Maintaining acid base balance presents a considerable challenge to the growing neonate. The infant must ingest protein for growth and development. The metabolism of sulfur containing amino acids leads to the production of protons that must be secreted by the kidney. In addition, the formation of hydroxyapatite for the mineralization of growing bone also leads to acid production. Thus, the growing infant must excrete approximately 2 to 3 mEq of acid per kilogram of body weight per day to avoid becoming acidotic. The mechanisms for excreting acid undergo complex maturational changes that predispose the neonate, and the premature neonate in particular, to a great risk for the development of acidosis. In addition, infants are susceptible to gastrointestinal disturbances that can lead to acidosis due to acute loss of bicarbonate in the stool. The kidney is then responsible for the production of new bicarbonate to restore the body’s acid base balance. There are also a number of inherited disorders in the kidney that affect acid secretion and lead to acid base disturbances in neonates. This review discusses the mechanisms by which the kidney is capable of excreting acid as well as the developmental regulation of these processes and the basis of inherited disorders of acidification.

We first review the mechanisms for acid excretion in the mature kidney. To excrete nitrogenous wastes, the kidney forms urine by a process of filtration in the glomerulus, followed by secretion and reabsorption in the tubules. The high glomerular filtration rate allows for clearance of the nitrogenous wastes but also places a large demand on the tubules to reabsorb solutes that are not to be excreted, such as bicarbonate. A normal adult will form about 150 L of glomerular filtrate each day. This fluid contains about 3,750 mEq of bicarbonate ions (25 mEq/liter) that must be reabsorbed to keep from losing any bicarbonate. The proximal tubule is responsible for the reabsorption of most of the solutes filtered by the kidney including 80% of the filtered bicarbonate.1,2 The mechanisms responsible for the reabsorption of bicarbonate by the kidney are illustrated in Figure 1.3 The mechanism for transporting bicarbonate from the lumen of the tubule to the blood stream involves several steps. First, hydrogen ions are secreted into the tubule lumen from the intracellular compartment. The main transporter for accomplishing this is the sodium-proton (Na+/H+) exchanger on the luminal membrane of the proximal tubule, which has been designated NHE3.4 The driving force for the Na+/H+ exchanger is generated by the low intracellular sodium concentration that is maintained by the basolateral sodium-potassium ATPase. One turnover of the Na+/H+ exchanger will result in one sodium ion entering the cell and one proton (H+) exiting the cell into the lumen of the tubule. In the adult proximal tubule, there is also a proton pump (H+-ATPase) that derives its energy directly from the hydrolysis of ATP. It has been estimated that about two-thirds of proton secretion in the adult proximal tubule occurs via the Na+/H+ exchanger and about one third via the H+-ATPase.5,6

After entering the lumen of the tubule, the proton combines with bicarbonate to form carbonic acid (H2CO3), which will dissociate in the presence of carbonic anhydrase (C.A.) to water and carbon dioxide as in equation 1:

\[
H^+ + HCO_3^- \rightleftharpoons \text{carbonic anhydrase} \rightarrow H_2O + CO_2
\] (1)
The water and carbon dioxide generated can then enter the proximal tubule cell where they recombine, in the presence of carbonic anhydrase, to form carbonic acid.\textsuperscript{3} In the cell the reaction then runs in the reverse direction to generate a proton and bicarbonate. The protons then can be secreted into the lumen and the bicarbonate exits across the basolateral membrane and into the blood stream. (Enclosed circles represent ATPases; Open circles represent transporters that utilize secondary transport).

The water and carbon dioxide generated can then enter the proximal tubule cell where they recombine, in the presence of carbonic anhydrase, to form carbonic acid.\textsuperscript{3} In the cell the reaction then runs in the reverse direction to generate a proton and bicarbonate. The bicarbonate exits the cell across the basolateral membrane and then goes into the blood stream, which is mediated by a sodium-bicarbonate co-transporter.\textsuperscript{7,8} Thus, bicarbonate transport is closely linked to sodium reabsorption in the proximal tubule. The thick ascending limb of Henle and the distal convoluted tubule reabsorb the remaining 20% of the filtered bicarbonate not reabsorbed by the proximal tubule.\textsuperscript{3}

