Renal Physiology

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Key Points

1. The kidney has wide array of responsibilities that range from regulation of fluid volume and osmolarity, management of electrolytes, elimination of endogenous and exogenous toxins, metabolic functions (for example, the production of hormones), and acid base balance.
2. The nephron is the functional unit of the kidney that is responsible for filtration, reabsorption, and secretion of compounds that the body must manage in order to maintain homeostasis.
3. The kidney receives approximately 20–25% of cardiac output.
4. The nephron is the functional unit of the kidney.
5. The glomerulus is responsible for filtration. The specialized filter allows water to flow freely through, but acts as a barrier for particles based on both size and charge. Within the glomerulus, the amount of filtered fluid will depend on the counterbalancing forces of hydrostatic pressure and oncotic pressure.
6. Once the fluid filtered by the glomerulus exits Bowman's capsule it enters the proximal tubule, which is the major site of reabsorption. Through this process of reabsorption the proximal tubule regulates the extracellular fluid volume of the body, reclaims important electrolytes and nutrients, and regulates acid base balance.
7. The region from the corticomedullary junction to the papilla is characterized by an increasing osmolarity gradient that plays a critical role in the ability of the body to produce concentrated urine.
8. Water makes up approximately 50–60% of total body weight. The allocation of water within the body is divided into 2 basic compartments: intracellular and extracellular. Two-thirds of total body water is located in the intracellular compartment with the remaining one-third located in the extracellular space. The extracellular space is further divided into the intravascular space containing 25% of extracellular fluid and the interstitial space comprised of the remaining 75% of fluid. Thus, regulation of the body's fluid status is largely accomplished through its ability to manipulate serum sodium.
9. The bicarbonate/carbon dioxide buffer system is the most important buffering system in the body. The contribution that the kidney makes in regulating acid base balance is accomplished through its handling of bicarbonate. The kidney has a variety of tools that it can employ in order to compensate for different acid-base disturbances. As with any compensatory response, the effects are not able to completely correct for the original acid-base disturbance and take some time to fully develop.
10. The glomerular filtration rate (GFR) represents the cumulative functioning of all of the nephrons in the kidney and serves as an indicator of global renal function. This value represents the amount of plasma that is filtered through all the glomeruli per unit of time. A normal value for GFR ranges from 120 to 130 mL/min/1.73m^2. This value will vary from person to person depending on factors such as age, sex, and race. GFR cannot be measured directly. Instead we must rely on surrogates that act as representative markers of filtration. These markers can either be produced endogenously by the body or be introduced exogenously. Although we use GFR as a measure of renal function, the degree of injury to nephrons does not translate to a proportional decrement in the GFR.
11. Inulin clearance remains the gold standard for determination of GFR. The convenience associated with the use of serum creatinine has led to its widespread use as an indicator of renal function. Creatinine is a product of skeletal muscle breakdown and is freely filtered by the kidney without being reabsorbed. Its serum levels correlate inversely with GFR. However, creatinine undergoes secretion by the proximal tubule in varying amounts depending on body conditions. It is not an ideal renal biomarker to assess glomerular filtration rates.
12. Creatinine clearance is not usually calculated based on plasma and urinary creatinine levels in clinical practice. Instead, an estimated GFR (eGFR) is calculated with equations that incorporate laboratory results with demographic data.
13. Cystatin C is a compound that is generated by nucleated cells and released into the blood. Because serum levels vary inversely with GFR like creatinine, it can be used to estimate GFR. While cystatin C levels are not impacted by factors such as diet and muscle mass in the manner serum creatinine levels are, they can be affected by conditions affecting cell turnover rate such as high doses of steroids and thyroid dysfunction.
14. The difficulties inherent in determining global renal function make it challenging to identify when the kidney has sustained an injury or is under stress. The current consensus definition for acute kidney injury has been described by the Kidney Disease Improving Global Outcomes (KDIGO) group. The classification system according to the KDIGO criteria describes 3 stages of acute renal injury. These stages are generally differentiated from one another based on 2 variables: increases in serum creatinine and changes in urine output.
15. Excretion of drugs is managed by both the glomerulus and the proximal tubule. The glomerulus filters cations and smaller molecules. The proximal tubule manages those compounds that are either too large or protein bound to be effectively filtered by the glomerulus. Multi-specific drug transporters then import the compounds into the peritubular cell.

