CHAPTER

7 Renal Acid–Base Transport

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ACID–BASE HOMEOSTASIS

The body maintains systemic acid–base homeostasis (a pH in the range of 7.35 to 7.45) in two ways: (1) through chemical buffering in extracellular fluid (ECF) and intracellular fluid (ICF), and (2) through physiologic regulation controlled by the metabolic, renal, and respiratory systems. The central nervous and respiratory systems control CO_2 tension (PCO_2), and the kidneys regulate the plasma HCO_3^- concentration. Buffers in the ECF and ICF guard against acid and base retention. These processes serve to dispose of carbonic and nonvolatile acids on a daily basis and pathologic quantities of acid and alkali as needed. This chapter briefly reviews the role of chemical buffers, but focuses primarily on the renal transport and regulatory processes that maintain acid–base balance.

Buffer Systems

The body's buffer systems keep the blood from becoming too basic or too acidic by combining with or releasing H^+, and thus are comprised of a base (i.e., an H^+ acceptor) and an acid (i.e., an H^+ donor). The most prodigious base is bicarbonate (HCO_3^-) and the most common acid is carbonic acid (H_2CO_3). Because buffer systems attenuate system changes, acid or base loads in the presence of a buffer cause smaller changes in the pH than if the buffer were absent. During homeostasis, the extracellular H^+ concentration ($[H^+]_e$) is constant. H^+ concentration can be expressed either directly as $[H^+]$ or indirectly as pH.

When CO_2 is dissolved in water, H_2CO_3 is formed according to the reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \quad (1)$$

The rate of this reaction, in the absence of the enzyme carbonic anhydrase, is slow, with a half-life of about 8 seconds at 37°C. The importance of carbonic anhydrase in bicarbonate equilibrium in the kidneys and lungs are discussed in the following paragraphs. The major portion of CO_2 remains as dissolved CO_2; only about 1 part in 1,000 forms H_2CO_3, a nonvolatile acid. Because H_2CO_3 is a weak acid, it dissociates rapidly to yield H^+ and HCO_3^-.

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad (2)$$

Because the concentration of H_2CO_3 remains low and proportional to the concentration of dissolved CO_2, Eqs 1 and 2 can be combined and treated as a single reaction:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (3)$$

The equilibrium constant for this reaction is given by

$$K = \frac{[H^+]\,[HCO_3^-]}{[CO_2]\,[H_2O]} \quad (4)$$

Defining $K' = K[H_2O]$ as the apparent equilibrium constant and using Eq. 4,

$$K' = \frac{[H^+]\,[HCO_3^-]}{\alpha_{co2}\,PCO_2} \quad (5)$$

Taking logarithms of both sides of Eq. 5 and recognizing that $pK' = \log_{10}(K')$, the familiar Henderson-Hasselbalch equation is derived:

$$pH = \frac{pK' + \log_{10}[HCO_3^-]}{(\alpha_{co2}Pco_2)} \quad (6)$$

Using $pK' = 6.1$ in Eq. 6, the Henderson equation is derived, which may be used in the clinical interpretation of acid–base data:

$$[H^+](nmol/L) = \frac{24\ PCO_2\ (mm\ Hg)}{[HCO_3^-]\ (mM)} \quad (7)$$

Physiologic Processes that Protect the Plasma Bicarbonate

Three physiologic processes mitigate changes in the HCO_3^-/CO_2 ratio: (1) metabolic regulation, (2) respiratory regulation, and (3) renal regulation. Metabolic regulation is

of minor importance in terms of the overall physiologic regulation of acid–base balance. Some enzymes are regulated by changes in blood pH. For example, the activity of phosphofructokinase, the pivotal enzyme in the glycolytic pathway, is inhibited by low pH and enhanced by high pH.

Because, under most circumstances, respiratory CO_2 excretion and CO_2 production are matched, the usual steady state arterial PCO_2 ($PaCO_2$) is maintained at 40 mm Hg. Underexcretion of CO_2 produces hypercapnia, and overexcretion produces hypocapnia. Production and excretion are again matched but at a new steady state PCO_2. Therefore, $PaCO_2$ is regulated primarily by neurorespiratory factors and is not subject to regulation by the rate of metabolic CO_2 production. Hypercapnia is primarily the result of hypoventilation, not increased CO_2 production. Increases or decreases in PCO_2 represent derangements of control of neurorespiratory regulation or can result from compensatory changes in response to a primary alteration in the plasma HCO_3^- concentration.

Sources of Endogenous Acids

Pathologically, acid loads may be derived from endogenous acid production (e.g., generation of ketoacids and lactic acids) or loss of base (e.g., through diarrhea) or from exogenous sources (e.g., ammonium chloride or toxin ingestion). Under normal physiologic circumstances, a daily input of acid derived from the diet and metabolism confronts the body. The net result of these processes amounts to about 1 mEq of new H^+ per kilogram per day entering the ECF.[1–4]

Sulfuric acid is formed when organic sulfur from methionine and cysteine residues of proteins are oxidized to SO_4^{2-}. The metabolism of sulfur-containing amino acids is the primary source of acid in the usual Western diet, accounting for approximately 50%. The quantity of sulfuric acid generated is equal to the SO_4^{2-} excreted in the urine.

Organic acids are derived from intermediary metabolites formed by the partial combustion of dietary carbohydrates, fats, and proteins as well as from nucleic acids (uric acid). Organic acid generation contributes to net endogenous acid production when the conjugate bases are excreted in the urine as organic anions. If full oxidation of these acids can occur, however, H^+ is reclaimed and is eliminated as CO_2 and water. The net amount of H^+ added to the body from this source can be estimated by the amount of organic anions excreted in the urine.

Phosphoric acid can be derived from hydrolysis of PO_4^{3-} esters in proteins and nucleic acids if it is not neutralized by mineral cations (e.g., Na^+, K^+, Mg^{2+}). The contribution of dietary phosphate to acid production is dependent on the kind of protein ingested. Some proteins generate phosphoric acid, whereas others generate only neutral phosphate salts.[1–4] Hydrochloric acid is generated by the metabolism of cationic amino acids (lysine, arginine, and some histidine residues) into neutral products. Other potential acid or base sources in the diet can be estimated from the amount of unidentified cations and anions ingested.

Potential sources of bases are also found in the diet (e.g., acetate, lactate, citrate) and can be absorbed to neutralize the acid loads from the three sources just mentioned. The net base absorbed by the gastrointestinal tract is derived from the anion gap (AG) of the diet minus that of the stool. Acid production is partially offset by HCO_3^-, which is produced when organic anions combine with H^+ and are oxidized to CO_2 and H_2O or when dibasic phosphoesters combine with H^+ during hydrolysis. The gastrointestinal tract may modify the amount of these potential bases reabsorbed under particular circumstances of acidosis or growth. It has been confirmed in patients ingesting an artificial diet that urinary net acid excretion is equal to urinary [(SO_4^{2-}) + organic A^- + dietary phosphoester-derived H^+].[1,2,5] Therefore, in summary, the metabolism of certain proteins, nucleic acids, and small fractions of lipids and certain carbohydrates generate specific organic acids that cannot be burned to CO_2 (e.g., uric, oxalic, glucuronic, hippuric acids). In addition, the inorganic acids H_2SO_4 and H_3PO_4, derived respectively from sulfur-containing amino acids and organophosphates, are excreted by the kidneys or the gastrointestinal tract.

Impact of Daily Metabolism on Acid–Base Balance

Human subjects ingesting a typical Western diet are confronted, under most physiologic circumstances, with an acid challenge. Metabolism generates a daily load of relatively strong acids (lactate, citrate, acetate, and pyruvate), but under physiologic circumstances, in the steady state, metabolic production and consumption are matched. However, if production and consumption rates become mismatched, organic acids can accumulate (e.g., lactic acid accumulation with anoxia or ischemia). These acids are buffered by HCO_3^- in the ECF, causing a decline in plasma HCO_3^- concentration as the organic acid concentration increases. During recovery, these organic acids reenter the metabolic pathways to CO_2 production, the removal of H^+, and the generation of HCO_3^- unless the organic anions are excreted (e.g., ketonuria), and thereby are no longer available for regeneration of HCO_3^-.

Both metabolic and renal regulatory mechanisms protect a normal HCO_3^- concentration in blood (25 mEq per liter) despite the daily addition of acid (or alkali) to the ECF. Although the buffering capacity of the body is magnified severalfold by respiratory adjustments in $PaCO_2$, primary changes in $PaCO_2$ may result in acidosis or alkalosis, depending on whether CO_2 is elevated above or depressed below the normal value: 40 mm Hg (*respiratory acidosis* and *respiratory alkalosis*), respectively. A primary change in the plasma HCO_3^- concentration as a result of metabolic production or the retention or excretion of an acid or base by the kidney evokes a ventilator response because a decrease or increase in pH is sensed by chemoreceptors in the circulation and signals the respiratory center to increase or decrease minute ventilation. The respiratory response to acidemia (increase ventilation

and decrease PCO_2) or alkalemia (decrease in ventilation and increase in PCO_2) thereby blunts the change in blood pH that would occur otherwise. Such respiratory alterations that adjust blood pH toward normal are referred to as *secondary* or *compensatory* alterations, because they occur in response to primary metabolic changes. While helpful, respiratory compensation to metabolic acidosis or alkalosis is never sufficient to return blood pH to normal. Therefore, the kidneys must play a very significant role in adjustments to metabolic disturbances by altering bicarbonate reabsorption and net acid excretion.

