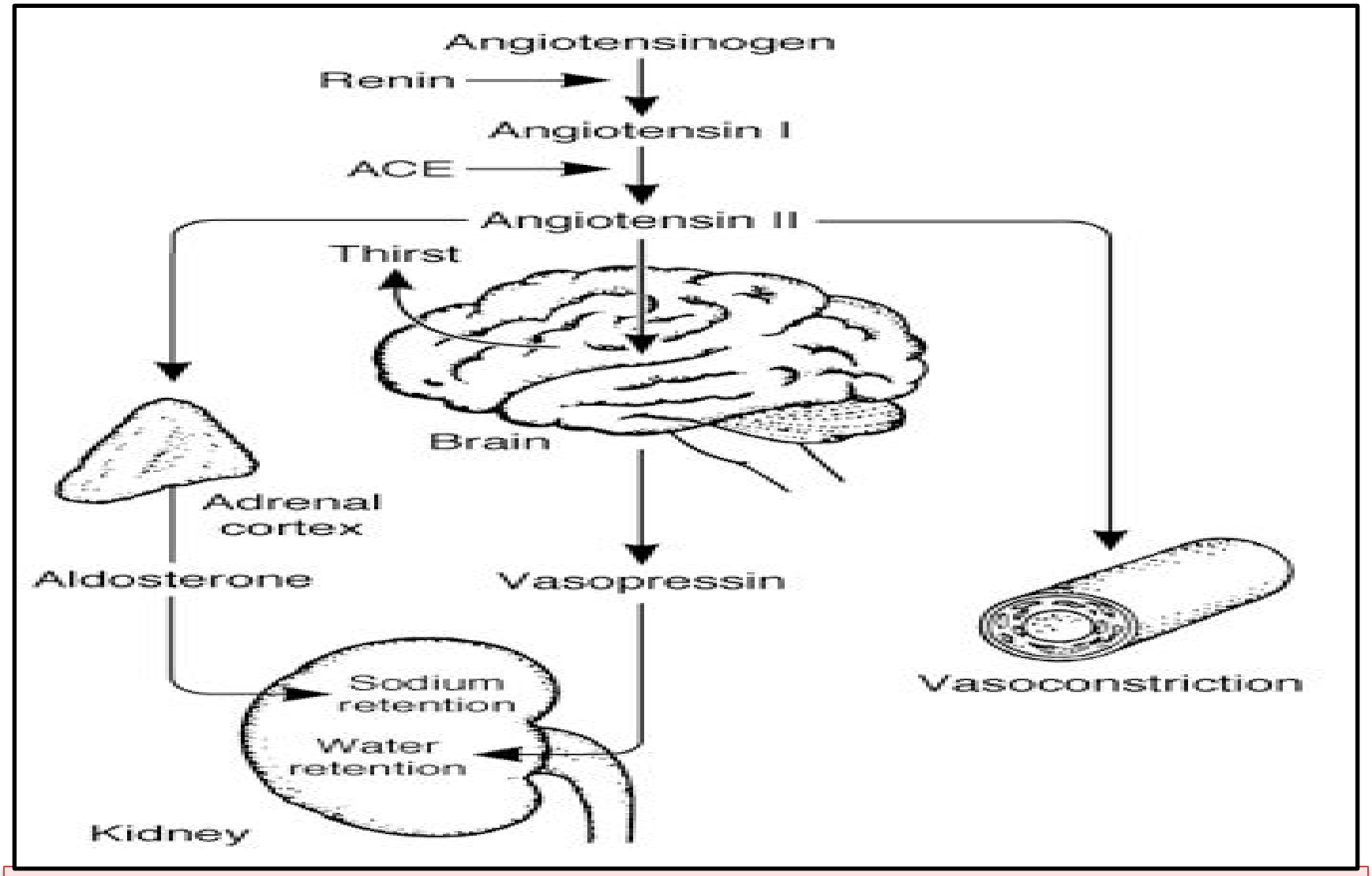
- Conversely, when the plasma becomes hypotonic, vasopressin secretion is decreased and "solute-free water" (water in excess of solute) is excreted.
- In this way, the tonicity of the body fluids is maintained within a narrow normal range.
- In health, plasma osmolality ranges from 280 to 295 mosm/kg of H_2O, with vasopressin secretion maximally inhibited at 285 mosm/kg and stimulated at higher values .

DEFENSE OF VOLUME

- The volume of the ECF is determined primarily by the total amount of osmotically active solute in the ECF.
- Since Na^+ and Cl^- are by far the most abundant osmotically active solutes in ECF, and since changes in Cl^- are to a great extent secondary to changes in Na^+, the amount of Na^+ in the ECF is the most important determinant of ECF volume.
- Therefore, the mechanisms that control Na^+ balance are the major mechanisms defending ECF volume.
- There is, however, a volume control of water excretion as well; a rise in ECF volume inhibits vasopressin secretion, and a decline in ECF volume produces an increase in the secretion of this hormone.
- Volume stimuli override the osmotic regulation of vasopressin secretion.

DEFENSE OF VOLUME

- Angiotensin II stimulates aldosterone and vasopressin secretion.
- It also causes thirst and constricts blood vessels, which help to maintain blood pressure.
- Thus, angiotensin II plays a key role in the body's response to hypovolemia .
- In addition, expansion of the ECF volume increases the secretion of ANP and BNP by the heart, and this causes natriuresis and diuresis .



- Defense of ECF volume by angiotensin II. ACE, angiotensin-converting enzyme.