Once the compounds have been imported into the cell they are shuttled across into the tubular lumen. Variability in the clinical effects of drugs in different individuals may be partially explained by different variations or polymorphisms of these transporter proteins. Changes in the makeup of these proteins may lead to differences in rates of transport, which may affect drug efficacy and or toxicity.

22.1 Anatomy, Blood Flow, Glomerular Filtration, Tubular Reabsorption and Secretion

22.1.1 Renal Anatomy and Blood Flow

Before we drill down into events occurring at the cellular level, a quick review of the basic make up and architecture of the kidney is needed [1]. The kidneys live in the retroperitoneal space at the level between the T12-L2 vertebrae and are surrounded by Gerota's fascia. Because of its proximity to the liver, the position of the right kidney is more caudal than that of the left kidney. Below Gerota's fascial plane lies a peritoneal fat pad. The organ itself is surrounded by the renal capsule, which is composed of fibrous connective tissue. Three main areas comprise the kidney: the outer renal cortex, renal medulla, and renal pelvis. The nephron is the functional unit of the kidney that is responsible for filtration, reabsorption, and secretion of compounds that the body must manage in order to maintain homeostasis. Each kidney contains roughly 1 million nephrons. The component parts of the nephron include the glomerulus, the proximal tubule, loop of Henle (comprised of thin and thick limbs), the distal tubule, the connecting tubule, and finally the collecting ducts (Fig. 22.1) [2]. We will discuss the specific functions and characteristics of these areas subsequently. The renal cortex is the region that contains the glomeruli. Most glomeruli are located along the outer renal cortex and thus referred to as cortical nephrons. Juxtamedullary nephrons are located further inside the kidney in the area adjacent to the renal medulla. The differences between these 2 nephron types are not limited to their placement in the renal cortex but also includes modifications in the structure of the loop of Henle. Cortical nephrons have a short descending limb and the thick ascending limb begins shortly after the hairpin turn. The entire nephron remains mainly located in the renal cortex. In contrast, juxtamedullary nephrons have long descending and ascending limbs and dive deeper into the renal medulla. The thick ascending limb of these longer loops begin at the border of the inner and outer medulla. The renal medulla looks like an area organized into little pyramids. These pyramids are composed of the tubules draining urine into the renal pelvis. The renal pelvis then empties into the ureter.

The kidney receives approximately 20–25% of cardiac output. Blood enters the kidney from the aorta via the renal arteries, which divide into interlobar arteries. Each of these

