3 Renal Tubular Development

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Organization of the Nephron

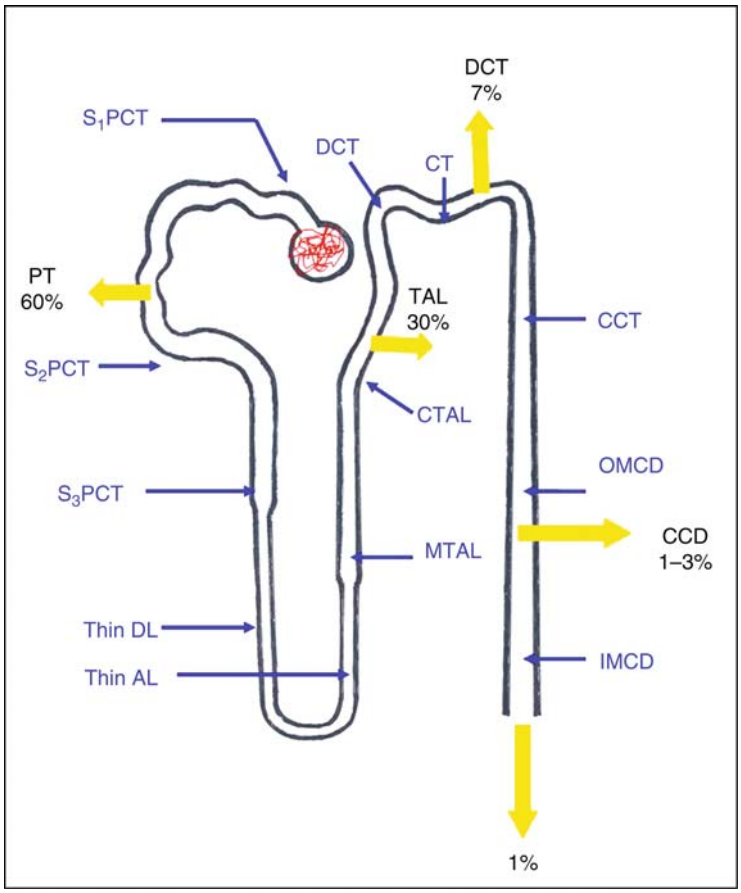
The kidney is faced with the enormous task of maintaining a constant composition and volume of the extracellular fluid. The adult ingests nutrients and water and generates waste products that must be eliminated to maintain this balance. In other words, the amount of electrolytes that are ingested and absorbed must be eliminated and the waste products from metabolism must also be excreted. This challenge is all the more complex as our dietary intake is quite variable from day to day. Despite this variable intake, there is virtually no change in the volume or composition of the extracellular fluid volume from day to day.

There are two possible ways that our kidney could balance ingestion and excretion. The kidney could be a secretory organ, from where all the excess solutes and water would be excreted by tubular secretion. This would be very inefficient and require an enormous amount of energy. In addition, in times of a disturbance in the extracellular fluid volume such as a high salt intake or volume loss from diarrhea, for example, the regulatory systems necessary to maintain a constant extracellular fluid volume and composition while excreting waste products would be very complex. On the other hand, the kidney could filter an enormous quantity of extracellular fluid, which would be very efficient in removing waste products, and reclaim the desired salt, organic solutes and water. The mammalian kidney actually uses both mechanisms to perform its job, which is necessary for our survival on land. The adult kidney filters ~150 l of isotonic fluid a day and reclaims most of it, leaving the waste products to be excreted. In addition, there are secretory processes for solutes such as organic anions and cations in the proximal tubule and secretory mechanisms to excrete the excess acid generated from metabolism in the distal nephrons, which aid in maintaining homeostasis.

To accomplish the remarkable task of reclamation of the necessary solutes and water in the filtered load, the mammalian kidney has evolved into a highly specialized organ with one million units called nephrons. Each nephron is a tube consisting of epithelial cells and is divided into 12 specialized segments as shown in *Fig. 3-1*. The epithelial cells allow the vectorial transport of solutes. The proximal tubule is responsible for the bulk reclamation of solutes and for the secretion of organic cations and anions. Approximately two-thirds of the glomerular filtrate is reabsorbed by the proximal tubule in an isotonic fashion. Virtually all of the organic solutes, as well as the majority of bicarbonates, phosphates, and chlorides, are reabsorbed in this segment. The proximal tubule is divided into S_1, S_2, and S_3 segments, on the basis of the rates of transport of some solutes, and morphological changes that occur down the proximal tubule. The nephron makes a hairpin turn, which aids in the generation of concentrated urine. The length of the thin descending and ascending limb is variable among species, with desert rodents having very long thin limbs as they need to conserve water and excrete very concentrated urine. The length of the thin ascending and descending limbs increases as one goes from the superficial cortex down to the medulla. The thin descending limb expresses aquaporin 1 on the apical and basolateral membranes and is very permeable to water (255), but is impermeable to solutes. This results in a concentrated fluid in the medulla with a very high sodium chloride content, providing a passive driving force for sodium chloride diffusion in the thin ascending limb. The thin ascending limb is impermeable to water but has a high permeability to NaCl (145). The chloride channel (CLC-K1) in the thin ascending limb is developmentally regulated (160). There is no expression in the fetus and until the end of the first week of life, in rats. There is a correlation between CLC-K1 and urinary osmolality, suggesting the important role of this channel in generating a hypertonic medulla (160). Diffusion of NaCl causes a high interstitial osmolality. This loop structure, along with the thick ascending limb, generates the countercurrent multiplication system that results in a medullary osmolality far greater than that of blood (162, 258). The importance of the passive properties of the thin limbs in this counter current system is exemplified in mice which are deficient in the water channel designated aquaporin 1, and do not express aquaporin 1 in the thin descending limb (182). The urine osmolality of aquaporin 1 deficient mice is greater than plasma, but far less than control mice expressing

Figure 3-1

This cartoon depicts the nephron with its 12 segments. Shown in blue are the nephron segments. S_1, S_2, S_3 PCT depict the three segments of the proximal tubule. The loop of Henle consists of the thin descending limb (thin DL) and thin ascending limb (thin AL), the medullary (MTAL) and cortical thick ascending limb (CTAL). The distal convoluted tubule is comprised of the (DCT), connecting tubule (CT) and the initial cortial collecting tubule. The collecting duct is made up of the cortical collecting tubule (CCT), outer medullary (OMCD) and inner medullary collecting duct (IMCD). Shown in yellow is the percentage of sodium reabsorbed by the proximal tubule (PT), thick ascending limb (TAL), distal convoluted tubule (DCT) and cortical collecting duct (CCD). One percent of the filtered sodium is excreted.



aquaporin 1 (182). Unlike control mice, aquaporin 1 knock-out mice cannot increase their urine osmolality in response to water deprivation.

The thick ascending limb is the segment responsible for ~30% of sodium chloride transport and has a vital role in generating a concentrated medulla. Apical sodium chloride absorption is mediated by the bumetanide sensitive cotransporter. One of the unique features of this segment is that it is impermeable to water and thus the fluid leaving this segment is hypotonic to blood. In addition, this segment has a very high paracellular permeability to cations and is responsible for much of calcium and magnesium transport. The distal convoluted tubule is responsible for ~5–10% of NaCl transport. NaCl transport in this segment is mediated by the thiazide sensitive cotransporter. Active transcellular calcium and magnesium transport also occurs in this segment.

The rest of the distal tubule is separated into the connecting tubule and the cortical, outer and inner medullary collecting tubule. These segments are responsible for potassium secretion, final urinary acidification and water absorption, the latter mediated by the action of vasopressin. While the fraction of salt transport and renal acidification is only a fraction of that in other

nephron segments, the collecting tubule is responsible for the final modulation of the tubular fluid. Thus, the final composition of urine and significant regulation of transport occur in this segment.

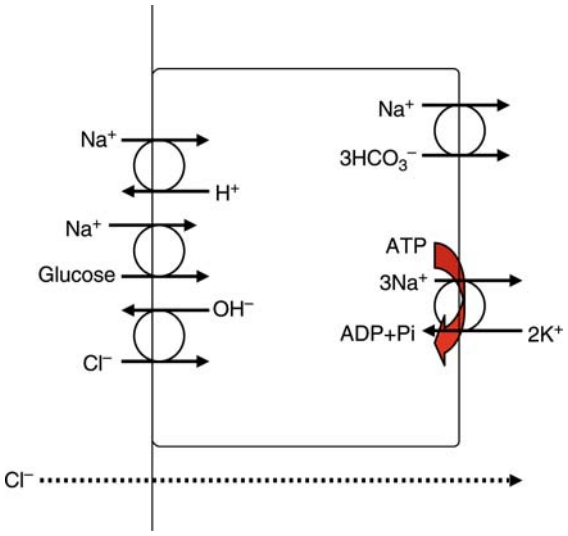
Principals of Membrane Transport

The cells along the nephron are quite different in the various nephron segments, as will be discussed in the subsequent sections. The cells in each nephron segment are poised for vectorial transport. The apical and basolateral membranes are, by and large, a lipid bilayer which would be impermeable to water and solutes if there were not specific proteins to facilitate transport across the apical and basolateral membranes. In addition, many transporters are regulated to adjust their rate of transport to meet the physiologic changes in volume status or concentration of solutes in the extracellular milieux.

The reabsorption of solutes along the nephron is characterized by active and passive transport processes. A typical cell is shown in *Fig. 3-2.* It should be appreciated that if all active transport was inhibited along the nephron, we would excrete urine with the composition and volume of the glomerular ultrafiltrate. Passive transepithelial transport is, by and large, the result of gradients generated by active transport. Most active transport is the result of the basolateral Na^+-K^+-ATPase. This transporter pumps three sodiums out of the cell in exchange for two potassium ions. The pump utilizes ATP and it is an example of primary active transport. This pump is vital to the generation of the low intracellular sodium and high intracellular potassium concentration as well as the negative intracellular potential difference across the apical and basolateral membranes. Both the low intracellular sodium and this potential difference can provide a driving force for secondary active transport. For example, in *Fig. 3-2*, the reabsorption of glucose via a sodium-dependent transporter utilizes both the sodium gradient and the relative negative cell potential to bring glucose to the cell. The Na^+/H^+ exchanger on this cell is electroneutral and utilizes the sodium gradient to secrete protons and reabsorb sodium. Both the sodium glucose and the Na^+/H^+ exchanger are secondary active transport processes dependent on the basolateral Na^+-K^+-ATPase. The secretion of protons will cause the luminal pH to drop, providing a favorable driving force for the Cl^-/OH^- exchanger, an example of tertiary active transport. Thus, in secondary and tertiary active transport, the transporters do not utilize ATP directly; however, inhibition of the ATP-dependent Na^+-K^+-ATPase would bring these transport processes to a halt.