As noted above, the kidney must not only reclaim the filtered bicarbonate but must secrete the acid generated from the metabolism of protein (and bone formation in the growing child). This is accomplished by secretion of protons in the collecting duct. First, we need to realize the problem with secreting acid as protons. If the urine pH (-log[H\textsuperscript{+}]) can be reduced to 4 (actually 4.5 is about the lowest attainable urine pH in man) it would be necessary to produce 500 L of urine for an adult to excrete 50 mmol of acid. Thus, the kidney must be able to excrete acid with buffers so that the urine can contain 50 mmol of acid in a small volume at a pH of 4.5.

To excrete acid in a small volume of urine the kidney produces ammonia.\textsuperscript{3,9} Ammonia is primarily generated in the proximal tubule by the deamination of glutamine by the enzyme glutaminase and is then protonated in the distal tubule to form ammonium.\textsuperscript{9} There are a few other enzymes involved in the process of ammoniagenesis, but glutaminase is by far the most important for producing ammonia and can be stimulated 10-fold by acidosis. Thus, most of the acid we excrete daily is in the form of ammonium chloride. A smaller fraction of the acid is buffered by phosphate and sulfate. This is referred to as titratable acidity.\textsuperscript{3} Net acid excretion in the adult is the sum of ammonium and titratable acidity, which matches precisely the amount of acid generated from metabolism and new bone formation.

The excretion of the protons by the collecting duct is depicted in Figure 2. The intercalated cells of the collecting duct are divided into 2 types.\textsuperscript{3} The $\alpha$-intercalated cells contain an H$^+$/ATPase in the luminal membrane for the secretion of protons. When the acid is secreted, bicarbonate is generated in the cell and must be excreted across the basolateral membrane into the blood stream. This occurs through a chloride-bicarbonate exchanger, which is designated AE1 (Anion-Exchanger 1). The activity of the $\alpha$-intercalated cell increases during acidosis. The $\beta$-intercalated cells secrete base into the lumen and acid into the blood stream. These cells become active when a patient becomes alkalotic and needs to correct the alkalosis.

**Maturation of Acid Excretion**

Neonates have a serum bicarbonate that is lower than that of adults. The lower level of serum bicarbonate is caused by a lower bicarbonate threshold, the maximal capacity of the kidney to reabsorb bicarbonate.\textsuperscript{10} In the neonate as in the adult, most of bicarbonate reabsorption occurs in the proximal tubule. The rate of neonatal
proximal tubule bicarbonate reabsorption is approximately one third that of the adult proximal tubule. The lower rate of bicarbonate reabsorption is not due to a back leakage of transported bicarbonate from the bloodstream into the lumen but due to a lower rate of bicarbonate reabsorption.

All of the transporters responsible for the reabsorption of bicarbonate have a lower activity in the neonatal proximal tubule when compared to the adult. The basolateral sodium-potassium ATPase, responsible for maintaining the low intracellular sodium concentration, has about one half the activity compared to that of the adult. The apical Na\(^+\)/H\(^+\) exchanger has a much lower activity and is in much lower abundance on the apical membrane of the neonatal proximal tubule. There appears to be no activity of the H\(^+\)-ATPase in the proximal tubule of the neonate. In addition, the basolateral sodium-bicarbonate cotransporter is in lower abundance in the neonatal proximal tubule compared to that of the adult. Thus, all of the active transport steps for the reabsorption of bicarbonate are underdeveloped in the neonate.

The factors that induce the maturation of proximal tubule bicarbonate reabsorption are currently under investigation. It is clear that one of the major hormones that stimulate the development of bicarbonate transport are glucocorticoids. Neonates are relatively glucocorticoid deficient during the first weeks of life. There is a developmental increase of glucocorticoids that precedes the increase in bicarbonate transport. Indeed, if pregnant rabbits are injected with glucocorticoids prior to giving birth, the neonates have a proximal tubule bicarbonate transport rate that is comparable to that of the adult proximal tubule. Glucocorticoids affect several steps in the regulation of transport. Thyroid hormone also affects the development of bicarbonate transport but plays a much less important role.