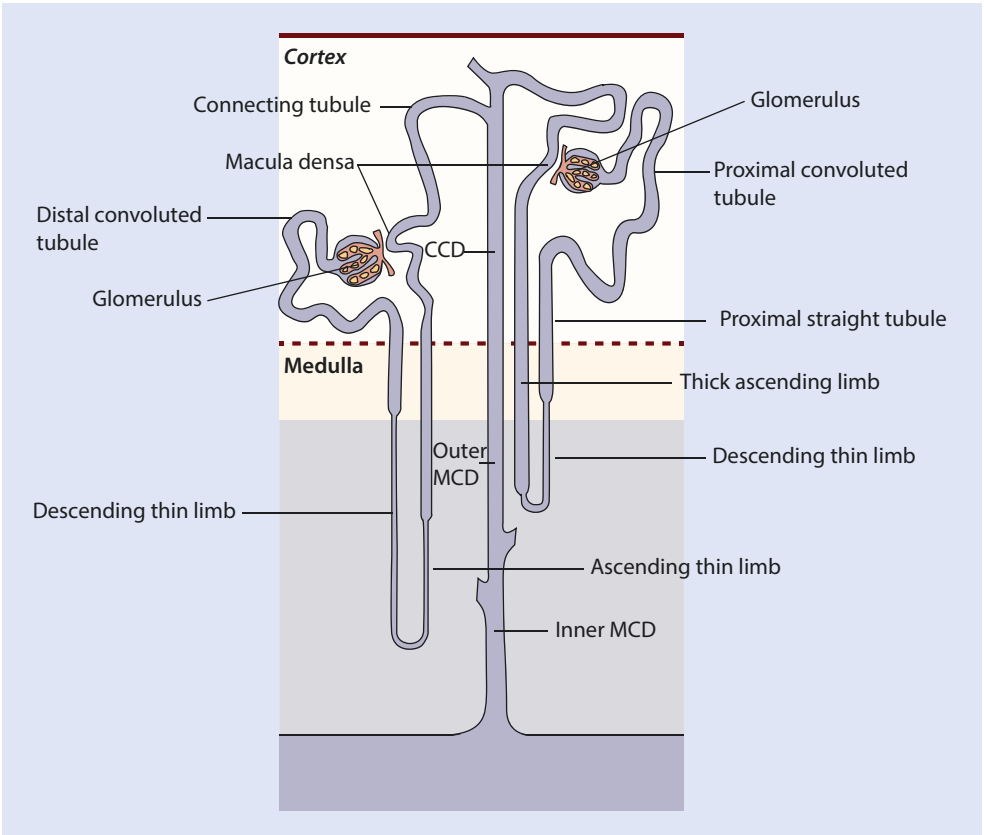


Fig. 22.1 Schematic of nephron. *CCD* cortical collecting duct, *MCD* medullary collecting duct (Adapted from [2])

interlobar arteries travels through areas in which the renal cortex invaginate into the medulla between the medullary pyramids. Each interlobar artery is further divided into arcuate arteries, which travel parallel to the base of the renal pyramids at the junction of the renal cortex and medulla. Again, further division occurs into interlobular arteries that keep dividing until they become an afferent arteriole supplying a single nephron, which begins with the specialized capillary bed of the glomerulus. Blood exits the glomerulus via an efferent arteriole, before entering the peritubular capillary system. Drainage of the peritubular capillary system occurs via a small venue with venous drainage flowing back along a parallel path to the arterial system before finally emptying into a single renal vein and the vena cava. Of note the left renal vein is longer than the right renal vein. This anatomical difference does have clinical implications. For example, donor nephrectomies can be done laparoscopically if the left kidney is being harvested because of this difference, whereas an open procedure must be performed for right donor nephrectomy.

22.1.2 The Glomerulus

Filtration begins in the glomerular capillary bed. This specialized capillary bed resides in a space called Bowman's capsule. The glomerular filter itself is made of 3 component parts: the endothelial cells of the glomerular capillary, the glomerular basement membrane, and specialized cells called podocytes or visceral epithelial cells (Fig. 22.2) [3, 4]. The endothelial cells are different from those in other capillary beds in the body. They have fenestrations of roughly 70–100 nm in diameter that serve as a "size" filter. The glomerular basement membrane is a extracellular matrix of proteins created by fusion of the endothelial cell and podocyte basement membranes.

Podocytes provide support to the glomerular capillary complex and also form another slit type of filtration barrier by weaving together the little foot-like processes that extend from one cell to another. These foot-like processes support the glomerular capillary by wrapping around them and weave together with the foot processes of adjacent podocytes. The areas between the adjacent "feet" form the slit filtration barrier that makes up the third component of the overall glomerular filtration barrier. Healthy podocytes are very important for normal functioning of the glomerulus. Unfortunately, they have a limited ability to repair or regenerate themselves.

The glomerular barrier allows water to flow through freely, but acts as a barrier for particles based on both size and charge. Flow of albumin and anionic particles is restricted while small neutral particles and cations traverse the barrier freely.

Mesangial cells are another important cell type located in Bowman's capsule (Fig. 22.3) [4, 5]. These cells play a number of important roles in the glomerular apparatus. One of the primary roles they serve is in providing a support structure for the glomerular capillary network. They form a central tuft within the glomerulus and secrete different types of collagen, fibronectin, and other compounds that form a mesangial extracellular matrix. Mesangial cells help to support the structure of the glomerular loops and help to provide protection from high hydrostatic pressures. The mesangial cells have contractile properties that aid them in this task. Although the major regulation of blood flow to the glomerulus occurs as a result of changes in the efferent and afferent arterioles, the mesangial cells are thought to have the ability to fine tune intraglomerular blood flow. The extracellular matrix produced by glomerular cells also have important immunologic and homeostatic functions. Production of free radicals, cytokines, and chemokines all occur when these cells become activated. Mesangial cells appear to "talk" with podocytes and endothelial cells through mechanisms that are