RENAL PARTICIPATION IN ACID–BASE HOMEOSTASIS

Although temporary relief from changes in the pH of body fluids may be derived from chemical buffering or respiratory compensation, the ultimate defense against the addition of nonvolatile acid or of alkali resides in the kidneys. The addition of a strong acid (e.g., HA) to the ECF titrates plasma HCO_3^-:

$$HA + NaHCO_3 \rightarrow NaA + H_2O + CO_2 \quad (8)$$

The CO_2 is expired by the lungs, and body HCO_3^- declines. This process occurs constantly as endogenous metabolic acids are generated. To maintain a normal plasma HC O_3^- in the face of the constant accession of metabolic acids, the kidneys must (1) conserve the HCO_3^- present in the filtered load (through reclamation), and (2) regenerate the HC O_3^- decomposed by reaction with metabolic acids (Eq. 8).

The first function of the kidneys in maintaining acid–base homeostasis is to reclaim or reabsorb filtered HCO_3^-. The kidneys filter approximately 4,000 mEq of bicarbonate daily (defined as the product of the glomerular filtration rate [GFR] and the plasma HCO_3^- concentration), and the excretion of any significant portion of this leads to metabolic acidosis. The renal tubules essentially reabsorb all of the filtered HCO_3^-, or may excrete excessive HCO_3^- when needed, to maintain the normal plasma HCO_3^- concentration of 25 mEq per liter.

HCO_3^- reclamation is accomplished principally in the proximal tubule. There is a subsequent contribution by the loop of Henle and a minor contribution by more distal nephron segments. Under most circumstances, the filtered load of HCO_3^- is absorbed almost completely, especially during an acid load. Nevertheless, when less acid is generated or if the plasma HCO_3^- concentration increases above the normal value of 25 mEq per liter, HCO_3^- will be excreted efficiently into the urine.

The generation of acid in the body by metabolism is referred to as *net acid production*. Because a typical Western diet generates fixed acids at 50 to 70 mEq per day, net acid excretion must be affected to maintain acid–base balance. Therefore, net acid excretion approximates 50 to 70 mEq per day. If acid production remained stable and unabated by net acid excretion, metabolic acidosis would ensue. Conversely, an increase in net acid excretion above the level of net acid production results in metabolic alkalosis. Each milliequivalent of net acid excreted corresponds to 1 mEq of HCO_3^- returned to the ECF. This process is called HCO_3^- regeneration and is necessary for replacing the HCO_3^- lost by the entry of fixed acids into the ECF or, less commonly, replacing the HCO_3^- excreted in stool or urine.

To prevent metabolic acidosis, this excreted acid must be recovered. The kidney recovers this acid through the second process, HCO_3^- regeneration, which is represented by the renal output of acid or **net acid excretion (NAE)**, or the sum of ammonia (NH_3)/ammonium (NH_4^+) plus excreted titratable acids (TA) minus any bicarbonate excreted.

$$\text{Net acid excretion (NAE)} = (U_{NH_4^+}V) + (U_{TA}V) - (U_{HCO_3^-}V) \quad (9)$$

where V is urinary volume per 24 hours and U refers to the concentration of the moiety in the urine.

TA excretion in the urine is represented by buffers that are filtered at the glomerulus and then titrated to acid moieties in the tubule lumen. Because renal H^+-secretory mechanisms cannot generate such steep pH gradients between the cell (pH ~7.3) and lumen, excretion of 70 mEq of acid per day requires that most of this acid be buffered in the luminal fluid.

Buffers with a *pK* between 5.0 and 7.4, the pH range of the luminal fluid, are most effective chemically. In this regard, the most abundant and effective buffer in the urine is the phosphate buffer pair, $H_2PO_4^- - HPO_4^{2-}$, with a p*K* of 6.9. The excretion of phosphate, the major titratable buffer, is usually regulated in accordance with phosphate homeostasis. Phosphate excretion increases modestly in metabolic acidosis, but the magnitude of the increase is limited by the filtered load of phosphate and, therefore, is significantly regulatable. In general, the buffers responsible are the $NH_3 - NH_4^+$ and titratable buffer, such as $HPO_4^{2-} - H_2PO_4^-$, systems. Physiologically, the most significant of these is the NH_3–NH_4^+ system.

Ammoniagenesis

Ammoniagenesis by protein catabolism is balanced by the generation of new HCO_3^- through renal NH_4^+ and TA excretion (Fig. 7.1A). The products of these neutral reactions are HCO_3^- and NH_4^+. Most ammoniagenesis occurs in the proximal tubule. NH_4^+ produced by the metabolism of amino acids reacts with HCO_3^- or forms urea and thus has no impact on acid–base balance. A portion of this NH_4^+ is diverted to glutamine synthesis, the amount of which is regulated by pH. Acidemia promotes and alkalemia inhibits glutamine synthesis. Each glutamine molecule metabolized produces two NH_4^+ and two HCO_3^- molecules (Fig. 7.1A) (water, CO_2, and glucose are also formed). This breakdown occurs as follows: glutamine is transported into the proximal

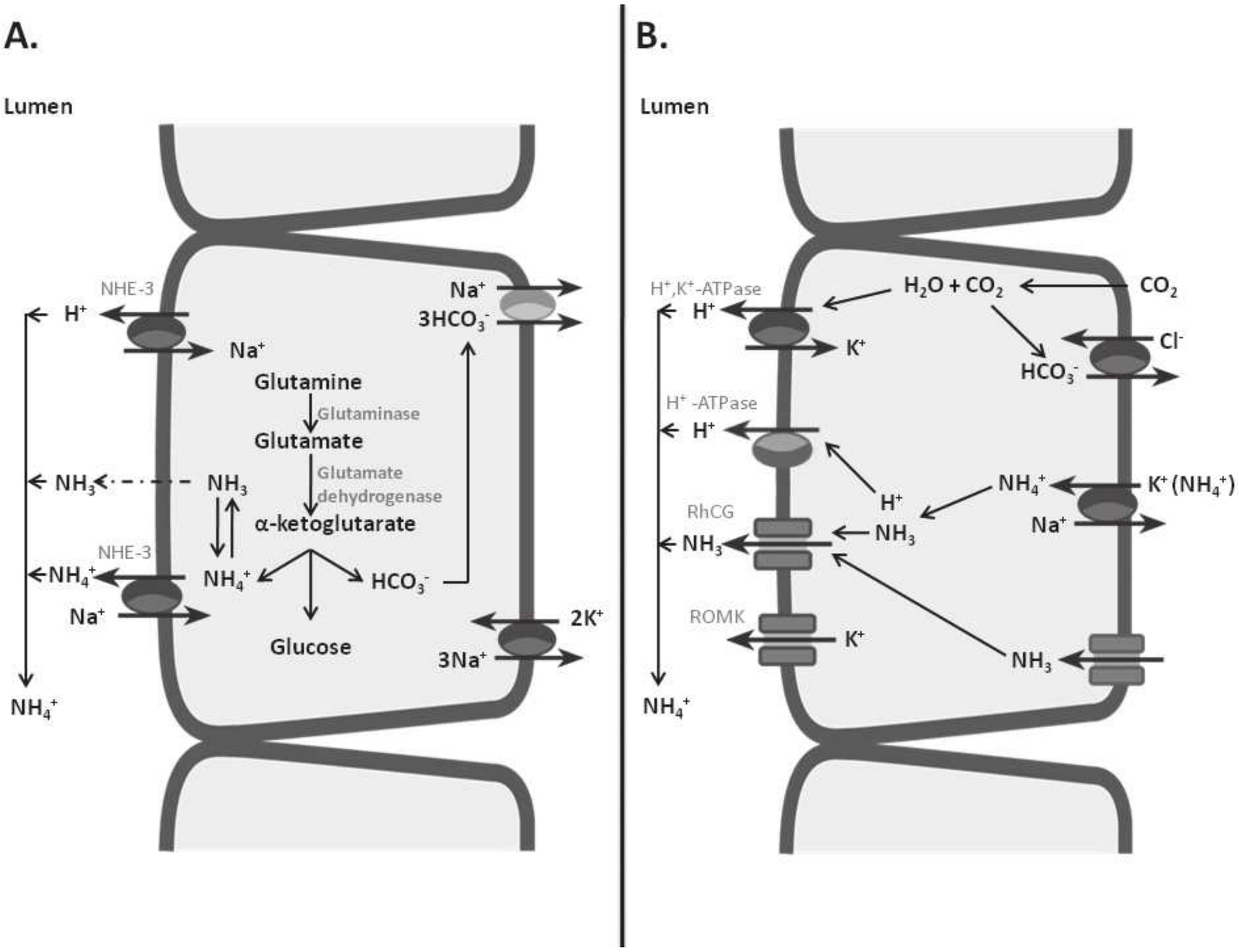

FIGURE 7.1 Cell models of ammonia synthesis and excretion pathways. **A:** A proximal convoluted tubule. Ammonia is derived from glutamine precursors to produce 2 NH_4^+ and 2 HCO_3^- molecules through an enzymatic pathway activated by acidemia and hypokalemia and inhibited by alkalemia and hyperkalemia. **B:** A type A intercalated cell in the collecting tubule. Ammonium enters across the basolateral membrane through the substitution of K^+ for NH_4^+ in K^+ conductance and is secreted across the apical membrane via renal outer medullary potassium (ROMK) or RhCG (see text). In both **A** and **B**, NH_3 diffusion coupled with H^+ secretion traps NH_4^+ in the tubule lumen.