If glucocorticoids can hasten the development of transport in the kidney tubules, why don’t we treat all premature infants with these drugs? There is mounting evidence that there can be considerable detrimental effects of prenatal glucocorticoids. Rats that were treated with dexamethasone prenatally during specific times during gestation were found to develop hypertension when they became adults. In addition, examination of their kidneys revealed an increase in glomerulosclerosis. While one cannot and should not make any conclusions about humans from these studies, it is possible that prenatal glucocorticoids may have adverse effects in humans if given at particular times during renal development.

The collecting duct is divided into the cortical collecting duct and the inner and outer medullary collecting duct. Data from perfused tubules from rabbit kidneys indicate that the cortical collecting duct has a decreased ability to secrete acid in the neonate when compared to the adult. This is because of the fact that there is about one half the number of intercalated cells in the cortical collecting duct of the neonate as compared to the adult. The medullary collecting duct in the neonate has transport rates that are comparable to that of the adult and the number of intercalated cells is also comparable. So the overall developmental change in the collecting duct is that the neonatal collecting duct has a lower rate of transport than the adult collecting duct.

There is also evidence for developmental changes in renal carbonic anhydrase activity. There are 2 major isoforms of carbonic anhy-
drase in the kidney that are designated C.A. II, which is located in the intracellular compartment, and C.A. IV, which is located on the luminal membranes of the tubules, primarily the proximal tubules. In maturing rabbits, carbonic anhydrase IV has been shown to increase its activity and parallels the developmental increase in bicarbonate reabsorption in the proximal tubule. Thus, there is a lower activity rate of this enzyme in the neonatal kidneys that may contribute to the lower rates of acid secretion. More recently, carbonic anhydrase II was also shown to have lower abundance in neonatal rats. This isoform appears to be critical for the function of the intercalated cells of the collecting duct and may be responsible for the developmental increase in the collecting ducts ability to excrete acid. However, when studied in humans, it appears that there is not much difference in carbonic anhydrase activity between the neonate and adult kidneys. Neonatal human kidneys do have more carbonic anhydrase activity in the juxtamedullary region where the nephrons are more developed than the superficial cortical region. Thus, it is unclear in humans if the differences in carbonic anhydrase activity between neonates and adults are critical for the development of acidification.

One other aspect of acidification that is important in the development of acidification is ammoniagenesis. As we discussed earlier, most of our acid is secreted in the form of ammonia. Without this crucial buffer, it is very difficult to excrete large amounts of acid in a relatively small volume of urine. The enzymes for ammoniagenesis are present in the neonate, but the rates of ammonia production are somewhat lower than that of the adult. The primary enzyme, glutaminase, has a lower activity in the neonatal kidney. The renal content of glutamine as a substrate is also lower in the neonatal kidney than the adult kidney. There is a higher concentration of glutamate in the neonatal kidney that probably inhibits the action of glutaminase. In addition, while the adult kidney can increase ammonia production by 10-fold during acidosis, the neonate cannot. Thus, when neonates become acidic (for instance with diarrhea) it takes them much longer to recover from the acidosis.

The neonate, as the adult, must excrete acid generated form metabolism in the form of ammonia and titratable acid. While adults need to excrete about 1 mEq/kg per day of acid, neonates need to excrete 2 to 3 times this amount because of their protein intake and the formation of new bone. As we have just seen, the transport mechanisms for the excretion of acid undergo complex developmental changes. We will next review how the neonate manages to survive and grow and also review some of the disturbances that affect neonates.

Acid Base Disturbances

So how does the growing infant survive with these developmentally low rates of acid excretion? Acid-base balance is maintained in part by the ingestion of base equivalents contained in the mother’s milk. The mineralization of bone places a huge demand on the kidney to excrete acid, which it probably could not keep up with. Thus, the ingested base equivalents help keep the growing infant in acid base balance at a time when its kidney’s ability to excrete acid is underdeveloped.

Premature neonates have tubules which are much less mature that that of the term neonate. Therefore, very premature neonates can have a generalized proximal tubule transport disorder known as the Fanconi syndrome. They will have glucosuria with normal serum glucose levels, aminoaciduria, and lower serum bicarbonate levels. Administration of bicarbonate does little to improve growth in very premature neonates.