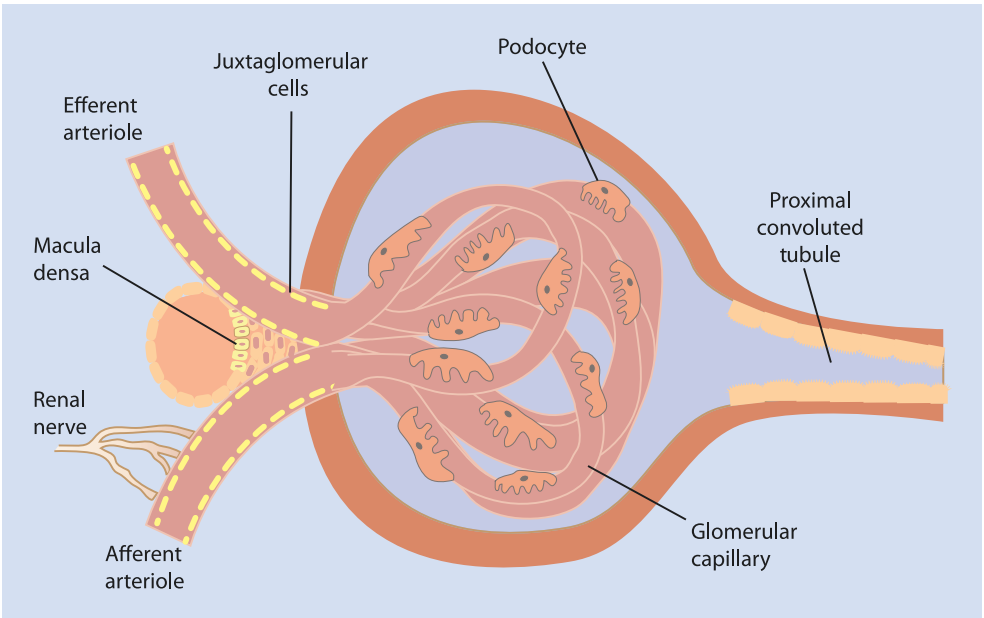


Fig. 22.2 Cross section of glomerulus (Adapted from [3])