tubule across the apical and basolateral membranes and is then transported into the mitochondria. In the mitochondria, an NH_4^+ molecule is formed when glutamine is deaminated by glutaminase to glutamate. When glutamate is then deaminated to α-ketoglutarate, a second NH_4^+ molecule is formed. Finally, the α-ketoglutarate molecule is metabolized in the Krebs cycle to CO_2, H_2O, glucose, and the two HCO_3^- molecules. Glutamine deamination in the kidney is highly regulated by systemic pH, so that acidemia augments and alkalemia inhibits NH_4^+ and HCO_3^- production. Hepatic regulation of NH_4^+ metabolic pathways appears to facilitate glutamine production when NH_4^+ excretion is stimulated by acidemia or, conversely, blunts glutamine production when excretion is inhibited by alkalemia.[5]

Renal Excretion of Ammonia/Ammonium

The ultimate control, however, resides in the renal excretion of NH_4^+, because the NH_4^+ must be excreted to escape entry into the hepatic urea synthetic pool. Hepatic urea synthesis would negate the new HCO_3^- realized from α-ketoglutarate in the kidney. Thus, ammoniagenesis does not contribute to acid–base homeostasis until the urine is acidified and the HCO_3^- produced by glutamine metabolism is added to the ECF. In order for this to occur, the ammonia synthesized in the proximal tubule has to be transported to the terminal nephron segments and is then excreted by the kidney (the pathway is described in detail in the following paragraphs). This ammonia transport occurs through both nonionic diffusion (for NH_3) and ionic transport (for NH_4^+). NH_3 quickly and easily diffuses across the plasma membrane to compartments of lower pH, where it is rapidly converted to NH_4^+, thus leading to accumulated NH_4^+ in acidic compartments. NH_4^+ can diffuse across tight junctions or can substitute on transporters such as the apical membrane Na^+–H^+ (NHE-3) antiporter (as Na^+–NH_4^+ exchange).[6] In general, NH_3 has a higher membrane permeability than NH_4^+, but the concentration of NH_4^+ exceeds that of NH_3 by approximately 100-fold at a pH 7.4 (pK 9.3). Thus, the relative

permeability of the two moieties defines whether either crosses membranes by nonionic NH_3 diffusion or ionic NH_4^+ transport; for example, if the NH_4^+ moiety is transported in a specific nephron segment as in the thick ascending limb of the loop of Henle by substitution for potassium on the Na^+–K^+–$2Cl^-$ cotransporter.

The first step of ammonia transport and urinary excretion following ammoniagenesis in the proximal tubule is for the ammonia to enter the proximal tubule lumen, and it can do so in two ways. Because the luminal pH is lower than cell pH, much of the NH_3 is diffused into the tubule fluid and is converted into NH_4^+. The apical membrane Na^+-H^+ antiporter also plays an important role in NH_3–NH_4^+ transport (Fig. 7.1A).[7,8] Transport mechanisms for NH_3–NH_4^+ in more distal segments function to ensure that the NH_3– NH_4^+ issuing out of the proximal tubule fluid is excreted into the urine in a regulated fashion.

From the lumen, ammonia enters the medullary interstitium (Fig. 7.2) via the thick ascending limb of the loop of Henle. If ammonia did not enter the interstitium, it would return to the distal nephron and diffuse back into the blood, thereby failing to be excreted in the urine. The thick ascending limb of the loop of Henle also creates a "corticomedullary concentration gradient" for NH_3–NH_4^+. The NH_3–NH_4^+ transported into the medullary interstitium accumulates at a higher concentration by countercurrent multiplication and can reenter the collecting tubule for a final excretion in a more regulated way. Nonionic diffusion of NH_3 into the medullary interstitium is not possible because the thick ascending limb's apical membrane is highly impermeable to NH_3.[9] NaCl transport enables the ionic transport of NH_4^+ into the interstitium. NH_4^+ can substitute for K^+ on the Na^+–K^+–$2Cl^-$ cotransporter, or it can be transported via the renal outer medullary potassium (ROMK) channel for transport across the apical membrane (Fig. 7.1B). Alternatively, lumen-positive voltage can drive NH_4^+ diffusion across the tight junction. NH_4^+ that enters the thick ascending limb cell can exit the basolateral membrane by nonionic diffusion driven by cell-to-interstitial NH_3 concentration gradient. Once in the interstitium, the NH_3

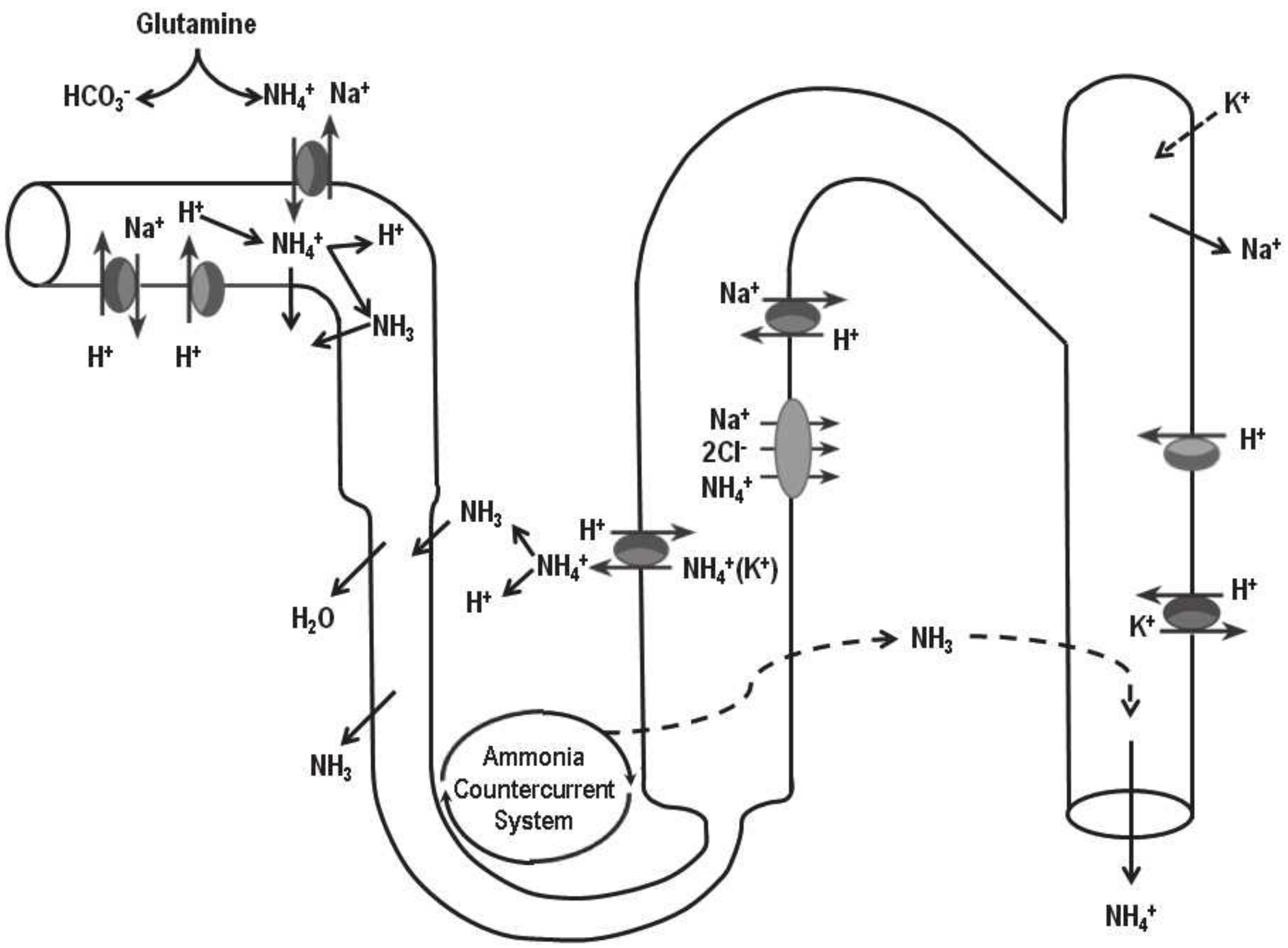

FIGURE 7.2 Nephron sites of ammonia production, secretion, transport, and ultimately, excretion. Note that approximately 75% of ammonia/ammonium delivered to the loop of Henle is transported into the medullary interstitium and is accumulated through countercurrent multiplication, thus resulting in higher concentrations from the cortex to the medulla. In acidosis, both the production of ammonia/ammonium and the transport, multiplication, and excretion of NH_4^+ is augmented. Therefore, the physiologic response of the kidney to an acid load is to increase bicarbonate reabsorption and ammonia production and excretion. The resulting urine should demonstrate a pH $<$5.5 and a higher concentration of ammonium. If these conditions are met, a normal gap metabolic acidosis would be nonrenal in origin.

will again accept a H^+, forming NH_4^+. Furosemide, which inhibits the Na^+–K^+–$2Cl^-$ cotransporter and secondarily inhibits the lumen-positive voltage, significantly inhibits all three mechanisms of thick ascending limb NH_3–NH_4^+ absorption.[6,10,11]

Through these processes, NH_3–NH_4^+ accumulates in the renal medulla (Fig. 7.2). The countercurrent system concentrates the solute toward the tip of the medulla and preserves the cortical-to-medullary gradient. Blood in the descending vasa recta has lower NH_3–NH_4^+ concentrations than the surrounding interstitium, allowing net entry. Conversely, blood in the ascending vasa recta has a higher NH_3–NH_4^+ concentration than the surrounding interstitium, causing net efflux. In patients with an intact urinary concentrating system and medullary architecture, the net result is a steep gradient, with the highest concentrations of NH_3–NH_4^+ in the inner medullary interstitium.