Figure 3-2

A proximal tubule cell which shows the Na^+-K^+ ATPase on the basolateral membrane, an example of primary active transport. Na^+-K^+ ATPase decreases the intracellular sodium to about 10 mEq/l and increases the intracellular potassium to approximately 140 mEq/l. The pump is electrogenic with a cell negative potential of about 60 mV. The sodium gradient provides the driving force for the apical Na^+/H^+ exchanger and both the sodium gradient and the potential difference provide the driving force for the apical sodium glucose transporter. The secretion of protons via the Na^+/H^+ exchanger results in the driving force for the Cl^-/OH^- exchanger which is an example of tertiary active transport. Chloride is shown transversing the paracellular pathway. Bicarbonate is exiting the basolateral membrane via a sodium bicarbonate cotransporter.



In addition to active transport, a substantive amount of passive transport occurs between the cells across the tight junction. Active transport along the nephron will generate ion and solute gradients between the lumen and peritubular fluid. Depending on the permeability properties of the tight junction, passive absorption or secretion can occur. In the cell depicted in *Fig. 3-2*, there is passive chloride transport across the paracellular pathway. It has become apparent that the characteristics of the tight junction vary along the nephron. The tight junction creates the primary permeability barrier to the diffusion of solutes across the paracellular pathway. Occludin and claudin proteins are localized to junctional fibrils and are transmembrane components of tight junctions (4, 199, 200). These tight junction fibrils or strands are a major factor determining the permeability properties of the

paracellular pathway (4, 75, 75, 76, 76). The claudin family of tight junction proteins now numbers 24. Occludin has a ubiquitous distribution and is not responsible for the differential permeability properties in the various nephron segments. The claudin isoforms present at the tight junction of various epithelia determine the resistance and the permeability properties of the epithelia (4, 75, 75, 76, 76). The distribution of claudin isoforms varies along the nephron and is responsible for the unique permeability properties of each nephron segment.

The final form of passive transport is called solvent drag. Solvent drag has been postulated to be responsible for a small fraction of transport in the proximal tubule. The reabsorption of solutes could result in water movement that could entrain or carry solutes with it. For this to occur, the solute should have a low reflection coefficient or high sieving coefficient (sieving coefficient = 1/refection coefficient). In other words when a solute is entrained in fluid and hits a membrane or tight junction, it could pass through it and be transported or bounce off and not be transported. Direct measurements of solute drag in the proximal tubule of neonates and adults have shown that it contributes to a negligible fraction of transport (79, 146, 230, 233).

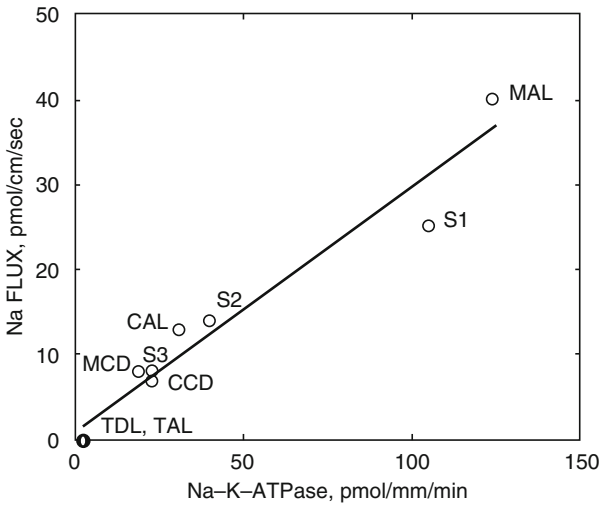
Maturation of Na^+-K^+-ATPase along the Nephron

The Na^+-K^+-ATPase is located on the basolateral membrane of most tubules in the kidney. It is a heterodimer composed of an α and β subunit. There are four different α subunits and 3 β subunits on mammalian cells which have different functional properties (47) There is also evidence for a small γ subunit that is not required for Na^+-K^+-ATPase activity (47, 244). The γ subunit binds to the α subunit, stabilizes the enzyme, and plays a regulatory role in enzyme activity (47, 244). The adult kidney expresses the α1 and ß1 isoforms of the Na^+-K^+-ATPase (100, 214). The α subunit is the catalytic subunit and has the cation, ATP and ouabain binding sites (47). The β subunit is the regulatory subunit and is essential for the function of the enzyme (47, 189). Several hormones that regulate sodium transport along the nephron act, at least in part, by regulating Na^+-K^+-ATPase activity (42, 47, 99, 109, 154, 188, 208, 264–268, 311).

The Na^+-K^+-ATPase is responsible for lowering the intracellular sodium concentration and establishing the negative cell potential difference. Thus, the Na^+-K^+-ATPase provides the driving force for sodium transport across the nephron. As shown in *Fig. 3-3*, there is a direct relationship between sodium transport and Na^+-K^+-ATPase activity factored per millimeter of tubule along the nephron (111). Neonates have a lower renal Na^+-K^+-ATPase activity than adults (10, 11, 109, 271, 274, 325). As will be discussed, there is a developmental increase in sodium transport in each nephron segment, which is paralleled by an increase in Na^+-K^+-ATPase activity as shown in *Fig. 3-4*.

Figure 3-3

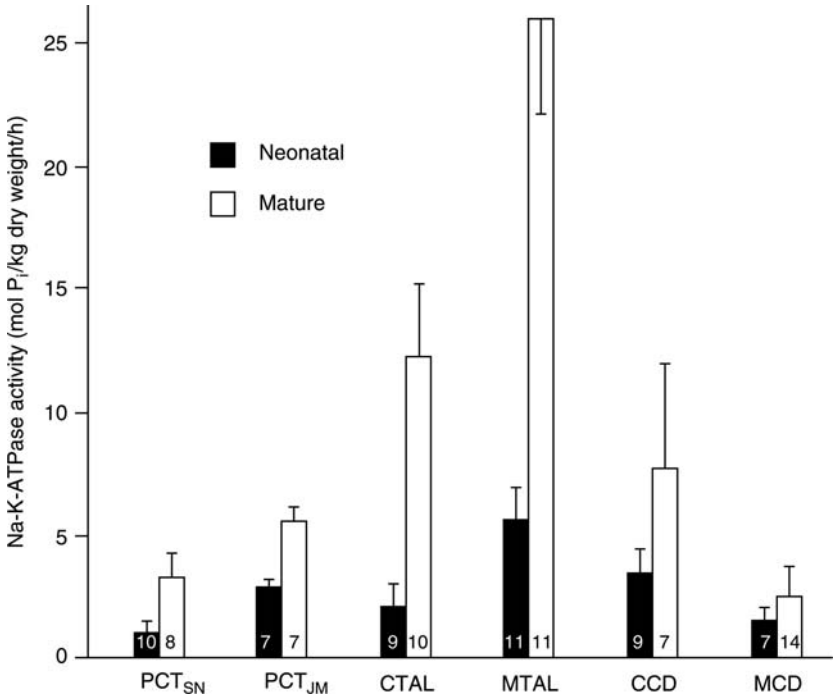
Sodium transport is plotted against Na^+-K^+ ATPase activity. As is shown, the rate of sodium transport in various nephron segments parallels Na^+-K^+ ATPase activity (111).



The parallel maturational increase in sodium transport with Na^+-K^+-ATPase activity (274) along with the striking relationship between sodium transport and Na^+-K^+-ATPase activity (111), suggests that the maturational increase in apical sodium transport may contribute to the postnatal increase in Na^+-K^+-ATPase activity. In cell culture studies an increase in intracellular sodium caused a stimulation in Na^+-K^+-ATPase activity (126, 166) as well as an increase in the α subunit mRNA and membrane pump density (80). In addition to in vitro studies, there is evidence that a chronic increase in Na^+/H^+ exchanger activity induced by metabolic acidosis, increased Na^+-K^+-ATPase activity, an effect that was blocked by coadministration of the Na^+/H^+ exchange inhibitor, amiloride (108). Finally, there is a postnatal increase in both serum thyroid hormone and glucocorticoid levels with age (28, 131, 132, 323). Both glucocorticoids and thyroid hormone have been shown to increase Na^+-K^+-ATPase activity (11, 68, 70, 71, 112, 208).

Figure 3-4

Na^+-K^+ ATPase activity is shown in nephron segments of neonates and adults. As is demonstrated, there is a maturational increase in Na^+-K^+ ATPase activity in every nephron segment (271).



Proximal Tubule Transport

Proximal tubule transport is characterized by a phenomenon called threshold, which is depicted in *Fig. 3-5.* It is the threshold that keeps our serum bicarbonate at 25 mEq/l. If we were to ingest bicarbonate and try to raise the serum bicarbonate level, we would have a bicarbonaturia and our serum levels would return to 25 mEq/l as long as we were euvolemic. Our serum glucose is set by other factors well below the threshold level. As shown in *Fig. 3-5*, if we increased the serum glucose level, we would reabsorb more glucose until the load of glucose delivered to the proximal tubule exceeded its ability to reabsorb glucose and we would have glucosuria.

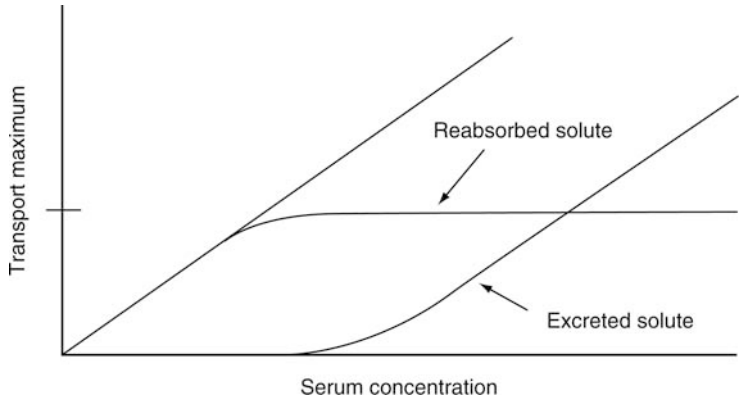
In the adult kidney there is a parallel change in proximal tubule transport with alterations in glomerular filtration rate. This phenomenon has been designated glomerular tubular balance. If this did not occur, an increase in glomerular filtration rate would swamp the distal nephron with solutes and water and there would be a huge natruresis and diuresis. A similar phenomenon must occur during postnatal development. There must be a parallel increase in proximal tubule transport with the maturational increase in glomerular filtration rate. If this did not occur the neonate would die of dehydration when the glomerular filtration rate increased after birth.

In the neonate there is a concomitant increase in tubular transport to accommodate or balance the increase in glomerular filtration rate (140, 163, 305). However, glomerular tubular balance is not present in the fetus (198). Renal development is characterized by centrifugal maturation. The surface nephrons are relatively immature compared to the juxtamedullary nephrons. These immature nephrons with short proximal tubules have glomerular tubular imbalance (198). This is clinically relevant as neonates born before 34 weeks of gestation can have glucosuria and very premature neonates can have significant salt wasting (12).