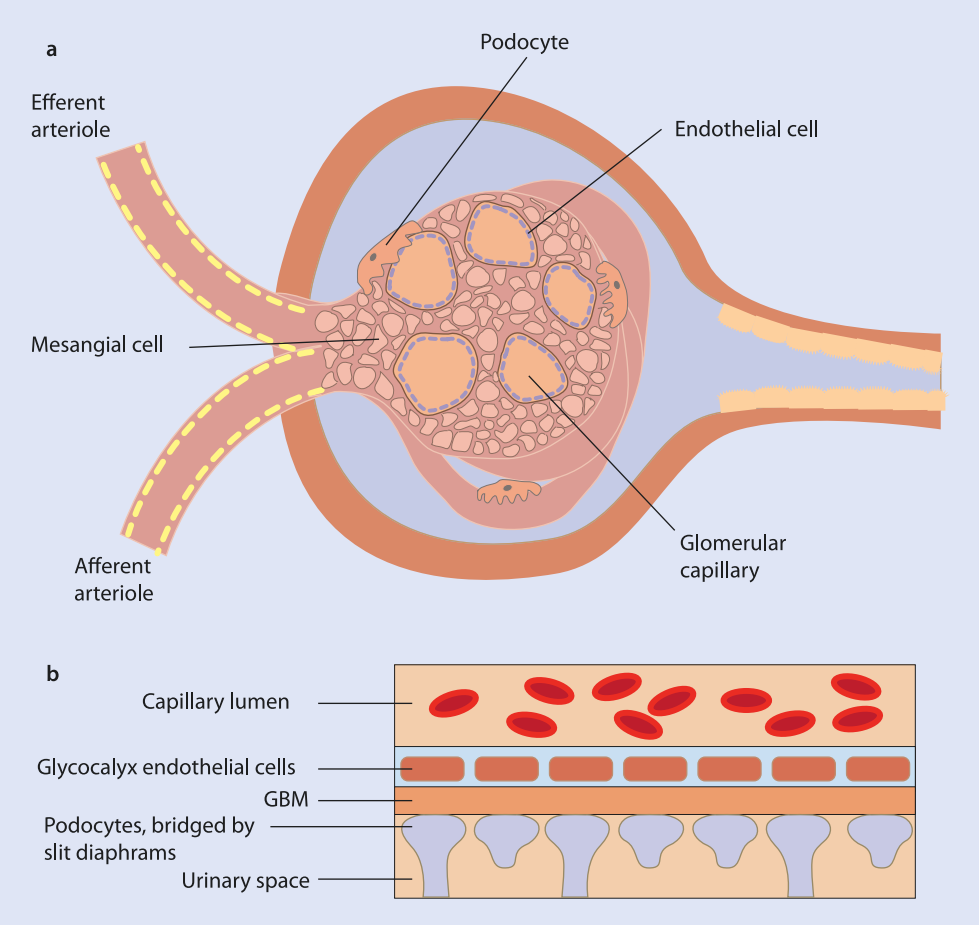


Fig. 22.3 **a** Cross section of glomerulus illustrating relationship between mesangial cells, podocytes, and endothelial cells. **b** Representation of the 3-part filtration barrier comprised of the podocytes, glomerular basement membrane (GBM), and endothelial cells (Adapted from [5])

still being investigated [6]. These interactions prove to be important during disease processes as dysfunction in one cell type can result in alterations in the others.

Two classic glomerular diseases that are associated with mesangial cell function are immunoglobulin A (IgA) nephropathy and diabetic nephropathy. In IgA nephropathy, mesangial cells are injured by the deposition of IgA, which results in mesangial cell proliferation and increased production of the mesangial matrix components through the work of cytokines and growth factors. In turn this activation appears to result in changes in podocyte function leading to protienuria. Patients who develop diabetic nephropathy demonstrate pathologic changes in mesangial cells as well, as demonstrated by expansion of the mesangial matrix, mesangial cell hypertrophy and proliferation, and the development of inflammation. These changes contribute to the global renal dysfunction seen in this condition.

What determines the rate of flow through the glomerulus? Within the glomerulus, the amount of filtered fluid will depend on the counterbalancing forces of hydrostatic pressure and oncotic pressure. Thus at the beginning of the glomerular capillary, the hydraulic force of fluid will overcome the oncotic pressures working to keep fluid within the capillary bed. As fluid exits the capillary, protein concentration and thus oncotic pressure will increase until the two forces balance and no further net filtration of fluid occurs. Another factor affecting flow is represented by ultrafiltration coefficient, K_f. This value represents factors such as the surface area available for filtration, and the "leakiness" of the capillary filter. One final component affecting the glomerular filtration rate (GFR) will depend on the rate of flow of fluid through the glomerular capillaries. When fluid is flowing slowly through the glomerulus, the point of equilibrium between the hydrostatic forces driving fluid out into Bowman's capsule and the oncotic pressure created by plasma proteins may occur earlier along the capillary path. Thus, for the remaining portion of the capillary bed left to traverse, no net filtration occurs. When fluid travels more quickly it takes more time for this point of equilibrium to occur. Thus, although there may be less net filtration at one single point, the longer path and thus greater time that filtration occurs leads to a net increase in the amount of filtered fluid. Differing resistances in the afferent and efferent arterioles modulate this rate of flow depending on hormonal factors, drugs, and other vasoactive substances.

22.1.3 Proximal Tubule

Once the fluid filtered by the glomerulus exits Bowman's capsule it enters the proximal tubule, which is the major site of reabsorption. Through this process of reabsorption the

proximal tubule regulates the extracellular fluid volume of the body, reclaims important electrolytes and nutrients, and regulates acid base balance [7]. These proximal tubule cells can be thought of as bulk processing centers. They reabsorb the majority of the substances that were filtered, with the fine tuning of many processes accomplished further downstream of this component of the nephron.

To understand the manner in which proximal tubule cells accomplish all of the aforementioned tasks, we must first understand the general makeup of the tubular cells in the nephron. Each cell type, whether they be in the proximal tubule or in the collecting duct, is oriented with two interfaces: one to the tubular fluid, and the other with blood from peritubular capillaries. The membrane lying at the interface with the tubular fluid is referred to as the apical membrane, while the basolateral membrane refers to the blood-cell interface. Many cellular processes in the nephron are powered by a protein transporter complex known as the Na^+-K^+-ATPase either directly or indirectly. The Na^+-K^+-ATPase uses the energy achieved from ATP hydrolysis to transport three molecules of sodium out of the cell while moving two molecules of potassium into the cell's interior. This mechanism is referred to as "active transport." In most cells along the nephron this process occurs along the basolateral membrane. The net effect of this process results in the establishment of a concentration gradient such that the interior of the cell has a lower sodium concentration and higher potassium concentration compared to the exterior. The combination of the ratio of three positive cations removed from the cell for the only two imported, as well as subsequent conductance of potassium back out of the cell, results in the establishment of a negative intracellular voltage. The energy potential created by this concentration gradient and voltage difference is then leveraged to transport many solutes. Examples of the use of this strategy recur repeatedly along subsequent areas in the nephron. In the following sections, we will discuss the individual mechanisms the proximal tubule is thought to use to accomplish the reabsorption of NaCl, water, bicarbonate, amino acids, phosphates and important metabolites. The proximal tubule is also involved in the excretion of drugs and endogenous toxins, but this will be addressed in the section on renal drug excretion.