Ultimately, NH_3–NH_4^+ is transported from the medullary interstitium into the lumen of the collecting tubule for final urinary excretion (Figs. 7.1B and 7.2). The apical and basolateral membranes of the medullary collecting tubule are permeable to NH_3, allowing nonionic diffusion to be driven by the low pH of the collecting tubule fluid. In addition, NH_4^+ can be transported from the medullary interstitium into the collecting tubule cell on the Na^+, K^+-ATPase.[12] Once inside the cell, the dissociation of NH_4^+ into NH_3 and H^+ provides a source of H^+ for transport into the luminal fluid by the vacuolar H^+-ATPase. H^+ secreted into the luminal fluid will combine with secreted NH_3 to form NH_4^+, maintaining the gradient for nonionic NH_3 diffusion into the luminal compartment, which has a much lower pH, and this way NH_4^+ is "trapped" in tubule fluid and is swept into the collecting system. Medullary interstitial NH_3–NH_4^+ concentrations are determined by the rate of ammonia synthesis in the proximal tubule, the efficiency of ammonia transport into the lumen of the proximal tubule and subsequently into the medullary interstitium in the thick ascending limb, and the efficiency of countercurrent trapping of ammonia in the medullary interstitium. Both ammonia synthesis and each step in the highly integrated response by the nephron to excrete ammonia are upregulated by metabolic acidosis and chronic hypokalemia, and are inhibited by alkalosis and hyperkalemia (see Chapter 18).

The Rh glycoprotein (RhCG/Rhcg), which is expressed not only in both principal and intercalated cells but also in the distal convoluted tubule, connecting tubule, initial collecting tubule, cortical collecting duct, and the outer and inner collecting ducts, is now thought to be critical for the excretion of ammonia by the kidney (Fig. 7.1B).[13,14] RhCG expression is greater in A-type intercalated cells than in principal cells but is not detectable in B-type intercalated cells.[15] RhCG is thought to play a role in renal ammonia excretion because of some recent genetic studies. Biver and colleagues[16] found that global RhCG deletion impaired ammonia excretion in the urine and decreased basal ammonia excretion in response to metabolic acidosis. Similar findings were observed when RhCG was deleted only from the collect ducts.[17] In summary, that both intercalated and principal cells express RhCG and that metabolic acidosis increases RhCG in both cell types suggests that RhCG contributes to transepithelial ammonia secretion.[16]

Therefore, the sum of TA and ammonia/ammonium excretion is represented, as noted previously, as net acid excretion (Eq. 9). All three components of net acid excretion are dependent on the operation of an H^+ secretory mechanism into the tubule fluid. Therefore, increases in the rate of H^+ secretion will increase urinary ammonium and TA excretion and will lower urinary HCO_3^- excretion. A protein-rich diet leads to acid production, so the kidneys must both reclaim the filtered bicarbonate and also excrete an acid equivalent to that produced in the diet. In this way, in a steady state, net acid production equals net acid excretion, and acid–base balance is maintained. In the presence of nonprotein-rich diets, the tubules do not reclaim all of the filtered bicarbonate and they decrease production of new bicarbonate in order to maintain acid–base homeostasis.

SPECIFIC MECHANISMS OF H^+–HCO_3^- TRANSPORT ALONG THE NEPHRON

How the transport of H^+ and HCO_3^- in the nephron occurs is generally defined by the specific characteristics of each epithelium and cell type within the nephron segments. Bicarbonate reabsorption is largely the responsibility of the proximal tubule and net acid secretion is largely of the distal tubule. A number of transport mechanisms across either the apical (for H^+) or basolateral (for alkali) membranes have been identified and are detailed in the following paragraphs.

Apical Membrane H^+-Transport Mechanisms

Renal tubule cells use both primary and secondary transport mechanisms for active H^+ secretion. A primary transport couples the metabolism to a transport mechanism, whereas a secondary transport couples the transporter to the metabolism, which generates a concentration gradient for a solute.

There are two mechanisms for primary active H^+ secretion, both of which are directly related to the metabolism of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (P_i). The first and quantitatively most important mechanism involves the vacuolar H^+-ATPase, or V-type ATPase, a multisubunit protein complex that secretes H^+,[18] and is electrogenic, thereby generating a positive charge in the tubule lumen. The ability of the H^+-ATPase to affect H^+ secretion depends on where it is located; when it is in the proximal tubule, it minimally mediates H^+ secretion into the lumen, but in type A (acid secreting) intercalated cells of the collecting tubule, it significantly mediates H^+ secretion into the lumen. This

mechanism is able to adapt as needed by increasing mRNA and protein abundance to protect against metabolic and respiratory acidosis.

The second mechanism for primary active H^+ secretion involves P-type ATPases. The P-type ATPases, which are isoforms of the H^+–K^+–ATPase ($HK\alpha_1$, the gastric isoform, and $HK\alpha_2$, the colonic isoform), participate importantly in H^+ transport by the collecting tubule. These P-type ATPases are coupled to ATP metabolism, have a phosphorylated high-energy intermediate, and possess α and β subunits. Both the gastric and colonic isoforms are expressed on the apical membranes of type A intercalated cells (IC) in the cortical collecting tubule, and the outer and inner medullary collecting tubules.[19–24] These transport processes are electroneutral, mediating H^+ secretion across the apical membrane into the tubule lumen in exchange for K^+ entry (into the cell). Both isoforms react to metabolic or respiratory acidosis by increasing in abundance and activity. Alternatively, the two types of H^+-transporters are also located on the basolateral membranes of type B (bicarbonate secreting) ICs in the cortical and outer collecting tubule.[25–29] The H^+-ATPase or H^+, K^+-ATPase in this location mediates H^+ transport across the basolateral membrane into the interstitium.

The secondary active H^+ transporter is an isoform of the Na^+-H^+ antiporter, NHE-3. Although there are eight known isoforms of the Na^+-H^+ antiporter, NHE-3 (*SLC9A3*) is the most important for bicarbonate reabsorption. It is located in the S_1 and S_2 proximal tubule and thick ascending limb of the loop of Henle on the apical membrane and mediates H^+ secretion into the tubule fluid.[30–33]

Basolateral Bicarbonate Transport

Two transporters mediate passive base efflux along the nephron. One transporter is the electroneutral Cl^-/HCO_3^- exchanger, which carries HCO_3^- out and Cl^- into the cell across the basolateral membrane in the S3 segment of the proximal tubule, the thick ascending limb of the loop of Henle, and the cortical and medullary collecting tubules. This Cl^-/HCO_3^- exchanger is a member of the anion exchanger 1 (AE1) family and is a truncated form of the red cell AE1 Cl^-/HCO_3^- exchanger,[34–37] or kAE1 (*SLC4A1*). The mRNA lacks the first three exons of red cell mRNA,[38] but the net result of this truncation is a protein with similar transport characteristics but altered cytoskeletal interactions. Cl^- enters the cell in exchange for HCO_3^- and exits the basolateral membrane through a Cl^- conductance.[39,40]

The second is the sodium bicarbonate cotransporter (NBC), of which there are four isoforms. The NBCe1 (*SLC4A4*) isoform is the most important for base efflux, mediating the majority of base efflux (~80%) out of the cell and into the interstitium.[41–44] It is present on the basolateral membrane of the proximal tubule and is electrogenic because it transports three base molecules with one Na^+ ion ($Na^+/n\ HCO_3^-$ or *SLC4A4*).[41–44]

SEGMENTAL CONTRIBUTION TO BICARBONATE ABSORPTION AND ACIDIFICATION OF THE URINE

Proximal Tubule

The proximal tubule reabsorbs approximately 80% of the filtered HCO_3^- load; the HCO_3^- concentration at the end of the proximal tubule is approximately 8 mEq per liter and the pH is 6.7.[45,46] The reabsorption rate is a function of proximal tubule segmentation, so the rate of HCO_3^- reabsorption is highest in the early segment (S_1), decreases in the midsegment (S_2), and is lowest in the terminal segment (S_3).

The specific mechanisms responsible for H^+–HCO_3^- transport in the proximal tubule are shown in Figure 7.3. Approximately two-thirds of H^+ secretion is mediated by the apical membrane Na^+–H^+ antiporter, and the remaining one-third is mediated by the vacuolar H^+–ATPase.[47–49]

The active secretion of H^+ into HCO_3^--rich glomerular ultrafiltrate by the NHE-3 results in the formation of H_2CO_3. Luminal carbonic anhydrase (type IV) facilitates the conversion of H_2CO_3 to CO_2 and H_2O and prevents the generation of limiting pH gradients across the proximal convoluted tubule.[50] CO_2 diffuses readily into the cell where, under the influence of cytoplasmic carbonic anhydrase (type II), it forms H_2CO_3 that dissociates rapidly to H^+ and HCO_3^-, which are transported, respectively, across the apical and basolateral membranes. Studies from Weinman's group[51] have clearly established that NHERF-1 (sodium-hydrogen exchange factor 1) is required for a protein kinase A (PKA)–associated downregulation of NHE-3 activity. NHE-3 has a specific requirement for NHERF-1 for cAMP-mediated phosphorylation and inhibition. In addition to H^+ secretion via NHE-3, an apical H^+-ATPase is also responsible for a small but significant fraction of bicarbonate reclamation in the proximal tubule.