The proximal tubule reabsorbs 60% of the glomerular filtrate in an isoosmotic fashion. Due to the fact that the proximal tubule has a relatively high permeability to many ions, even solutes which are not actively transported by this segment get absorbed by the paracellular pathway. The luminal fluid concentration of magnesium, which is not actively transported, would rise over two-fold by the end of the proximal tubule as over half of the fluid is reabsorbed. This does not happen because magnesium is passively reabsorbed across the paracellular pathway as

Figure 3-5

This figure depicts the concept of renal threshold. As the delivered load increases to the tubule either by an increase in the serum concentration or an increase in glomerular filtration rate, the amount of solute absorbed increases. At some point, the renal tubular absorption reaches a maximum, called the threshold for that solute, and any further increase in the filtered load is excreted.



the magnesium concentration rises above that in the peritubular capillaries.

Glucose Transport

Glucose is reabsorbed solely by the proximal tubule. Physiologic studies have demonstrated that the S_1 proximal tubule reabsorbs glucose through a high capacity–low-affinity transporter, while in the late proximal tubule (S_3) glucose transport is via a low capacity–high affinity transporter (22). Similar axial heterogeneity of glucose transport kinetics was validated using cortical brush border membrane vesicles to measure apical membrane transport and outer medullary brush border membrane vesicles which contain vesicles from the S_3 segment (316). The high capacity–low affinity sodium dependent glucose transporter on the apical membrane is designated as SGLT-2 (332). This removes the bulk of the glucose from the glomerular ultrafiltrate. The low capacity–high affinity transporter is designated SGLT-1 (130, 143). The glucose that is transported by the tubule exits across the basolateral membrane by facilitative diffusion. As shown in *Fig. 3-6*, SGLT-2 transports one sodium with one glucose molecule while SGLT-1 transports two sodium with each glucose molecule. A defect in SGLT-1 causes glucose-galactose malabsorption as this transporter is also present in the intestine (95, 96, 193, 195). Some patients with familial glucosuria, a benign condition, have a mutation in SGLT2 (63, 184). This axial arrangement of glucose transporters results in reabsorption of virtually all the filtered glucose.

Sodium-dependent glucose reabsorption results in a positive charge entering the proximal tubule cell. This charge leaves a lumen negative transepithelial potential difference. This negative potential provides a driving force for the absorption of an anion or the back diffusion of a cation (sodium) across the paracellular pathway. Thus glucose transport can result in a net absorption of sodium chloride with sodium moving into the cell with glucose and chloride across the paracellular pathway. Whether sodium is recycled or chloride is reabsorbed is dependent on the relative sodium/chloride permeability of the paracellular pathway.

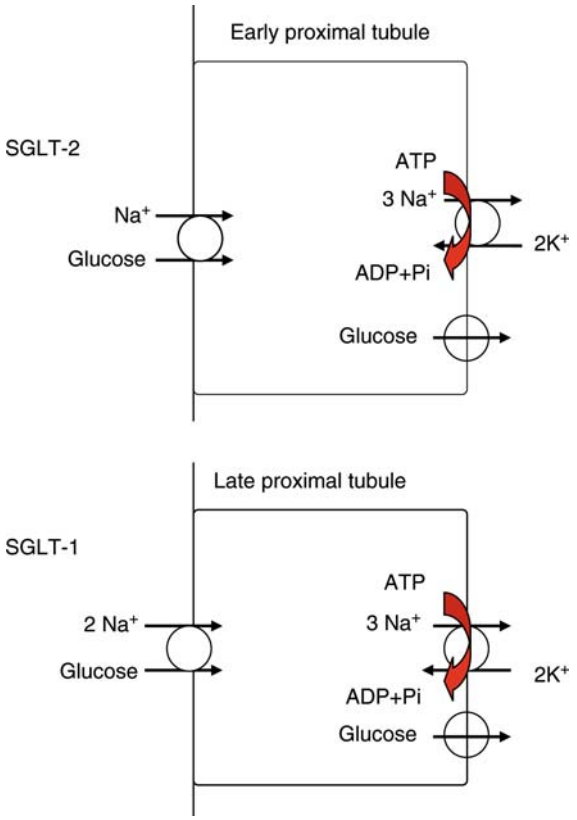
Numerous studies using various techniques and animal species have shown that the fetus and neonate transport glucose at a slower rate than the adult (13, 36, 106, 198, 273). These studies are of clinical relevance as premature neonates can have glucosuria (12, 127, 317). Despite the fact that the glomerular filtration rate is about 100th of that of the adult and the filtered load delivered to the neonatal nephron is also about 100th of that of the adult, the filtered load exceeds the reabsorptive capacity for glucose transport in the premature neonate. Thus there is a time during development when glomerular tubular balance is not present.

Amino Acid Transport

All amino acid transport occurs in the proximal tubule. Unlike most cells, which have amino acid transporters to provide substrates for protein synthesis, the proximal tubule mediates the vectorial transport of amino acids

Figure 3-6

Diagram of glucose transport in the proximal tubule. The early proximal tubule has SGLT-2 on the apical membrane which is a high capacity, low affinity transporter, while in the late proximal tubule the low capacity high affinity transporter, SGLT1 is on the apical membrane. Glucose exits the cell across the basolateral membrane by passive diffusion.



from the filtrate to the blood. The basic principal for transport is similar to glucose transport. The uptake of amino acids is sodium-dependent and electrogenic, with the basolateral exit mediated by facilitated passive diffusion. While there are 20 amino acids that are utilized in the synthesis of proteins, there are not 20 different amino acid transporters on the apical and basolateral membranes. Since some amino acids are similar in structure or charge, there is promiscuity among the classes of transporters. We will briefly discuss the three major classes of amino acid transporters.

The neutral amino acids include leucine, valine, isoleucine, methionine, phenylalanine, tyrosine, cysteine, glutamine, alanine, glycine, serine, histidine, tryptophan and proline. Transport of these amino acids is electrogenic with one sodium being transported with the amino acid across the apical membrane. B^0 AT1 (SLC6A19) has recently been cloned (158, 283). This transporter is expressed on the proximal tubule (250), and transports all neutral amino acids, though there is greater affinity for valine, leucine, isoleucine, and methionine (49). Mutation of SLC6A19 causes Hartnup disease which is an autosomal recessive disorder of variable expression, characterized by a pellagra-like rash, cerebellar ataxia, psychological and neurological disturbances (60, 158, 283). There are other neutral amino acid transporters but their transport properties have been less well characterized (60, 243) (250).

The acidic amino acids are aspartate and glutamate. They are transported across the apical membrane of the proximal tubule in an electrogenic fashion where two sodium ions are transported for each of these negatively charged amino acids (150, 257). Brush border membrane vesical studies have shown that there are at least two apical transporters for glutamate, one with a high substrate affinity and one with a low affinity (330). The high affinity transporter has been cloned and designated EAAC1 (149). EAAC1 is expressed on the apical membrane of the proximal tubule (288). Eaac-1 knockout mice have a dicarboxylic aciduria proving the importance of this transporter in acidic amino acid transport (218). The basolateral transport of glutamate is via a sodium dependent cotransporter, indicating that the intracellular glutamate levels must be very high in the proximal tubule to provide an adequate driving force for sodium exit across the basolateral membrane (256). In addition there is a sodium independent aspartate/glutamate transporter which is localized to the basolateral membrane designated AGT1 (186).

The basic amino acids lysine and arginine utilize the same amino acid transporter as cystine. There are a number of basic amino acid transporters (280). rBAT is a cystine/dibasic amino acid transporter expressed along the proximal tubule but predominantly in the S_3 segment (43, 103). rBAT protein is undetectable in the fetal kidney and is expressed at very low levels even after weaning (110). Mutations in SLC3A1, the gene encoding rBat, results in type I cystinuria (65, 66, 222, 223). B^{0+} AT is a recently cloned cystine transporter/dibasic amino acid transporter (102, 105). Unlike rBAT, B^{0+} AT is predominantly expressed in the early proximal convoluted tubule but it overlaps with the expression of rBAT (103, 201). While both rBAT and B^{0+} AT can function as cystine/dibasic amino acid transporters, they likely function in vivo as a heterodimer (219) (103).

Neonates have a generalized aminoaciduria which is more pronounced in premature neonates (59, 89, 279). While there have been numerous studies examining amino acid transport using a number of species, most

have utilized kidney slices or slurries of renal tubules. These studies are complicated by the fact that one is looking simultaneously at cellular uptake, often from a collapsed tubule, metabolism of the amino acid and basolateral exit. Examinations of glycine transport in tubule suspensions and kidney slices of neonates and adults have provided disparate results. Glycine uptake has been shown to be the same in neonates and adults in some studies (241, 252) and lower in neonates than adults in others (19, 191). The few studies looking at brush border membrane during development, a more direct way of studying uptake transport across the apical membrane, have shown lower rates of transport in the neonate, compared to the adult (73, 192).

Organic Acid Transport

In addition to filtration and reclamation, the kidney has the ability to secrete some substances through organic anion transporters. There are five organic anion transporters on the basolateral membrane (OATS) of the proximal tubule (20, 167, 282, 339, 344). Different OATS have different substrate specificities. Organic acids transported by OATS include prostaglandins, uric acid, nonsteroidal anti-inflammatory drugs, β lactam antibiotics, antiviral medications, para-amino hippurate, probenecid, uric acid, bumetanide, salicylates, methotrexate and many others (167, 282, 339). Many of these substances are protein bound in the blood which limits their excretion by glomerular filtration. The basolateral uptake of OATS is an example of tertiary active transport (167, 282). OATS take up organic anions into the cell predominantly in exchange for α-ketoglutarate. The α-ketoglutarate that is exchanged for the organic acid enters the cell via an α-ketoglutarate transporter. The energy for this whole process is the basolateral Na^+-K^+-ATPase. The organic acid transported inside the cell must exit across the apical membrane to enter the primordial urine. The mechanism for this is less well understood but includes members of the multidrug resistance protein family (167, 282, 339), which has been localized to the apical membrane of the proximal tubule (318).

Para-amino hippurate is almost totally removed from the blood with one pass through the kidney and is used as a measure of renal blood flow. Para-amino hippurate has been used to assess the maturation of renal blood flow in humans and to determine the maturational changes in organic anion transport. Studies in humans have shown that there is a maturational increase in para-amino hippurate secretion with adult values being attained at about 2 years of age (64, 253). Para-amino hippurate secretion is less in premature than term neonates (101).

There are a number of factors that could contribute to the maturational increase in organic anion secretion. Since organic anion secretion requires an organic anion transporter (OAT), a sodium dependent organic acid cotransporter and the Na^+-K^+-ATPase to mediate intracellular transport of the organic acid and an apical secretory mechanism to secrete the organic acid, a developmental paucity in any of these transporters, compared to the adult, could be the rate limiting step. OAT1 and OAT2 have been shown to be present in the late gestation fetus and mRNA and protein expression increase during postnatal development (207, 217).

One of the unique features of organic anion transport is that it can be induced prematurely during renal development by itself or another organic anion (134–136, 276). This is not true of adult animals where organic anions do not cause a stimulation in transport (134). In vitro microperfusion studies demonstrated that there was an intrinsic increase in the rate of transport with postnatal age in rabbits and that pretreatment with penicillin increased the rate of para-amino hippurate secretion in vitro (276). The mechanism of this induction of organic anion transport by organic acids is unclear.