Another common problem for neonates is the loss of bicarbonate due to gastrointestinal disturbances. The pancreas secretes bicarbonate into the small intestine to neutralize the stomach acid. When the baby develops diarrhea, much of this bicarbonate is lost and the infant becomes acidic. Several aspects of development make this a severe problem in the infant. First, the growing infant is dependent on base equivalents in the mother’s milk to help maintain acid base balance. When the infant has diarrhea, milk is often withheld from the neonate, which deprives him of this source of base. Second, while the ammonia production of the kidney of the neonate may help keep him in balance under normal conditions, unlike the adult, there is limited ability of the neonatal kidney to increase NH₃ production to replace
the bicarbonate that was lost in the gastrointestinal tract. Thus, it will take the infant much longer than an adult to recover from the loss of bicarbonate. And last, the tubules’ (both proximal and distal) ability to secrete protons is also somewhat limited, which will again cause the infant to take a long time to recover from the acidosis.

These same factors probably also limit the infant’s ability to recover from acidosis generated by sepsis (ie, lactic acidosis) or from the administration of amino acids in the TPN. Arginine in the TPN is a source of HCl. One must remember that large amounts of arginine in the TPN of premature infants can result in a metabolic acidosis.

**Inherited Defects in Acidification**

We will next consider some of the genetic defects that result in acid-base disturbances. In general, defects that interfere with the reabsorption of bicarbonate in the proximal tubule or with the secretion of protons in the collecting duct lead to renal tubular acidosis. This form of metabolic acidosis is characterized by a low bicarbonate concentration and an elevated chloride concentration, thus the anion gap in these patients is normal. A discussion of metabolic acidosis with an elevated anion gap is beyond the scope of this review.

The proximal tubule reabsorbs not only bicarbonate, but glucose, phosphate and amino acids. Most disorders that cause a defect in proximal tubule acidification also involve these other solute transporters and are referred to as the Fanconi syndrome. These solutes are all transported by sodium coupled transporters, thus, the common factor to these transport processes is the luminal membrane sodium concentration gradient. So the Fanconi’s syndrome results from a defect in maintaining the luminal membrane sodium concentration gradient.

The most common inherited defect that causes this is cystinosis. Cystinosis is a defect in the lysosomal transporter for cystine and leads to accumulation of cystine in the lysosomes. This then leads to depletion of ATP as the energy source for the sodium-potassium ATPase and the sodium concentration gradient is dissipated. Thus, all of the lumen transporters that are sodium coupled have a lower rate of transport, which results in bicarbonaturia, glucosuria, phosphaturia, and aminoaciduria. The other defects that lead to proximal tubule acidosis probably have the same mechanism and include tyrosinemia, galactosemia, and inherited fructose intolerance. Rarely, proximal tubule acidosis can be inherited without the Fanconi syndrome. Recently, a defect in the sodium-bicarbonate co-transporter was found that caused proximal tubular acidosis and eye defects.

Defects in the collecting duct’s ability to secrete protons lead to distal renal tubular acidosis. The primary inherited defect that has recently been characterized is in the H^+/ATPase. This transporter has multiple subunits. The most commonly inherited defect is in the β-subunit. This subunit is also found in the inner ear, so children with this defect have sensorineural hearing loss. Another form of distal RTA that is inherited as an autosomal dominant trait is due to a defect in the basolaterally located chloride-bicarbonate exchanger, AE1.

Another rare form of renal tubular acidosis is due to a defect in carbonic anhydrase. This causes a combined proximal and distal renal tubular acidosis. Because carbonic anhydrase is also involved in the mineralization of bone, these patients also develop osteopetrosis.

Renal tubular acidosis can also be acquired. The most common cause of this is due to administration of drugs. Aminoglycosides, in particular gentamicin, has been associated with proximal renal tubular acidosis and the Fanconi syndrome. Amphotericin B has been associated with distal tubular acidosis. It is thought that amphotericin B causes an increase in the permeability of the distal nephron so that the tubule cannot maintain the necessary hydrogen ion concentration gradient to excrete acid.

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