HCO_3^- generated in the proximal tubule cell exits passively across the basolateral membrane Na^+/n-HCO_3^- cotransporter, or NBCe1, encoded by *SLC4A4* which, as mentioned previously, transports the equivalent of three HCO_3^- ions out in parallel with one Na^+ out (Fig. 7.3).[41–44,52,53] This is a passive process because this transporter carries a net of two negative charges, and the negative cell voltage provides a strong, favorable driving force for base efflux.

As noted previously, in addition to bicarbonate reabsorption, a second function of the proximal tubule in acid–base balance is to synthesize the NH_3 needed for HCO_3^- regeneration (see Fig. 7.1A) (see previous discussion on ammoniagenesis and ammonia/ammonium transport).

The Loop of Henle

Approximately 50% to 70% of the HCO_3^- delivered out of the proximal tubule is reabsorbed in the thick ascending limb of the loop of Henle.[54] The secretion of H^+ across the apical membrane is mediated by NHE-3 as in the proximal tubule (Fig. 7.4B).[30,54] A low cellular Na^+ concentration, as in the proximal tubule, is maintained by the

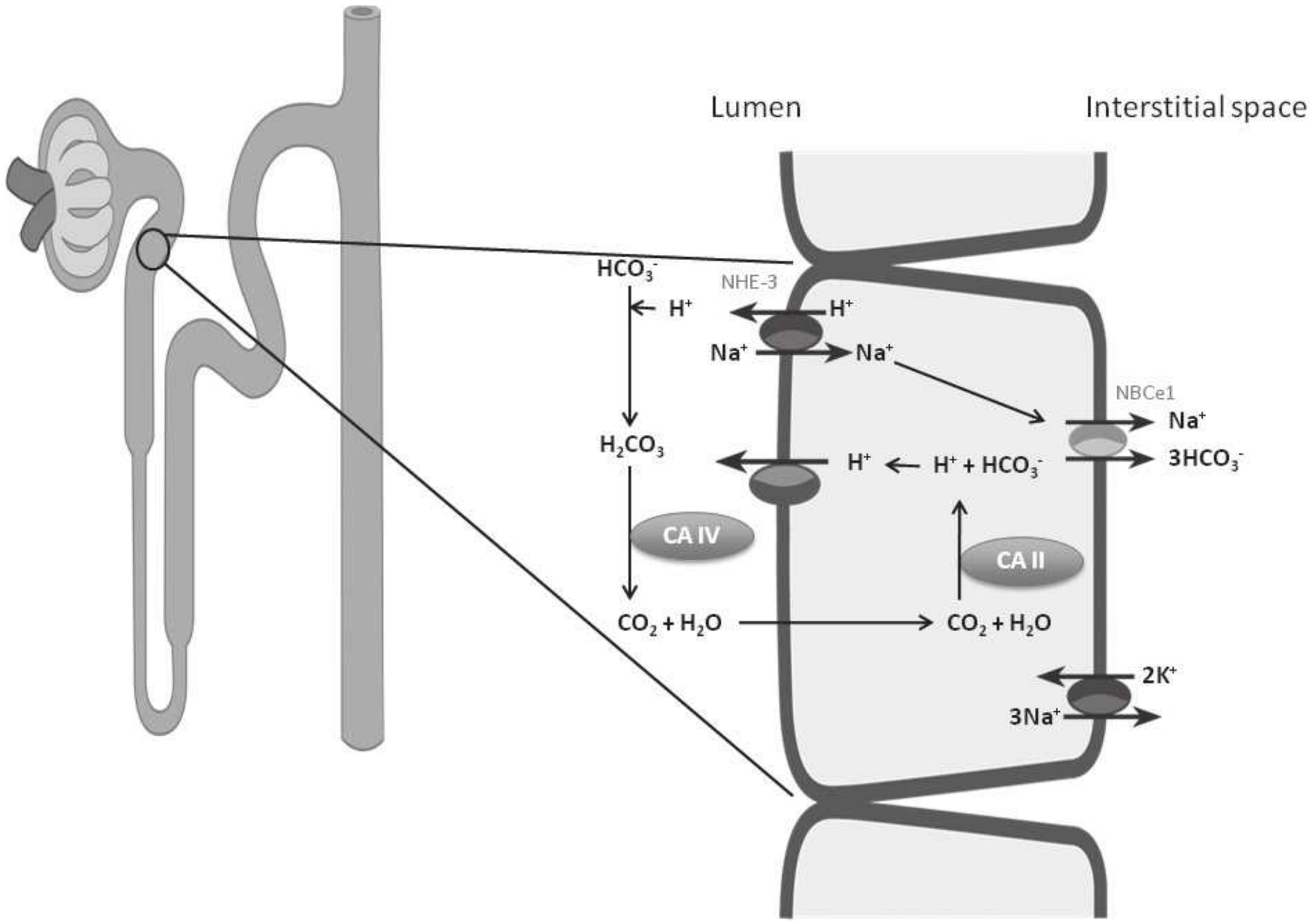

FIGURE 7.3 Aproximal convoluted tubule: the coupled apical and basolateral transporters responsible for bicarbonate reabsorption.

basolateral membrane Na^+, K^+-ATPase, to provide the driving force for operation of the Na^+/H^+ exchanger. Both AE1 (chloride–bicarbonate exchange) and NBC-2 or -3 (sodium–bicarbonate cotransport) are expressed on the basolateral membrane, where they mediate bicarbonate exit across the basolateral membrane.[55–57]

The Distal Tubule and the Collecting Duct

The distal tubule and the collecting duct are responsible for the final acidification of the urine. This process involves three steps: (1) the residual HCO_3^- in tubule fluid is reabsorbed; (2) the final titration of buffers is accomplished (TA excretion); and (3) the H^+ secreted traps ammonium in the lumen so that the majority of ammonia produced is excreted as NH_4^+ (Fig. 7.2). Stoichiometrically, excretion of NH_4^+ is necessary to regenerate the HCO_3^- lost in the buffering of acid products of metabolism. Therefore, each NH_4^+ excreted replenishes one HCO_3^- that is returned to the ECF via the renal vein. If the NH_4^+ is not excreted by the kidney, NH_3 is returned to the liver where each NH_3 molecule generates $2H^+$ in ureagenesis. Conversely, when the kidney is required to excrete alkali, the distal nephron is able to secrete HCO_3^-.[58]

The distal nephron, which begins at the macula densa, is a short segment that exhibits a small amount of amiloride-sensitive HCO_3^- reabsorption mediated by an apical membrane Na^+-H^+ antiporter isoform NHE2 (Fig. 7.4C).[29,59–61] Subsequent segments, including the connecting tubule, the initial cortical collecting tubule, and the cortical collecting tubule all possess ICs that mediate H^+–HCO_3^- transport. Type A ICs secrete H^+ into the tubule fluid and type B ICs secrete HCO_3^- across the apical membrane (Fig. 7.4D).

Type A ICs accomplish H^+ ion secretion through a process mediated by two ATP-dependent H^+ pumps, a V-type H^+-ATPase, and an H^+, K^+-ATPase (which is a P-type ATPase) (Fig. 7.5). There is abundant evidence supporting a role for both transporters. The H^+-ATPase has been localized by (1) the labeling of cells with antibodies against subunits of the vacuolar H^+-ATPase,[25,26] (2) the inhibition of H^+ transport by bafilomycin A_1, a specific inhibitor of vacuolar H^+-ATPases,[27,29] and (3) the electrogenicity of acidification in this nephron segment.[62] With regard to the latter, luminal acidification is associated with a lumen-positive voltage. Generation of a transepithelial voltage requires electrogenic or conductive processes on both apical and basolateral membranes. Because the IC does not possess significant apical membrane conductance,[39,40] electrogenic acidification requires an electrogenic H^+ pump.