Phosphate Transport

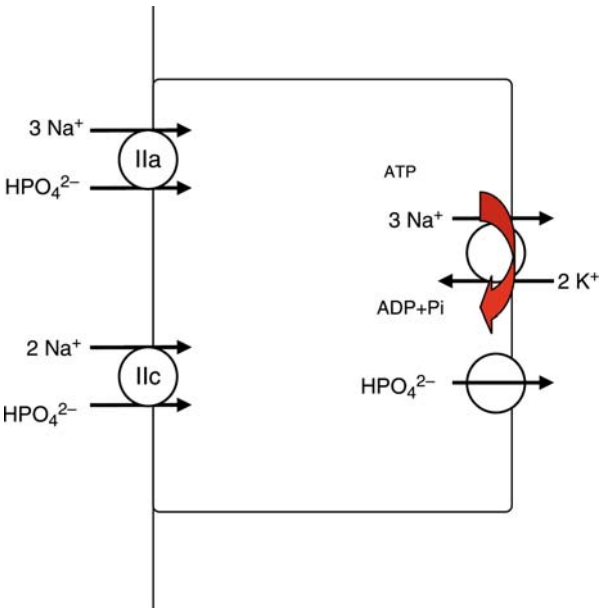
The adult ingests approximately 1–1.5 g of phosphorus a day and 80% of that is absorbed. The adult must be in neutral phosphorus balance: the amount of phosphorus absorbed from day to day has to equal that excreted. The phosphorus in our body is predominantly in the form of phosphate. At a pH of 7.4 there is a 4:1 ratio of HPO_4^{-2}/$H_2PO_4^{-1}$. The kidney maintains phosphate balance by its ability to regulate phosphate transport, which occurs predominantly in the proximal tubule. The main factors that regulate renal phosphate transport are dietary intake itself, and a number of hormones including parathyroid hormone, FGF-23, and growth hormone. Phosphate is essential for bone growth and 85% of our phosphate is in the bones. In addition, phosphate is involved in a myriad of enzymatic reactions and is present in nucleotides, phospholipids, and proteins. Unlike the adult, the neonate must be in positive phosphate balance for growth. The serum phosphate level is higher in the neonate than in the adult. This section will review phosphate transport and then discuss developmental changes which occur in transport and its regulation, which allow the neonate to be in positive phosphate balance.

The transporters involved in the regulation of phosphate transport are shown in *Fig. 3-7*. The first phosphate transporter cloned, designated NaPi-1, did not have the characteristics previously identified in physiologic studies and its function is still not clear (333) (44). There are two sodium-dependent phosphate transporters on the apical membrane of the proximal tubule, one designated NaPi-IIa (183), and the other NaPi-IIc (281). NaPi-IIa is an electrogenic transporter that transports three sodium ions with one phosphate HPO_4^{-2}, while NaPi-IIc transports two sodium ions with every phosphate and is electroneutral (107, 281). NaPi-IIa is regulated by PTH and dietary phosphate intake (155, 172, 173, 310). Phosphate exits the proximal tubule by a transporter which has not yet been identified and characterized. NaPi-IIb is the phosphate transporter on the intestinal apical membrane responsible for absorption of dietary phosphate (133).

The serum phosphate levels are higher in neonates than adults (77, 137). Since the glomerular filtration rate in the neonate is only a fraction of that of the adult, it is possible that this is the factor that is responsible for the relative hyperphosphatemia in neonates. However, early studies in human neonates found that the fraction of phosphate reabsorbed compared to the glomerular filtration rate was higher in neonates and infants than that in adults (77, 83, 137, 242). In the first 24 h of life, human neonates reabsorb over 95% of the filtered phosphate (137). This level drops to 90% later in the first week of life (77, 137). This fractional reabsorption of phosphate meets or exceeds the reabsorptive capacity of adults and older children eating a normal phosphate diet (242).

Figure 3-7

A proximal tubule cell reabsorbing phosphate is shown. The top apical phosphate transporter NaPi-IIa is the predominant phosphate transporter in adult rodents. NaPi-IIc is the predominant phosphate transporter on the apical membrane of neonatal rodents. NaPi-IIa is electrogenic while NaPi-IIc is electroneutral.



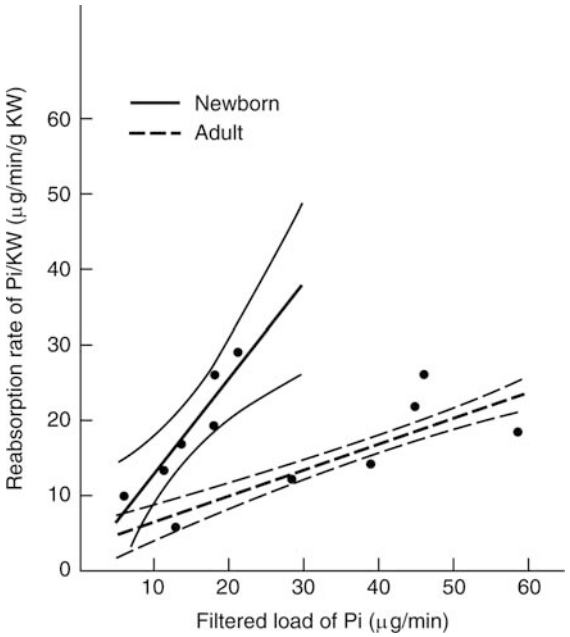
Animal studies demonstrated that young rats had a greater rate of phosphate reabsorption than adult rats (69). This was seen in rats that received parathyroidectomy, indicating that an altered response to parathyroid hormone was not the factor that caused the disparity in renal phosphate uptake (69). Finally, both young and adult rats responded to a low phosphate diet with an increase in the fractional reabsorption of phosphate; the magnitude of phosphate absorption was again higher in young animals (69).

While the above studies are consistent with an enhanced tubular reabsorptive capacity in neonates, this could be due to a higher reabsorptive capacity of the neonatal tubule, a diminished response to a phosphaturic factor or the result of an increased response to a substance which increases phosphate transport. To determine if there was an inherent increase in tubular phosphate transport, Johnson and Spitzer examined phosphate absorption in neonatal and adult kidneys perfused in vitro (147). As shown in *Fig. 3-8*, neonatal kidneys had a higher phosphate reabsorptive rate at any filtered load of phosphate which can only be due to an increased inherent rate of phosphate transport. In addition, they found that while addition of parathyroid hormone to the perfusate caused a phosphaturia in adult kidneys, there was no increase in phosphate excretion in neonatal kidneys. These studies directly demonstrate that neonates also have an attenuated effect of the parathyroid hormone, the primary factor regulating phosphate transport. A blunted effect of parathyroid hormone on phosphate reabsorption has also been demonstrated in young rats compared to adult rats in vivo (125, 328).

Micropuncture studies of neonatal and adult guinea pigs and rats have also demonstrated that there is a higher intrinsic rate of phosphate transport in young animals (153) (336). Studies using brush border membrane vesicles have demonstrated that the maximal rate (Vmax) of phosphate transport was several-fold higher in neonates than in adults, while there was no difference in the Km, the phosphate concentration at half maximal velocity (209). A low phosphate diet increased the Vmax of the

Figure 3-8

The rate of phosphate absorption by isolated perfused kidney preparation. As the filtered load increases, there is an increase in the reabsorptive rate in both the neonate and the adult kidney. At all filtered loads the rate of phosphate reabsorption per gram of kidney weight was higher in the neonate. From (147), with permission.



sodium phosphate transporter in adult guinea pigs, while a high phosphate diet had the opposite effect. There was no significant difference in Vmax in brush border membranes from neonates gavaged with different phosphate containing diets (209). As shown in *Fig. 3-9*, the 3-week-old rat has a maximal rate of phosphate absorption that was higher than that of adult rats (205). At all ages, there was an augmentation in the maximal capacity of phosphate reabsorption with a low phosphate diet (205). A greater effect of phosphate deprivation on brush border membrane vesicle phosphate uptake and NaP-IIa protein abundance was demonstrated in 4-week-old rats compared to older adult rats (336). Finally, the driving force for phosphate entry across the apical membrane may be greater in neonates. The intracellular phosphate concentration was almost 40% lower in kidneys measured using NMR (21).

Maturational studies examining the changes in NaPi-IIa expression have revealed that NaPi-IIa mRNA and protein are not detected in developing nephrons until there is a distinct brush border membrane (314). NaPi-IIa protein abundance was greater in brush border membranes from 13-day-old rats compared to 22-day-old rats (314). Others however have found that brush border membrane vesicle from suckling and adult rats had a slower rate of phosphate uptake than weanling rats (21 days old) (312). There was no change in NaPi-IIa mRNA abundance, but NaPi-IIa protein abundance from brush border membrane vesicles confirmed the transport findings that 21-day-old rats had the highest NaPi-IIa protein expression (312). Studies comparing 28-day-old rats to adult rats have also demonstrated greater brush border membrane NaPi-IIa protein abundance in young rats than in adults (336).

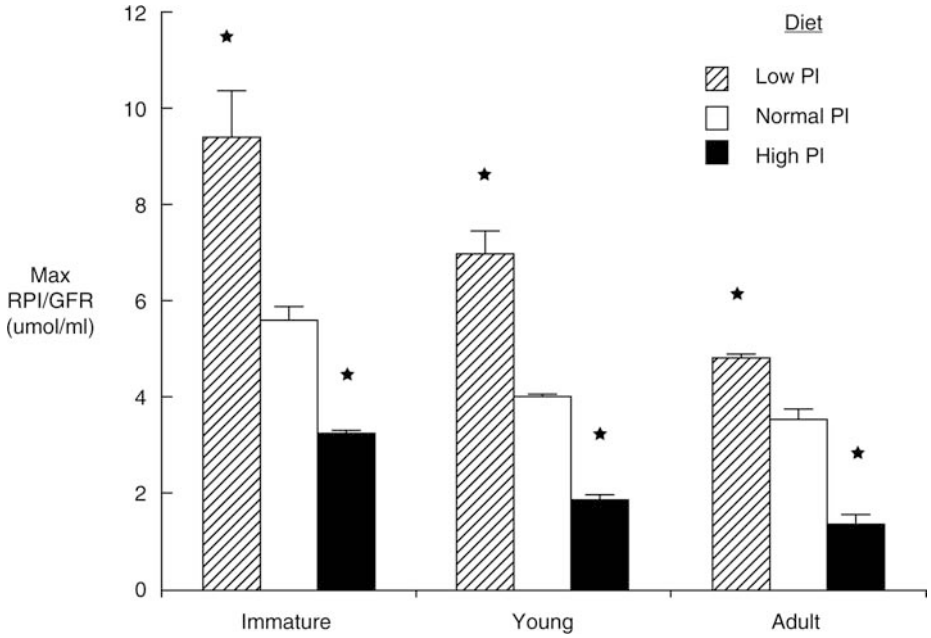
The high rates of phosphate transport in weanling rodents suggested that there may be a developmentally regulated transporter with greater expression at that of development (304). Indeed, NaPi-IIc was recently cloned and is a brush border phosphate transporter with its highest expression at the time of weanling in the rat (281). NaPi-IIc, like NaPi-IIa, is regulated by dietary phosphate uptake (281). The relative importance time of NaPi-IIa and NaPi-IIc may be quite different in humans. Patients with hereditary hypophosphatemic rickets with hypercalciuria, a rare autosomal recessive characterized by hypophosphatemia secondary to renal phosphate wasting and high vitamin D levels, have a mutation in the gene encoding NaPi-IIc, suggesting that NaPi-IIc may be the predominant renal phosphate transporter in humans and that NaPi-IIa cannot compensate for the loss of NaPi-IIc (40).

Growth hormone increases phosphate transport via stimulation of IGF-1 in the proximal tubule (228). Brush border membrane vesicle phosphate transport increased in dogs that were administered growth hormone compared to vehicle treated controls (122). While growth hormone is not a significant regulator of phosphate transport in the adult, this may not be the case in the growing animal. Administration of a growth hormone-releasing factor antagonist, which suppresses growth hormone secretion, has no effect on phosphate transport in adult rats, but significantly reduces phosphate absorption in young growing rats (124, 206, 338).

There are a number of hormones that have been shown to regulate phosphate transport including insulin (84), fibroblast growth factor-23 (34, 57, 291), frizzled-related protein 4 (41) and klotho (164, 227). These hormones may have differential effects on phosphate transport in the neonate and adult, but this is yet to be determined.

Figure 3-9

Age dependent maximal phosphate reabsorption is seen in 3–4 week old rats (immature), 6–7 week old rats (young), 12–13 week old rats (adult). All rats were parathyroidectomized. A low dietary phosphate intake stimulated phosphate absorption in all age groups. The maximal capacity for phosphate absorption was in the immature groups which need phosphate for growth. From (205), with permission.



Proximal Tubule Acidification

The proximal tubule reabsorbs 80% of the filtered bicarbonate. Luminal proton secretion is via the Na^+/H^+ exchanger and the H^+-ATPase. In the adult, one-third of proton secretion is via the luminal H^+-ATPase, and two-thirds is mediated by the luminal Na^+/H^+ exchanger that is designated NHE3 (24, 225). The secreted proton titrates the filtered HCO_3^- to generate H_2CO_3 which is converted to CO_2 and H_2O by luminal carbonic anhydrase (Carbonic anhydrase IV). CO_2 diffuses into the cell and combines with H_2O, which is facilitated by intracellular carbonic anhydrase (Carbonic anhydrase II) to regenerate H_2CO_3. H_2CO_3 dissociates into a proton that can be secreted across the apical membrane, and bicarbonate that exits the basolateral membrane via the basolateral $Na(HCO_3)_3$ symporter. The sodium which enters the cell via the Na^+/H^+ exchanger exits the basolateral membrane by the Na^+-K^+-ATPase, which provides the driving force for luminal proton secretion by the Na^+/H^+ exchanger. There are maturational changes in most of these processes that will be described below.

Neonates have a lower serum bicarbonate concentration than adults, which is the result of a lower threshold for bicarbonate (92). Premature neonates can have physiologic bicarbonate concentrations as low as 15 mEq/l (277). The lower bicarbonate threshold is mediated in large part by the lower rate of proximal tubule acidification. The fine tuning of renal acidification is mediated in the distal nephron which, by and large, is responsible for the secretion of acid from metabolism and new bone formation. Studies have shown that there is a maturational increase in bicarbonate absorption during postnatal development (30, 273), which accounts for the mutational increase in the bicarbonate threshold.

The greatest developmental changes in proximal tubule acidification occur on the apical membrane. There is a four-fold increase in Na^+/H^+ exchanger activity during postnatal maturation and an even greater increase in apical H^+-ATPase activity (23, 24, 284). Low levels of Na^+/H^+ exchanger activity have been measured in the fetus as well (37). Despite the fact that there was Na^+/H^+ exchanger activity on the apical membrane of the

neonatal rat at 1–2 weeks of age, as shown in *Fig. 3-10*, there was virtually no NHE3 on the brush border membrane (38, 284), the Na^+/H^+ exchanger on the apical membrane of the adult proximal tubule (45, 340).

NHE3 knock-out mice have been shown to have substantive proximal tubule apical membrane Na^+/H^+ exchanger activity (74). The apical Na^+/H^+ exchanger likely responsible for this non NHE3 Na^+/H^+ exchanger activity is NHE8, a recently discovered NHE isoform (116, 117). NHE8 has sodium dependent proton extrusion capabilities, which made it a potential candidate for the developmental isoform (345). As shown in *Fig. 3-10*, apical NHE8 was predominantly present in neonatal proximal tubules at a time when NHE3 was almost undetectable (38). Thus there is a developmental Na^+/H^+ exchanger isoform.

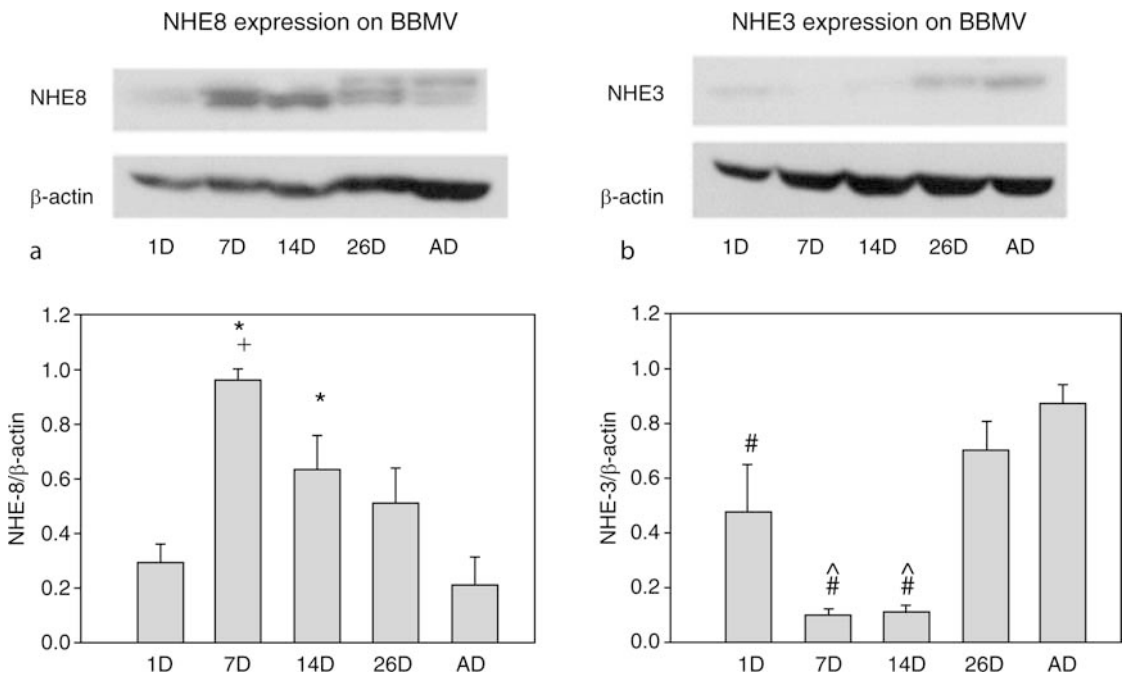
The $Na(HCO_3)_3$ symporter mediates bicarbonate exit in both the neonatal and adult proximal tubule. While there is a maturational increase in basolateral membrane $Na(HCO_3)_3$ symporter activity, it is relatively small compared to the magnitude of the change in Na^+/H^+ exchanger activity on the apical membrane (31). In addition to bicarbonate exit, the basolateral membrane Na $(HCO_3)_3$ symporter plays an important role in pH regulation of the proximal tubule cell (31).

Carbonic anhydrase which increases the rate of the interconversion of CO_2 and H_2O to Carbonic anhydrase II is located intracellularly in proximal and distal tubule acidifying cells and comprises ~95% of cell carbonic anhydrase activity. Carbonic anhydrase IV is on the apical and basolateral membrane of renal acidifying cells and comprises ~5% of carbonic anhydrase activity (275). Both carbonic anhydrase II and IV increase during maturation of proximal and distal acidification, but neither is likely a limiting factor causing the maturational increase in renal acidification in the proximal or distal tubule (335) (152, 278).

Since the developmental increase in renal acidification is due primarily to apical proton secretion, many studies have examined the cause for the increase in Na^+/H^+

Figure 3-10

Immunoblots of rat brush border membrane vesicles depict the changes in NHE8 (a) and NHE3 (b) protein abundance. As is seen, there is higher expression of NHE8 in the neonate than in the adult brush border membrane. The expression of NHE3 is highest in the adult. The higher NHE3 protein abundance at 1 day of age is likely the result of the surge of glucocorticoids at the time of birth. From (38), with permission.



exchanger activity. There is a substantive increase in both thyroid hormone and glucocorticoids during postnatal development and both hormones increase in parallel with the increase in proximal tubule Na^+/H^+ exchanger activity (28, 131, 132, 323). Administration of either glucocorticoids or thyroid hormone prior to the maturational increase in either hormone results in a precocious increase in exchanger Na^+/H^+ activity and NHE3 protein abundance (28–30). Both thyroid hormone and glucocorticoids increase NHE3 activity by increasing transcription (25, 67). Glucocorticoids have also recently been shown to increase the insertion of NHE3 into the apical membrane of proximal tubular cells by a posttranscriptional mechanism (48).

Interestingly, neither prevention of the maturational increase in glucocorticoids or thyroid hormone alone can totally prevent the postnatal increase in Na^+/H^+ exchanger activity and NHE3 mRNA and protein abundance (27, 28, 30, 287). Thus, there appears to be some redundancy in the postnatal maturational triggers for NHE3. As demonstrated in *Fig. 3-11*, prevention of the maturational increase in both hormones results in the total prevention of the postnatal increase in NHE3 mRNA, protein abundance and Na^+/H^+ exchanger activity (119).