Immunohistochemical studies show localization of the electroneutral H^+, K^+-ATPase to the apical membrane of a type A IC using antibodies against both the gastric ($HK\alpha_1$) and the colonic ($HK\alpha_2$) H^+, K^+-ATPase isoforms (Fig. 7.5).[63–65] Bicarbonate transport and potassium absorption are both

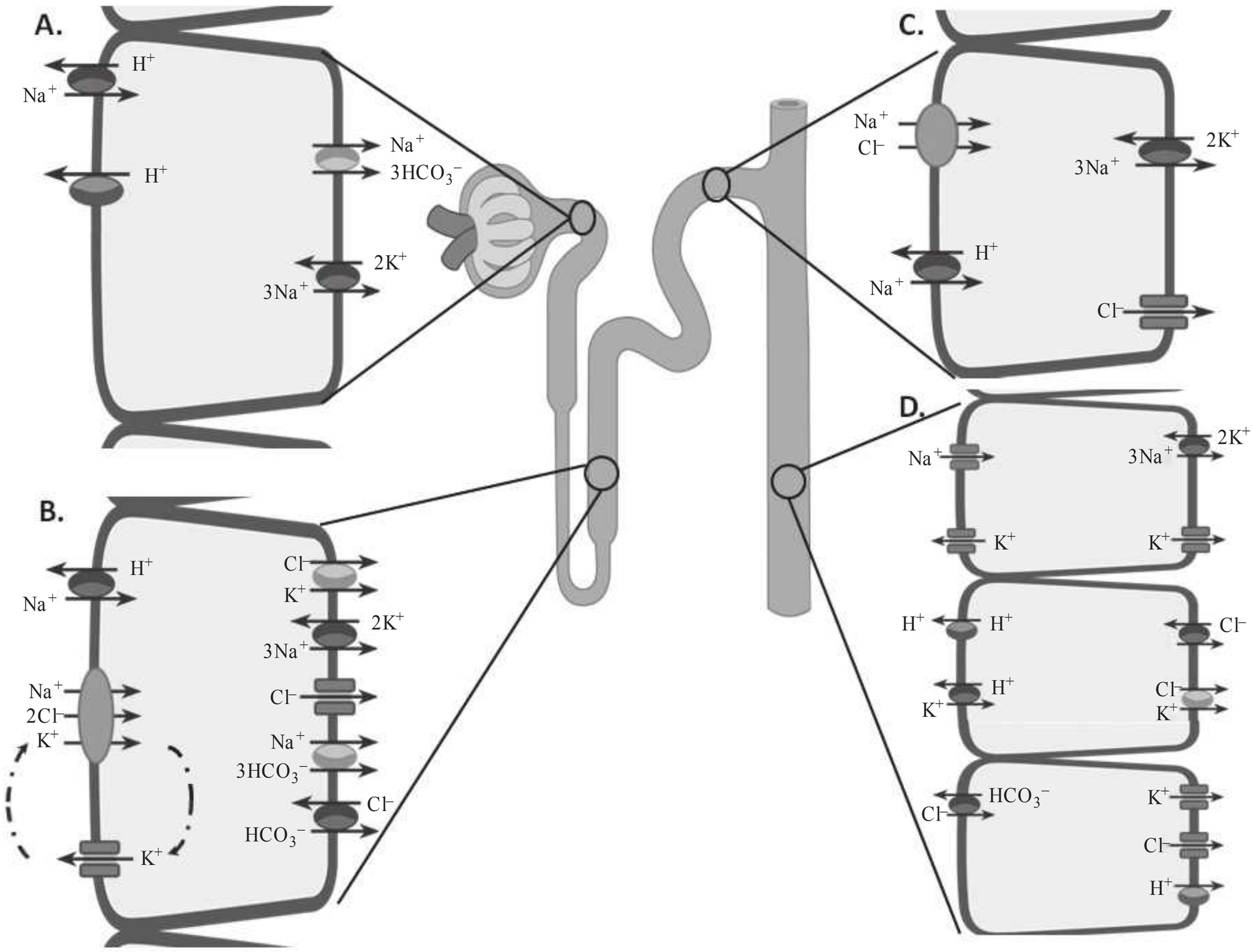

FIGURE 7.4 The H^+–HCO_3^- transport in the proximal tubule (*A*), the thick ascending limb of the loop of Henle (*B*), the distal convoluted tubule (*C*), and the collecting duct (*D*). Note that three cell types are displayed for the collecting tubule (*D*): the principal cell that does not participate directly in H^+ secretion, the type A intercalated or acid-secreting cell, and the type B intercalated or base-secreting cell.

inhibited by SCH 28080, a specific inhibitor of the gastric H^+, K^+-ATPase.[29,66–69]

The colonic isoform of H^+, K^+-ATPase, $HK\alpha_2$, is highly responsive to and upregulated by chronic potassium depletion. The site of regulation is most robust in the outer and inner medulla. The H^+, K^+-ATPase may play a highly significant role in potassium depletion, when its expression is significantly increased to minimize K^+ loss in the urine.[24,70] This regulatory response may explain the role of persistent chronic hypokalemia in the maintenance of metabolic alkalosis by maintaining H^+ secretion mediated by the H^+, K^+-ATPase, to prevent dangerous hypokalemia. In addition, both $HK\alpha_1$ and $HK\alpha_2$ increase abundance and activity due to either metabolic or respiratory acidosis. There is no specific inhibitor for $HK\alpha_2$ but Li and associates[71] in the DuBose laboratory have shown inhibition by intermediate concentrations of ouabain in vitro. Therefore, in summary, both the V-type H^+-ATPase and the H^+, K^+-ATPases contribute to H^+ secretion, although their relative contributions appear to be nephron segment–specific.

Type BICs secrete HCO_3^- into tubule fluid through the coupling of active secretion of H^+ across the basolateral membrane into the interstitium (Fig. 7.5) by both a vacuolar H^+-ATPase and an H^+, K^+-ATPase.[66] Therefore, these cells are mirror images of type A ICs and appear to be involved in protecting against metabolic alkalosis through a more efficient excretion of chronic bicarbonate loads. Type B ICs secrete HCO_3^- across the apical membrane into the collecting duct via a Cl^-/HCO_3^- exchanger. However, this Cl^-/HCO_3^- exchanger is not the same AE1 Cl^-/HCO_3^- exchanger found on the basolateral membrane of the proximal tubule. Rather, the anion exchanger pendrin, which is encoded by *SLC26A4*, is localized to the apical membrane of nonacid-secreting ICs in the kidney cortical collecting duct (CCD) (Fig. 7.5). Cl^- that enters the cell in exchange for HCO_3^- exits the cell through a basolateral membrane Cl^- conductance. This process does not generate a transepithelial voltage, because there is no net transcellular current.

The ability to secrete HCO_3^- disappears in the outer medullary collecting tubule. Here, the H^+ ion secretion is mediated by cells that are functionally similar to type A ICs.

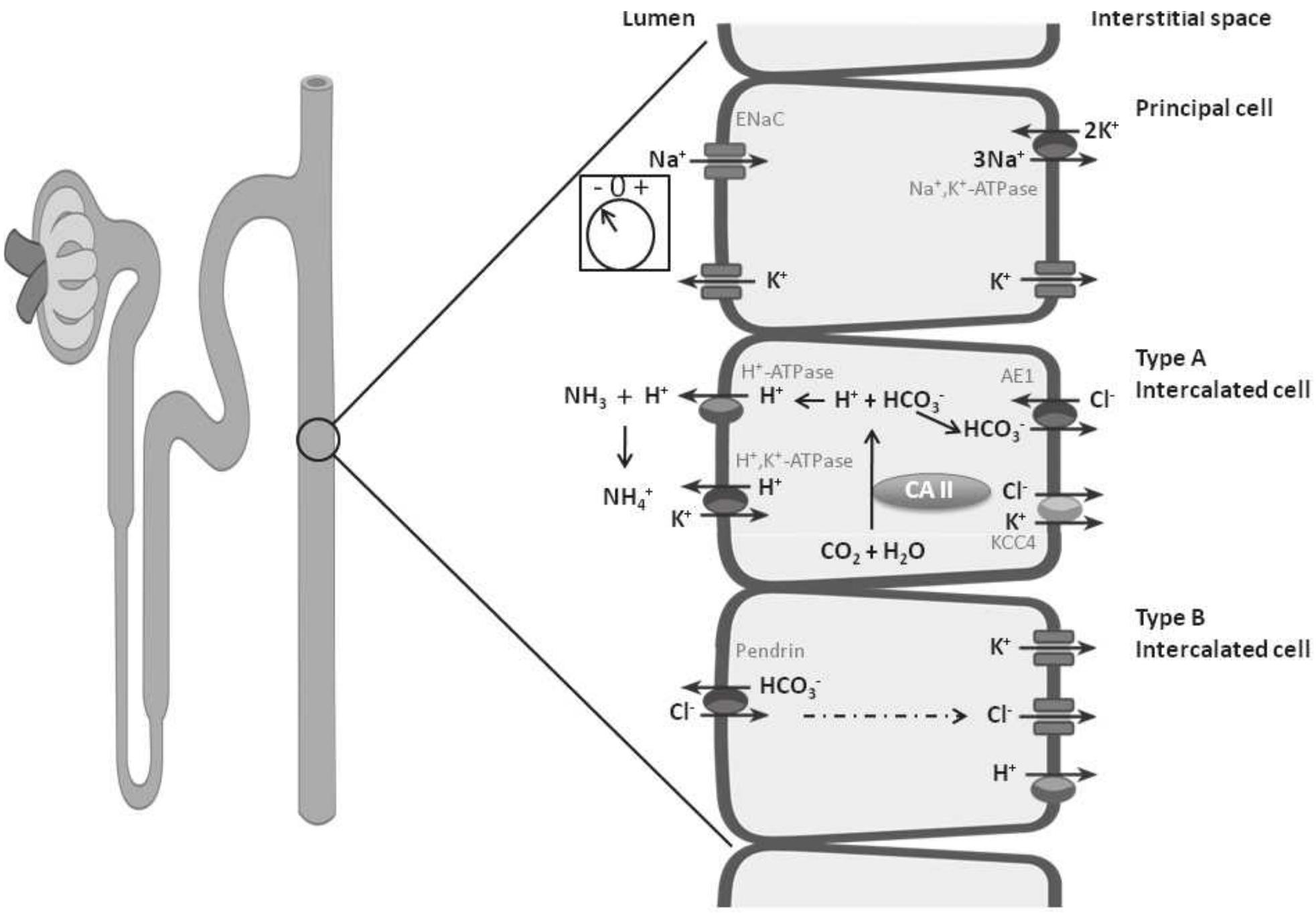

FIGURE 7.5 The H^+–HCO_3^- transport in the collecting tubule, which occurs in type A and type B intercalated cells.