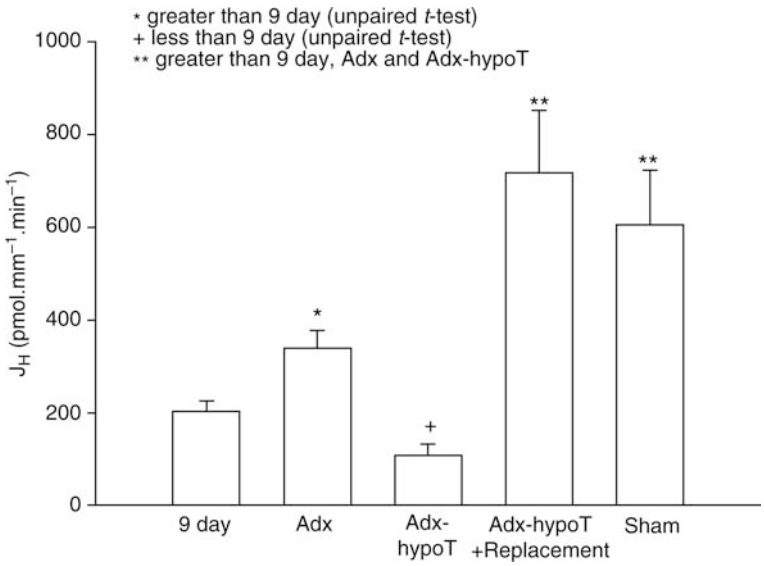
Proximal Tubule NaCl Transport

The glomerulus receives an ultrafiltrate of plasma and as shown in *Fig. 3-12*, immediately changes the luminal composition of the tubular fluid. The early proximal tubule reabsorbs glucose, amino acids and bicarbonate in preference to chloride (176, 239). The reabsorption of sodium with glucose and amino acids results in a lumen negative potential difference. This leaves the luminal fluid in the late proximal tubule with a higher chloride and lower bicarbonate concentration than that of the peritubular fluid.

The axial changes in luminal fluid create the potential for passive chloride diffusion across the paracellular pathway. The lumen to peritubular chloride gradient provides a driving force for the passive diffusion of chloride across the paracellular pathway. In the early proximal tubule, the transcellular reabsorption of sodium with glucose and

Figure 3-11

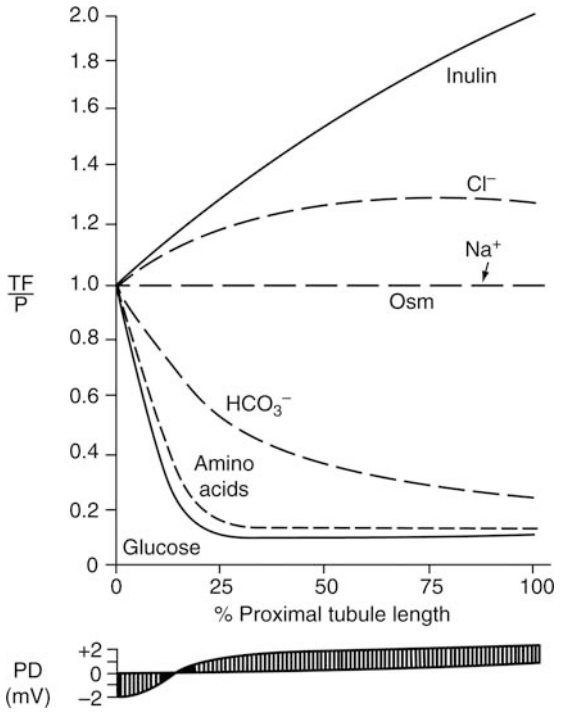
Apical Na^+/H^+ exchanger activity in perfused tubules in 9 day old, and adults that were adrenalectomized as neonates, adults that were adrenalectomized and hypothyroid since the neonatal period, adults that were adrenalectomized and hypothyroid (adx-HypoT) since the neonatal period but given thyroid and glucocorticoid replacement before study and sham controls. The 9 day old rats had a lower rate of Na^+/H^+ exchanger activity than the sham adult. Neonatal adrenalectomy did not totally prevent the maturational increase in Na^+/H^+ exchanger activity, but the maturational increase in Na^+/H^+ exchanger activity was abrogated in the adrenalectomized hypothyroid group. From (119), with permission.



amino acids results in a lumen negative potential difference as depicted in *Fig. 3-12*. This lumen negative potential provides a driving force for either chloride absorption or sodium secretion across the paracellular pathway. Whether chloride is absorbed or sodium is secreted is dependent on the relative permeabilities of chloride to sodium in the proximal tubule. The relative chloride to sodium permeability is higher in the adult than the neonate, so passive chloride transport secondary to the lumen negative potential difference in the early proximal tubule does not occur to a significant degree in the neonate (33, 230). As shown in *Fig. 3-12*, the luminal chloride gradient in the late proximal tubule results in a driving force for chloride diffusion across the paracellular pathway (33, 230). The diffusion of chloride across the paracellular pathway results in a lumen positive potential difference, and a driving force for the paracellular transport of sodium.

Figure 3-12

The axial changes in proximal tubular transport are depicted in this figure. The early proximal tubule preferentially reabsorbs glucose, amino acids and bicarbonate, leaving the luminal chloride solution higher than the blood in the peritubular capillaries. The early proximal tubule has a lumen negative transepithelial potential difference due to sodium dependent glucose and amino acid reabsorption. The higher luminal chloride concentration in the late proximal tubule provides a driving force for passive chloride absorption across the paracellular pathway, causing a lumen positive potential difference. From (239), with permission.



In the adult proximal tubule, approximately half of the sodium chloride is active and transcellular and half is passive and paracellular (3, 26, 287). Active chloride transport is mediated by parallel action of the Na^+/H^+ and Cl^-/base exchangers on the apical membrane (17, 286, 287, 290). It is still somewhat unclear what the nature of the base is as there is evidence for chloride exchange for hydroxyl, formate, and oxalate ions (14–16, 18, 165, 287). The rate of active transcellular chloride transport is lower in the neonate than in the adult. This is due to the lower rate of the apical Cl^-/base exchanger (285, 287), and the Na^+/H^+ exchanger discussed above (23, 24, 284). The driving force for active transcellular NaCl transport is the basolateral Na^+/K^+-ATPase that has lower activity in the proximal tubule (139, 271, 274). There are a number of factors that regulate proximal tubule NaCl transport, including renal nerves, dopamine, and angiotensin II, which are shown in *Table 3-1*. The serum levels of most hormones that regulate sodium absorption are equal or higher in the neonate than in the adult, but in general there is a blunted response to the action of most regulator hormones.

There are also changes in the properties of the paracellular pathway during postnatal development (1, 33, 230, 289). Most importantly, the permeability of the proximal tubule to chloride ions is less in the neonate than in the adult (33, 230, 289). The low permeability to chloride ions results in almost no passive paracellular chloride transport in the neonate (33, 230). As discussed, the permeability properties of an epithelium are determined by the expression of a family of proteins called claudins. The claudin proteins in the tight junction change during postnatal development. Claudins 6, 9, and 13 are present in the neonatal proximal tubule but not in the adult (1). The claudin isoform responsible for the low paracellular chloride permeability in the neonate as well as the factors that cause the claudin isoform changes during development are yet to be determined.

Of the potential factors that cause the maturational changes in paracellular chloride transport, only the thyroid hormone has been examined (32). Administration of thyroid hormone prior to the normal maturational increase results in an increase in chloride permeability. On the other hand, maintaining a hypothyroid state into adulthood prevents the maturational increase in chloride

Table 3-1

Comparison of the serum levels of hormones and their effect on sodium transport in neonates compared to adults

Hormone	Effect on urinary sodium excretion in adult	Neonatal serum level compared to adult	Effect of hormone on sodium in neonatal sodium excretion compared to adult	References
Renin		↑	Level responds appropriately in neonate	(88, 104, 114, 309)
Aldosterone	Increases distal sodium absorption	↑	↓	(8, 39, 104, 293, 307, 309, 319)
Atrial natriuretic peptide	Increases sodium excretion	↑	Probably (93) ↓	(58, 245, 251, 329)
Dopamine	Decreases proximal tubule sodium absorption	↑	↓	(9, 109, 151, 174)
PGE-2	Causes natriuresis and diuresis	Blunted synthesis in the medullary and cortical collecting tubule (269)	↓	(185, 215)

permeability. It is yet to be determined if thyroid hormone is the factor that causes the proximal tubule claudin isoform changes during postnatal development.

Proximal Tubule Water Transport

The proximal tubule reabsorbs most of the glomerular filtrate without a significant change in the luminal osmolality. For this to occur, the proximal tubule must be very permeable to water. Water movement is predominantly through the cell and not across the paracellular pathway (224, 231). The constitutively water permeable proximal tubule and thin descending limb have water channels on the apical and basolateral membranes (255). The isoform of this water channel was previously designated CHIP-28 but has been renamed aquaporin 1 (210–212, 226). Direct evidence of transcellular water transport comes from aquaporin 1 knock-out mice which have a marked decrease in proximal tubule sodium absorption (272). There is a paucity of water channels in the fetal kidney and an increase in expression of aquaporin 1 does not occur until birth (50, 303).

Direct measurements of water permeability have demonstrated that the neonatal rabbit proximal tubule has a higher water permeability that that of the adult (229). To determine the mechanism for the higher water permeability in neonatal rabbit tubules, studies were performed examining the water permeability of apical and basolateral membrane vesicles (202, 235, 234). Despite the higher transepithelial water permeability, the water permeability of the apical and basolateral membrane were lower in the neonate than the adult and there was less aquaporin 1 expression on both the apical and basolateral membranes of the proximal tubule of the neonate (234, 235). The apparent paradox between the higher water permeability in the neonatal proximal tubule and the lower water permeability of the apical and basolateral membranes was resolved with measurements of the contribution of the intracellular compartment to water movement in the neonatal and adult proximal tubule. The intracellular compartment was found to cause a large resistance to water flow and the neonatal proximal tubule intracellular compartment was less of a constraint to transcellular water movement than that of the adult (231).

The postnatal increase in glucocorticoids is a likely factor in mediating the above maturational changes in water permeability and aquaporin 1 expression. Administration of glucocorticoids to neonatal rabbits resulted in an increase in brush border membrane water permeability and aquaporin 1 expression (203). However, the developmental increase in thyroid hormone was shown not to be a factor mediating these postnatal maturational changes in water transport (204).

Thick Ascending Limb

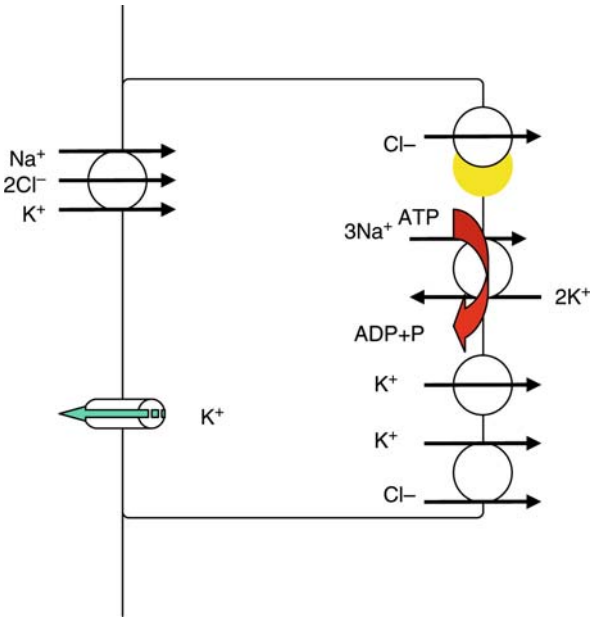
The thick ascending limb is responsible for the reabsorption of 30% of filtered NaCl. The thick ascending limb is

impermeable to water. The reabsorption of NaCl without water by the cortical and medullary TAL along with the distal convoluted tubule generates a luminal fluid with an osmolality of 50 mOsm/kg water. In the absence of ADH action on the collecting tubule, this dilute urine will be excreted. The medullary interstitial hypertonicity is largely generated by active NaCl reabsorption without water by that segment.

The reabsorption of NaCl is due to secondary active transport with the basolateral Na^+-K^+-ATPase generating a low intracellular sodium to provide a driving force for luminal sodium entry. A cartoon of a thick ascending limb cell is shown in *Fig. 3-13.* Sodium enters the cell via the furosemide or bumetanide sensitive $Na^+/K^+/2Cl^-$ cotransporter (128, 129). The $Na^+/K^+/2Cl^-$ cotransporter is electroneutral, yet there is a lumen positive transepithelial potential difference in the thick ascending limb (118). The positive luminal potential is generated by apical potassium recycling via the potassium channel ROMK1 (196, 341). With recycling of potassium back into the lumen, there is the net reabsorption of one sodium and two chloride ions which exit the cell across the basolateral membrane. Sodium exits the cell via the Na^+-K^+-ATPase. Chloride predominantly exits the cell via a chloride channel designated ClC-Kb (2, 156, 161). A subunit of this chloride channel designated barttin is important in the function of ClC-Kb (97). The lumen positive potential difference, generated by potassium secretion into the lumen, generates a driving force for the passive reabsorption of cations including Mg^{++} and Ca^{++}. The thick ascending limb has a very high permeability to magnesium and calcium ions, which results in a substantial fraction of filtered calcium and magnesium being reabsorbed passively across the paracellular pathway in this segment (56, 61, 62, 144, 248). The unique permeability properties of the thick ascending limb are due to the expression of claudin 16 which is mutated in familial hypomagnesemia with hypercalciuria and nephrocalcinosis where there is renal magnesium and calcium wasting (301).

Figure 3-13

The figure depicts a thick ascending limb cell. On the apical membrane is the $Na^+/K^+/2Cl^-$ cotransporter that is sensitive to loop diuretics and the potassium channel designated ROMK that is depicted as the green arrow. The basolateral membrane has a Na^+-K^+ ATPase, a KCl cotransporter, a chloride channel designated ClC-Kb, with its accessory channel designated barttin in yellow and a potassium channel. A loss of function mutation in the $Na^+/K^+/2Cl^-$ cotransporter, ROMK, ClC-Kb, or barttin results in Bartter's syndrome.



Mutations of the $Na^+/K^+/2Cl^-$ cotransporter (297), ROMK (179, 298), ClC-Kb (296) and barttin (46, 97) all cause Bartter's syndrome. Barttin is also expressed in the ear where it is a subunit of ClC-Ka and ClC-Kb. Mutations in barttin cause sensory neural hearing loss (46, 97).

Micropuncture studies of fluid from the early distal tubule showed that the osmolality of the fluid was significantly lower in the adult than in the neonatal rat (347). This is consistent with lower rates of sodium transport in the thick ascending limb but this study did not examine the latter parts of the diluting segment. Human neonates are able to dilute their urine to the same level as an adult (50 mOsm/kg water). This is vital as neonates ingest a hypotonic fluid, mother's milk. Sodium transport in the thick ascending limb has been directly examined in vitro and shown to be five-fold lower in the neonate than in the adult (138).

The $Na^+/K^+/2Cl^-$ cotransporter can first be detected in the mid-late gestation rat's thick ascending limb and macula densa (170). In the rat, there is a postnatal maturational increase in $Na^+/K^+/2Cl^-$ cotransporter, ROMK, Na^+-K^+-ATPase mRNA and protein abundance, but no change in ClC-K mRNA abundance (308). Administration of dexamethasone before the normal maturational increase at the time of weaning resulted in a premature increase in urinary concentrating ability and increase in $Na^+/K^+/2Cl^-$ cotransporter, Na^+-K^+-ATPase mRNA and protein abundance, but no change in ROMK protein abundance (308). There is also a postnatal maturational increase in thick ascending limb Na^+-K^+-ATPase activity

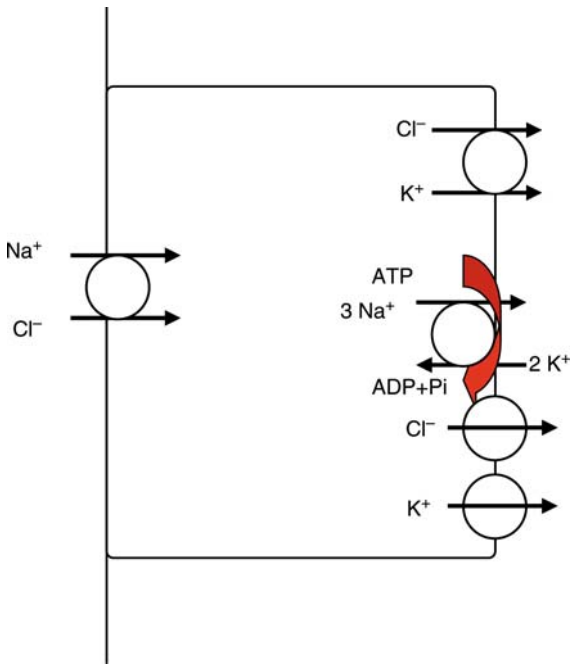
(238, 271) (87). The maturational increase in thick ascending limb Na^+-K^+-ATPase activity could be accelerated precociously by glucocorticoids and prevented by neonatal adrenalectomy (87, 237).

Distal Convoluted Tubule

The distal convoluted tubule reabsorbs approximately 7% of the filtered sodium. This segment is water impermeable and further reabsorption of salt without water occurs in this segment resulting in the nadir of luminal fluid osmolality. The transporters responsible for NaCl transport in the distal convoluted tubule are shown in *Fig. 3-14.* Sodium entry is via the thiazide sensitive cotransporter (94, 213, 292, 322). Mutations in the thiazide sensitive cotransporter cause Gitelman's syndrome (299, 300, 302). There is also evidence for parallel Na^+/H^+ and Cl^-/base exchange mediating sodium entry in the rat (324). The isoform of this Na^+/H^+ exchanger is NHE2 (72). Chloride exits the cell via a basolateral chloride channel ClC-K (178) and a KCl cotransporter KCNQ1 (175, 321). There is also a basolateral potassium channel (346). There is active transcelluar calcium and magnesium transport in the distal convoluted tubule but there is no information about the developmental expression and regulation of these transporters (81, 197).

Figure 3-14

Distal convoluted cell showing the transporters involved in active sodium reabsorption. The apical NaCl transporter is the thiazide sensitive cotransporter. Inactivating mutations in the thiazide sensitive cotransporter lead to Gitelman's syndrome.



The distal convoluted tubule is very difficult to study in vitro and in vivo. No studies have been performed to date, examining the relative abundance of the thiazide sensitive cotransporter in the neonate and adult. A study has been preformed that suggests that there is increased sodium absorption in 24- compared to 40-day-old rats (6, 115). Before detailing this study, it is important to know that studies have shown that the neonate is less able to excrete a salt load compared to the adult (5, 82, 115). For example, administration of isotonic saline equal to 10% of the dogs weight to an adult dog resulted in excretion of 50% of the salt load over 8 h but only 10% of the salt load was excreted in the 1–2 week old dog (115). This limited ability to excrete a sodium load was not due to the lower glomerular filtration rate in the neonate (115). Because sodium transport is less in the proximal tubule, thick ascending limb and collecting tubule, the nephron segment where there was avid neonatal sodium reabsorption was unclear. It must be stated that it was never considered that the volume of distribution of the saline infusion was greater in the neonate than the adult to account for these findings.

A micropuncture study suggested that the sodium retention in the neonate was the result of avid sodium reabsorption in the distal convoluted tubule (6). The tubular fluid to plasma Na/inulin (a volume marker) was greater in the early distal tubule in the 24-day-old neonate compared to the 40-day-old neonate due to decreased proximal and loop sodium absorption in the younger rat. This difference disappeared by the late distal tubule indicating that the 24-day-old rat had a higher rate of sodium absorption in that distal convoluted tubule. Volume expansion resulted in a higher distal delivery of sodium from the early proximal tubule of the 24-day-old rat, and there was less sodium remaining by the end of the distal nephron consistent with the distal convoluted tubule being the segment responsible for neonates' failure to excrete a sodium load compared to adults.

Urinary Concentration and Dilution

The urine exiting the distal convoluted tubule and entering the collecting duct has an osmolality of ~50 mOsm/kg water. Whether the urine will be of this osmolality or

maximally concentrated will depend on the presence or absence of vasopressin (ADH). Neonates can dilute their urine to nearly the adult level of nearly 50 mosm/kg water (7, 187, 249). Thus the neonates which imbibe mother's milk can excrete free water (169, 187, 249). However, unlike the normal adult where it is almost impossible to drink enough water to cause hyponatremia (30 l/day), neonates have a rather limited ability to generate and excrete free water and improperly mixed and dilute formula can result in hyponatremia (187).

The maximum urine osmolality of the human neonate is ~400–600 mOsm/kg water (123, 220, 334). The adult urine osmolality can be achieved at 1.5–2 years of age (334). There are many potential developmental factors that could limit the ability of the neonate to generate a maximally concentrated urine. The factors involved in urine concentration include:

1. The ability to sense an increase in serum osmolality or decrease in extracellular volume and secrete vasopressin.
2. There must be vasopressin receptors on the basolateral membrane of the collecting duct.
3. The collecting duct must be able to generate cAMP.
4. The loop of Henle must have generated a concentrated interstitium.
5. The architecture of the medulla must not limit the concentrating ability.
6. The collecting tubule must have aquaporins on the apical and basolateral membrane.
7. There must not be extracellular or intracellular mechanisms upregulated in the neonate, which limit urinary concentrating abilities.

During prenatal and postnatal maturation, there are anatomical changes which occur that likely affect urinary concentrating ability. There is an increase in the medullary capillary density, a decrease in medullary intersitial connective tissue, an increased presence and length of the thin limbs and the tubules become more tightly packed with cells in the loop decreasing in height with maturation (55, 315). The length of the papilla increased linearly from day 10 to day 40 in the rat (238). All these developmental anatomical changes are necessary for the counter current multiplication system to be maximally efficient. Accompanied by these anatomical changes are concomitant increases in the medullary sodium and urea concentration (238, 306). Administration of a high protein diet or urea to human neonates results in an increased ability to concentrate urine, implying that the ability of urea to accumulate in the medulla can be limited by dietary intake (90, 91). Adrenalectomy in neonatal rats prevented the maturational increase in urine osmolality while administration of glucocorticoids prior to the maturational increase caused a premature increase in urinary concentrating ability (238).