In some cases, these cells are designated as non-A and non-B intercalated cells. The inner stripe of the outer medullary collecting tubule is a high-capacity segment for H^+-secretion. The inner medullary collecting tubule has been difficult to study because of the difficulty in isolating this nephron segment for tubular perfusion. Nevertheless, studies in our laboratory provide evidence for a mechanism of H^+ secretion similar to that described for the type A ICs.[24,70] Moreover, it should be emphasized that this key segment is responsible for the final acidification of the tubule fluid and generates the low urinary pH typical of high acid excretion states.

INTEGRATED KIDNEY REGULATION OF URINARY ACIDIFICATION AND EXCESS BASE EXCRETION

The integrated response of heterogeneous nephron segments to regulate acid–base balance requires the regulation of transport in each of the nephron segment in concert (Fig. 7.4). In the following sections, the most important regulatory responses of H^+–HCO_3^- transport are emphasized.

Luminal HCO_3^- Delivery

In order to avoid metabolic acidosis or alkalosis, H^+ ion secretion and HCO_3^- reabsorption must work in concert. GFR increases lead to luminal flow rate increases, which in turn signal the tubules to secrete H^+ more rapidly. If the rate of acid excretion did not change, too little bicarbonate would be reabsorbed and the luminal pH would decrease too slowly. Alkaline tubule fluid stimulates apical membrane H^+ transporters.[72] The tubule flow rate can also directly regulate the rate of proximal tubule H^+ secretion, regardless of HCO_3^- concentration or luminal composition.[73]

Systemic pH

As noted previously, the kidneys respond to changes in systemic arterial pH (PCO_2) by either increasing urinary acidification (for acidosis) or by inhibiting acidification and increasing bicarbonate excretion (for alkalosis). Because changes in plasma pH cause changes in renal interstitial pH, the mechanisms influencing bicarbonate efflux on the basolateral membrane are also affected. Acidosis enhances HCO_3^- efflux and alkalosis inhibits basolateral membrane HCO_3^- efflux. Thus, in acidosis, cell pH decreases; whereas in alkalosis, it increases.[74] It is the change in intracellular pH that determines apical membrane H^+ ion secretion; relative acidity stimulates H^+ secretion, whereas a more alkaline pH reduces or inhibits H^+ secretion.

The Na^+-H^+ antiporter is activated by decreases in intracellular pH,[75] which also stimulates the insertion of H^+ transporters into the apical membrane.[76,77] These "reserved" H^+ ions in the apical membrane can enhance the rate of H^+

transport when needed. Long-term adaptations in tubular function result from chronic systemic pH changes. In the proximal tubule, chronic metabolic and respiratory acidosis enhances the proximal tubule capacity for H^+ secretion and ammoniagenesis.[78,79] These changes are mediated by parallel increases in apical membrane Na^+-H^+ antiporter and electrogenic basolateral membrane Na^+/n-HCO_3^- cotransporter activity.[80–83]

Such transporter adaptations are known to be a direct effect of pH because they can be experimentally induced by incubating cultured proximal tubule cells in acid media.[84] Enhanced activity of the apical Na^+-H^+ antiporter is mediated by the trafficking of NHE-3 to the apical membrane, and increasing whole cell NHE-3 abundance.[85] Several groups have demonstrated that endothelin-1 (ET-1) increases the activity of NHE-3 and that metabolic acidosis stimulates renal proximal tubule ET-1 expression.[86–89] Studies in ET_B receptor knockout mice demonstrate that the ET-1/ET_B signaling pathway mediates acid stimulation of NHE-3 activity.[90,91] In addition, a consensus sequence (KXXXVPKXXXV) in the second intracellular loop of the ET_B receptor appears to be responsible for ET-1/ET_B stimulation of NHE-3 activity.[92]

How the extracellular pH and the systemic acid–base status are sensed by renal epithelial cells and initiate a regulatory response has not been elucidated. Factors such as pH, PCO_2 and bicarbonate, along with possible hormonal stimuli[28] have all been proposed at one time or another. Early studies by Pitts dating to the 1940s indicated that metabolic acidosis induced by the infusion of 0.1 N HCl causes an increase in net acid excretion, predominately as ammonium excretion.[93,94] Schwartz and Al-Awqati[77] demonstrated that an increase in ambient PCO_2 stimulated proton secretion in isolated perfused proximal tubules and in the collecting ducts of rabbit nephrons. It was later shown that respiratory acidosis was associated with the apical insertion of the H^+-ATPase. Recent studies have suggested that the nonreceptor tyrosine kinases Pyk2 and c-Src may represent a possible signaling pathway involved in the increase in NHE-3 function in the proximal tubule in response to metabolic acidosis.[95–97] Although there is preliminary evidence that Pyk2 is expressed and may also function in the collecting tubule as a pH sensor, the signaling pathway has not yet been delineated (K. Fisher, personal communication, February 2012).

Recently, a small number of G protein-coupled receptors (GPCR) have been identified, the activation of which is stimulated by a reduction in extracellular pH, prompting them to be termed "proton-sensing" receptors. One of these putative proton-sensing GPCRs is the orphan receptor GPR4.[98] GPR4 is an upstream activator of cAMP-PKA signaling, and our preliminary studies implicate GPR4 in the upregulation of proton transporters in the collecting duct of the kidney. Together with existing literature on the activation of other proton translocating ATPases, these findings indicate the presence of an underlying signaling network involved in coordinating the renal adaptive response to acidosis.

Petrovic and colleagues[99] recently reported that deletion of GPR4 in vivo was associated with a reduction in net acid excretion and a relatively alkaline urine pH, parallel with a spontaneous nongap metabolic acidosis, thus providing evidence for an acidification defect in this model. Moreover, some of the features of the renal acidification defect in GPR4-/- resemble that which is reported for mice with the deletion of one of the genes that encodes for the rate-limiting bicarbonate transporter, AE1, in the kidney collecting tubule. Therefore, GPR4 appears to function as a pH sensor in the collecting tubule.

Recent studies have revealed that soluble adenylyl cyclase (sAC) is highly expressed in the collecting tubule and may colocalize with the H^+-ATPase in the apical membrane of type A ICs.[100] It has been suggested that sAC-regulated cAMP signaling may constitute a general sensing mechanism for regulating H^+-ATPase-mediated proton transport. Although parallels exist in the male reproductive tract,[101] how sAC functions to sense bicarbonate, primarily, and how it might function to play a regulatory role in the response to an acid intracellular or extracellular pH, which is pivotal for the renal defense against metabolic acidosis, has not been satisfactorily explained to date.

INTEGRATION OF KIDNEY RESPONSE TO DEFEND AGAINST ACID–BASE DISTURBANCES

As noted previously, a high-protein diet, such as that consumed by most Westerners, requires renal reclamation of most of the filtered bicarbonate as well as net acid excretion that is equivalent to net acid production. The ammonia produced in the kidney is derived from the synthesis of glutamine, and the amount produced is regulated by pH. Acidemia promotes and alkalemia inhibits glutamine synthesis.

Long-term changes in dietary acidity can cause chronic changes in H^+ and HCO_3^- secretion. For example, in the cortical collecting tubule, chronic increases in dietary acid result in an increased capacity for H^+ secretion into the lumen, whereas chronic increases in dietary alkali lead to an increased capacity for HCO_3^- secretion.[102] As noted previously, type A ICs mediate H^+ secretion and type B ICs mediate HCO_3^- secretion. The elegant studies of Schwartz and Al-Awqati[103] over many years have demonstrated that these cells can "switch" phenotypes based on chronic metabolic changes. For example, during metabolic acidosis, the number of type B ICs decreases and the number of type A ICs increases, but the total number of intercalated cells does not change.[104–107] This "switch" could be due to an extracellular protein called hensin.[103] Recent results demonstrate that acidosis induces the polymerization of this novel extracellular matrix protein as follows: The deposition of hensin in the matrix leads to the conversion of a HCO_3^--secreting epithelium to an acid-secreting epithelium. Hensin is deposited in the extracellular matrix of H^+-secreting type A ICs following acid incubation.[108] Alternatively, this "switch" could also be due to the exocytotic insertion of H^+-ATPase into the

apical membrane of type A ICs and the endocytic retrieval of the Cl^-/HCO_3^- exchanger from the apical membrane of the type B ICs.

As noted previously, the kidneys eliminate the acid that is produced daily and have the capacity to increase urinary net acid excretion (and, hence, HCO_3^- generation) in response to endogenous or exogenous acid loads. The renal excretion of acid is usually matched to the net production of metabolic and dietary acids, so little disturbance in systemic pH occurs.

As an acid load is incurred, the kidneys respond to restore balance by increasing the ammonium excretion (TA excretion has limited capacity for regulation). With continued acid loading, the renal net acid excretion increases over the course of 3 to 5 days (Fig. 7.2) but does not quite achieve the level of acid production. Progressive positive acid balance ensues, which is presumably buffered by bone carbonate.