Principal cells in the collecting tubule and the cells in the medullary collecting duct express aquaporin 2 on the apical membrane and aquaporins 3 and 4 on the basolateral membrane (35, 52, 159, 313, 326). Aquaporin three null mice have diabetes insipidus whereas aquaporin 4 null mice only have a small concentrating defect after water deprivation indicating that basolateral water movement is primarily via aquaporin 3 (180, 181). Vasopressin causes the intracellular vesicles containing aquaporin 2 to fuse with the apical membrane resulting in the insertion of aquaporin 2 into the apical membrane (326). There is a developmental increase in aquaporin 2 expression (35, 52, 254, 342, 343). The maturational increase in aquaporin 2 is accelerated by administration of synthetic glucocorticoids prior to the normal postnatal increase in plasma glucocorticoids (343). Neither aquaporin 3 nor aquaporin 4 are factors impairing urinary concentration in the neonate (35, 157).

The fetus and neonate respond to increases in serum osmolality, stress and hypovolemia with appropriate increases in plasma vasopressin levels. The fetal sheep has an increase in plasma vasopressin with an infusion of hypertonic saline and increase in plasma osmolality (168, 294, 331). Plasma vasopressin also increases in fetal sheep in response to volume depletion induced by hemorrhage or diuretics (85, 168, 247) Despite the fact that the fetal lamb can increase serum vasopressin levels, infusion of vasopressin resulted in a blunted increase in urine osmolality compared to adult sheep (246). It is hard to assess these issues in humans. However, comparison between a relatively stressful vaginal delivery compared to a cesarian section has consistently demonstrated higher vasopressin levels in neonates born vaginally (86, 121, 221, 240). However, there was no correlation with vasopressin levels and the degree of perinatal asphyxia in one study (221), while there was a correlation in another (86). In total, it appears that the fetus and neonate can respond to appropriate stimuli with vasopressin secretion.

There is a developmental increase in vasopressin receptors in the kidney, however vasopressin receptor abundance does not appear to be a limiting factor in urinary concentration in the neonate (216, 236). Exogenous administration of vasopressin has been show to increase the urine osmolality in fetal sheep showing that vasopressin action on the collecting tubule is a prenatal event (246). Vasopressin acts in the collecting tubule by increasing cAMP. There is some discrepancy about the

extent of cAMP production during postnatal maturation in comparison to adults. The sum of the data indicate that while vasopressin stimulates cAMP production in the neonate, the amount of cAMP generated is attenuated compared to the adult (113, 148, 236, 270).

The response of the collecting tubule to vasopressin has been examined in vitro (54, 141, 232, 295). The neonatal water permeability did not increase in response to vasopressin comparably to that seen in the adult (54, 141, 232, 295). Studies have shown that the phosphodiesterase activity is greater in the neonatal collecting tubule (232). The water permeability of the neonatal collecting tubule was identical to the adult in the presence of a phosphodiesterase inhibitor demonstrating that the predominant factor limiting the action of vasopressin in the collecting tubule was the enhanced degradation of cAMP generated by vasopressin in the neonatal collecting tubule (232). Vasopressin increases prostaglandin production in the collecting tubule which attenuates the vasopressin mediated increase in cAMP production (51, 53). Vasopressin mediated cAMP production, while less in the neonatal collecting tubule, increases to adult levels in the presence of indomethacin, a prostaglandin synthesis inhibitor (53). Thus, prostaglandin production by the collecting tubule may attenuate the effect of vasopressin in the neonatal tubule to a greater extent than in the adult tubule (53).

Distal Tubule Acidification

The distal nephron makes the final adjustment in distal acidification. The distal nephron, under normal circumstances, secretes the protons equal to that generated from metabolism and in children the protons liberated from the formation of bone. The cortical collecting tubule has two types of cells that are involved in renal acidification. The α-intercalated cell is responsible for proton secretion and is shown in *Fig. 3-15*. There is also a β-intercalated cell, which can secrete bicarbonate when the animal is alkalotic or eats a diet with alkali content (190). This is unusual for humans but not for some mammals which can ingest an alkali diet (194). The β-intercalated cell has the reverse polarity of the α-intercalated cell but the Cl^-/HCO_3^- exchanger on the apical membrane of the β-intercalated cell is different from that on the apical membrane of the α-intercalated cell.

The neonatal cortical collecting tubule has fewer and less differentiated intercalated cells than that of the adult segment (98, 260). The rates of both bicarbonate secretion and luminal acidification are less in the neonate than in the adult (194, 260, 263). The intercalated cells in the inner medullary collecting duct were much more differentiated in appearance and secreted protons at a rate comparable to the adult (194, 263). The collecting tubule also has a luminal H^+-K^+ ATPase which causes proton secretion and potassium absorption from the luminal fluid. It is activated under states of metabolic acidosis and hypokalemia (120). The H^+-K^+ ATPase can function at comparable rates in the neonatal cortical collecting tubule to that of the adult (78).

Cortical Collecting Tubule Sodium Transport

While relatively little sodium is reabsorbed by the cortical collecting tubule compared to the proximal nephron segments, it is the final nephron segment responsible for regulating sodium absorption and thus is vitally important for the regulation of sodium homeostasis. Sodium absorption occurs in the principal cell of the collecting tubule which is shown in *Fig. 3-15*. Sodium transport is through the epithelial sodium channel designated ENaC which has three subunits. The driving force for sodium entry across ENaC is the low intracellular sodium concentration and the potential difference across the luminal membrane generated by the basolateral Na^+-K^+ ATPase. The Na^+-K^+ ATPase undergoes a maturational increase in this segment but is not the limiting factor for the maturational increase in sodium absorption (271). The abundance of ENaC on the apical membrane is by and large determined by aldosterone in the adult, but aldosterone has a blunted effect in the neonate, despite the fact that there are higher serum levels in the neonate and ample aldosterone receptors (8, 39, 104, 293, 307, 309, 319).

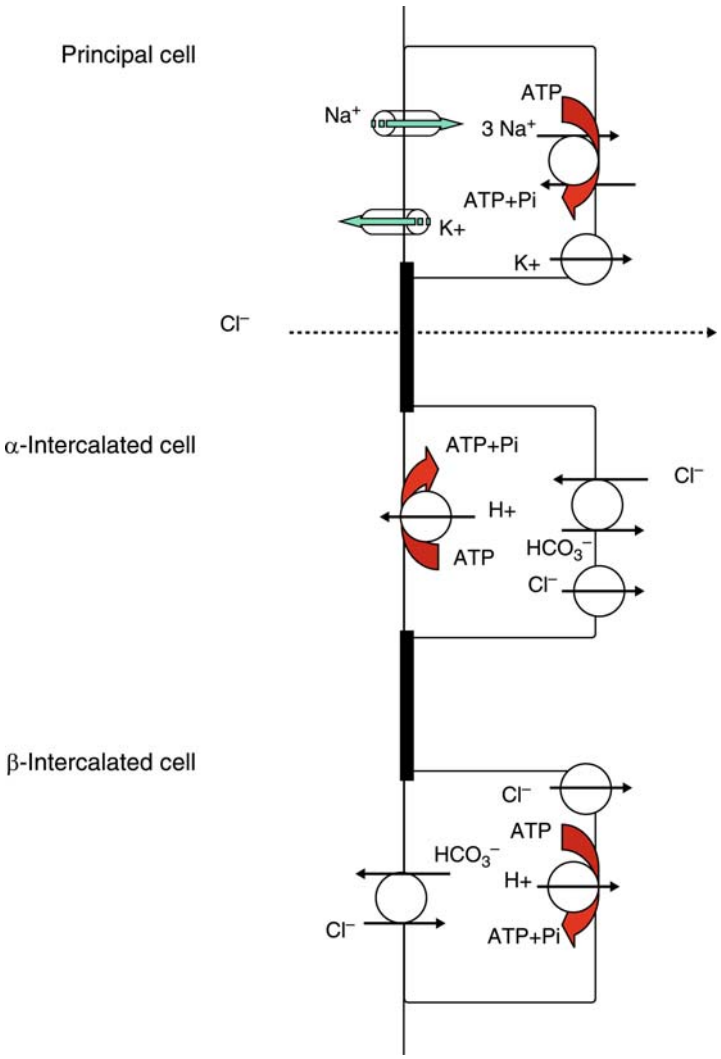
Sodium transport in the cortical collecting tubule increases with postnatal development (259, 319). This increase in sodium transport is paralleled by an increase in the apical expression of ENaC, as ENaC expression is the limiting factor in sodium absorption in this segment (261). ENaC is composed of α-, ß-, and γ-subunits and there is a developmental increase in mRNA and protein abundance of each during postnatal maturation (142, 261, 320, 327, 348).

Potassium Transport

Neonates have higher serum potassium levels than adults. Potassium is the predominant intracellular cation and neonates must be in positive potassium balance for

Figure 3-15

Three cells of the cortical collecting tubule are demonstrated. The principal cell is shown above with sodium entering the cell down its electrochemical gradient via a channel designated ENaC. This generates a lumen negative transepithelial potential difference. Potassium is secreted into the lumen down its electrochemical gradient via ROMK. Chloride is moving through the paracellular pathway down the electrical gradient generated by the lumen negative potential difference. The cell below is an alpha intercalated cell which secretes protons into the cell lumen via a H^+-ATPase. The bicarbonate generated in this process is secreted across the basolateral membrane via a Cl^-/HCO_3^- exchanger. The third cell is a beta intercalated cell which secretes bicarbonate ions in the face of metabolic alkalosis. This cell is the reverse of an alpha intercalated cell but the Cl^-/HCO_3^- exchanger is a different isoform.



growth, unlike adults, which excrete the quantity of potassium absorbed from their diet in their urine (120). Adult animals are more readily able to excrete an exogenous potassium load than is a neonate (177). Approximately half of the filtered potassium is reabsorbed in the proximal tubule of the adult and neonate by passive diffusion across the paracellular pathway (171). The loop of Henle reabsorbs 80% of the delivered potassium in the adult and only 45% in the neonate (171). Thus, the adult delivers approximately 10% of the filtered

potassium to the distal nephron while the neonate delivers 25%. The distal convoluted tubule, connecting tubule and cortical collecting duct are the sites of potassium secretion and final modulation of urine potassium excretion.

Potassium secretion in a principal cell is depicted in *Fig. 3-15*. Sodium enters the principal cell through the apical sodium channel down the favorable electrochemical gradient generated by the Na^+-K^+ ATPase on the basolateral membrane. This results in a lumen negative potential difference and a favorable electrochemical gradient for potassium secretion. While there is a maturational increase in the sodium channel and the Na^+-K^+ ATPase, these are not the limiting factors for potassium secretion. There are two potassium channels in the collecting tubule, one channel designated ROMK is on the apical membrane of principal cells and a flow-dependent channel that is activated by stretch designated maxi-K channel (120). There is a maturational increase in potassium secretion in cortical collecting tubules perfused in vitro (259). The secretion of potassium by principal cells is paralleled by the maturational increase in potassium channels (ROMK) on the apical membrane of the principal cell (262, 348). There is also a developmental increase in the maxi-K channel (337). As noted above and in *Table 3-1*, potassium secretion is regulated by aldosterone and there is resistance to the action of aldosterone despite higher serum levels in the neonate (8, 39, 104, 293, 307, 309, 319).

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