K^+ DEFICIENCY AND HYPERKALEMIA

Chronic K^+ deficiency leads to adaptations in H^+ ion secretion that are similar to those evoked by chronic metabolic acidosis. Thus, chronic K^+ deficiency leads to an increased capacity for transepithelial H^+ secretion by the proximal tubule through parallel increases in the activities of NHE-3 and the basolateral membrane Na^+–$3HCO_3^-$ cotransporter[109] and increases in activities of ammoniagenesis that are also stimulated by chronic metabolic acidosis (Fig. 7.1A).[110] The similarity between these two responses may be the result of chronic K^+ deficiency, which decreases intracellular pH and increases the NHE-3 transport rate.[111,112]

In the collecting tubule, chronic K^+ deficiency increases H^+ secretion through the insertion of H^+ pumps into the apical membrane of H^+-secreting cells (A-type ICs) and through a robust increase in the activity of the H^+, K^+-ATPase (Fig. 7.1B).[70,113–115] The result of the latter is to enhance H^+ ion secretion while, at the same time, to enhance K^+ absorption by the distal nephron. The stimulation of renal acidification in chronic K^+ deficiency explains the association of K^+ deficiency with chronic metabolic alkalosis and is primarily an adaptive upregulation of the H^+, K^+-ATPase, thus offering an explanation for the paradoxical increase in bicarbonate absorption in the face of chronic metabolic alkalosis with hypokalemia.[116] Furthermore, the metabolic alkalosis associated with hypokalemia cannot be corrected until the potassium deficiency is repaired.

Hyperkalemia appears to have the opposite effect on acid–base balance because hyperkalemia inhibits proximal tubule ammoniagenesis and ammonium transport. This decrease in ammonium excretion due to hyperkalemia contributes to the acidification defect characteristic of type 4 renal tubular acidosis.[117,118] Hyperkalemia not only suppresses ammoniagenesis, but also inhibits NH_4^+ reabsorption in the thick ascending limb, resulting in low NH_3 levels in the renal medulla, and, thus, a reduced capacity to buffer H^+ secreted in the collecting tubule.[118] Correcting hyperkalemia increases net acid excretion and helps correct the acidosis in disorders associated with hyperkalemia and the metabolic acidosis of renal origin.[20,114]

THE EFFECT OF MINERALOCORTICOIDS ON URINARY ACIDIFICATION

Mineralocorticoids such as aldosterone stimulate urinary acidification through a number of different mechanisms. Aldosterone binds to its intracellular mineralocorticoid receptor in collecting tubule cells and stimulates the rate of H^+ ion transport[119,120] by parallel increases in the activities of the apical membrane V-type H^+-ATPase[121] and the basolateral membrane Cl^-/HCO_3^- exchanger.[122] It also has indirect effects on H^+ secretion by increasing Na^+ reabsorption in the cortical collecting tubule (CCT) through increasing the open probability of the apical sodium channel in principal cells. This secondarily increases the transepithelial negative potential difference, which is an important driving force for H^+ secretion.[123]

Effective Arterial Volume

Effective arterial volume is an important regulator of renal acidification and is reviewed in the following paragraphs.

Extracellular Volume Expansion

The addition of sodium bicarbonate to the body results in prompt cellular buffering and respiratory compensation. However, as with an acid load, the kidneys have the ultimate responsibility for the disposal of base to restore a normal serum bicarbonate concentration. The renal response is more rapid with the addition of HCO_3^- than with acid ingestion. The speed and efficiency by which HCO_3^- can be excreted renders it challenging to cause more than mild metabolic alkalosis in a patient with normal renal function even with as much as 24 mEq per kilogram per day of sodium bicarbonate over several weeks.[124,125] Type B ICs in the collecting tubule are capable of HCO_3^-/Cl^- exchange (bicarbonate secretion). However, the role of HCO_3^- secretion in chronic alkali loading has never been quantitated precisely in whole organ clearance studies. Presumably, HCO_3^- secretion by the type B IC moderates the development of a more severe alkalosis, enhancing the HCO_3^- excretory response (Fig. 7.5).

The proximal tubule is principally responsible for HCO_3^- excretion when the blood HCO_3^- concentration increases. Absolute proximal HCO_3^- reabsorption does not increase in proportion to the load of HCO_3^- because alkalemia suppresses proximal acidification,[126] causing an increase in the HCO_3^- delivery to the distal nephron. The limited capacity of the distal nephron to secrete H^+ can be overwhelmed easily, and bicarbonaturia increases progressively. NH_4^+ and TA excretion are moderated in response to the increasing urine pH.[124,126]

Acute graded HCO_3^- loads that concomitantly increase ECF also function in human subjects to progressively increase urinary HCO_3^- excretion as the plasma HCO_3^- concentration increases.[126] In summary, an acute base load is excreted entirely when the kidney function is normal, and the blood HCO_3^- concentration is returned to normal within 12 to 24 hours because of the depression of fractional proximal HCO_3^- reabsorption. In addition to the suppression of the filtered HCO_3^- load reabsorption, direct HCO_3^- secretion by the CCT is another means for HCO_3^- disposal during metabolic alkalosis.[124]

The increased delivery of HCO_3^- out of the proximal tubule in response to an increased blood HCO_3^- concentration (filtered HCO_3^- load) in the setting of ECF expansion facilitates HCO_3^- excretion and the return of blood pH to normal. However, other factors may independently enhance distal H^+ secretion sufficiently to prevent HCO_3^- excretion and thus counterbalance the suppressed fractional proximal HCO_3^- reabsorptive capacity. Under these circumstances, the alkalosis is maintained. For example, in the setting of primary hyperaldosteronism, despite the expanded ECF, a stable mild alkalotic condition persists in most experimental models owing to augmented collecting duct H^+ secretion.[124] In such cases, concurrent hypokalemia facilitates the generation and maintenance of metabolic alkalosis by enhancing NH_4^+ production and excretion.[124,126] Moreover, chronic hypokalemia dramatically enhances the abundance and functionality of the H^+, K^+-ATPase in the medullary collecting tubule, thus increasing rather than decreasing bicarbonate absorption.[124,127–129] Enhanced nonreabsorbable anion delivery, as with drug anions, also increases the net collecting tubule H^+ secretion by increasing the effective luminal negative potential difference or by suppressing HCO_3^- secretion in the cortical collecting duct (CCD).

Extracellular Volume Contraction

The renal response to an increase in plasma HCO_3^- concentration can be modified significantly in the presence of ECF contraction.[128,130] Because the distribution volume of Cl^- is approximately equal to ECF, the depletion of the ECF is roughly equivalent to the depletion of Cl^-. The critical role of effective ECF and K^+ stores in modifying net HCO_3^- reabsorption has been demonstrated in numerous experimental models.

In most cases, metabolic alkalosis is maintained by decreases in effective arterial volume. This is due, in part, to volume-induced decreases in GFR (leading to a decrease in filtered HCO_3^- load).[131] Moreover, a decrease in effective arterial volume increases renal acidification by (1) decreasing paracellular HCO_3^- permeability in the proximal tubule, which inhibits HCO_3^- back leak[132]; (2) decreasing the distal delivery of Cl^-, thus inhibiting HCO_3^- secretion in the cortical collecting tubule (by limiting the apical membrane Cl^-–HCO_3^- exchange); and (3) secondarily increasing aldosterone and angiotensin II, as well as renal nerve activity. When taken together, these factors enhance HCO_3^- reabsorption and regeneration.

Deficiency of both Cl^- and K^+ is common in metabolic alkalosis because of renal and/or gastrointestinal losses that occur concurrently with the generation of the alkalosis.[129,130] With Cl^- depletion, the normal bicarbonaturic response to an increase in plasma HCO_3^- is prevented, and metabolic alkalosis can develop. Potassium depletion, even without mineralocorticoid administration, can cause metabolic alkalosis in rats and humans. When Cl^- and K^+ depletion coexist, severe metabolic alkalosis may develop in all species studied.

Two general mechanisms exist by which the bicarbonaturic response to hyperbicarbonatemia can be prevented by Cl^- and/or K^+ depletion: (1) As the plasma HCO_3^- concentration increases, there is a reciprocal fall in GFR and, if inversely proportional to the rise in the plasma HCO_3^- concentration, the filtered HCO_3^- load would not exceed the normal level. Accordingly, normal rates of proximal and distal HCO_3^- reabsorption would suffice to prevent bicarbonaturia. (2) Cl^- deficiency or K^+ deficiency increases the overall renal HCO_3^- reabsorption in the setting of a normal GFR and a high filtered HCO_3^- load. In this case, overall renal HCO_3^- reabsorption, and therefore acidification, would be increased. An increase in renal acidification might occur as a result of an increase in H^+ secretion by the proximal or the distal nephron or by both nephron segments.[124,127,129]

The augmented HCO_3^- absorption in distal nephron segments appears to be due to a primary increase in H^+ secretion that is independent of the delivered HCO_3^- load. Chronic hypokalemia dramatically enhances the abundance and function of the colonic isoform of the H^+, K^+-ATPase in the medullary collecting tubule, which may serve as a significant factor in the maintenance of chronic metabolic alkalosis.[70,115,127]

In summary, the physiologic response by the kidney to a base load associated with volume expansion is to excrete the base. Base is retained, however, if there is enhanced distal HCO_3^- reabsorption as a result of K^+ and/or Cl^- deficiency.

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