NaCl, Water, and Glucose Reabsorption

The electrochemical gradient, with the low intracellular Na^+ levels and negative intracellular lumen, powers the transport of both sodium chloride and water into the cell at the apical membrane. This is accomplished by multiple different exchangers as there is no transporter protein that reabsorbs NaCl as a single unit. Instead import of Na^+ is tied to organic compounds such as glucose, H^+, and sulfates. In a sense, Na^+ "catches a ride" with whatever transporter protein that can accommodate it. Chloride anion transport occurs through transporters tied to organic bases and other anions at the apical boarder and via a variety of transporters at the basolateral membrane. Also a significant amount of chloride is reabsorbed via the paracellular pathway. In this route, chloride rides down the concentration gradient in passive transport between the proximal tubule cells. The net effect of all these processes results in the reabsorption of 60–70% of the filtered NaCl and water as well as reabsorption of >99% of filtered glucose by the time the end of the proximal tubule is reached [7, 8]. Because water and solutes are reabsorbed together, the reabsorption occurring in the proximal tubule is generally isotonic in nature.

Regulation of the HCO_3^- Buffer

Bicarbonate represents one of the most important buffers in the human body. Variation in the levels of bicarbonate contribute to the maintenance of the acid base status, which needs to be regulated within a very precise range. The proximal tubule plays a major role in the regulation of bicarbonate, reclaiming 70–90% of filtered bicarbonate. Surprisingly, this is not accomplished by simply transporting sodium bicarbonate from the tubular lumen across the apical membrane. Instead, the proximal tubule secretes hydrogen ion into the tubular lumen through the work of NHE3, a protein that functions as an electroneutral Na^+/H^+ exchanger. Hydrogen ion is moved into the tubular lumen as the Na^+ is transported into the cell (Fig. 22.4).

This hydrogen anion can then follow 1 of 2 basic paths: (1) titrate another substrate that acts as an acid carrier that is ultimately excreted in the urine, or (2) undergo transformation into a form that leads to the reclamation and regeneration of bicarbonate. Together these 2 processes are referred to as "renal acidification" [7].

NHE3 (the Na^+/H^+ exchanger protein) not only secretes H^+ but also secretes the acid NH_4^+ directly into the tubular lumen. The path of renal acidification through titration of carriers that reside in the tubular lumen, mentioned previously, include compounds such as ammonium and the divalent form of hydrogen phosphate. When ammonium combines with the hydrogen ion, NH_4^+ is generated in the tubular lumen:

$$NH_3 + H^+ \rightarrow NH4^+$$

When hydrogen ion titrates phosphate to its monovalent form it functions as an acid that is secreted in the urine, much like the ammonium cation.

$$HPO_4^{2-} + H^+ \rightarrow H_2PO_4^-$$

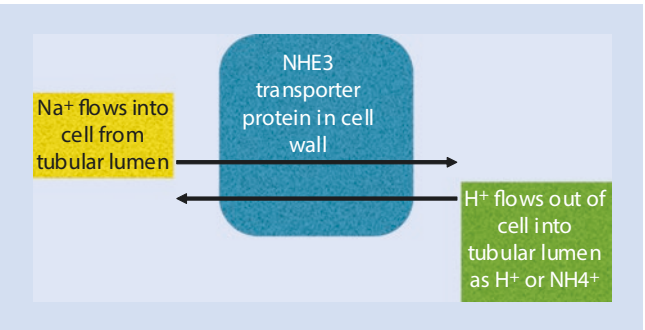


Fig. 22.4 Regulation of the HCO_3^- buffer

The second path of bicarbonate reabsorption can be accomplished a couple of different ways as well. The enzyme carbonic anhydrase catalyzes the combination of bicarbonate and hydrogen ion to form carbon dioxide, a substance that can easily diffuse across the apical cell membrane:

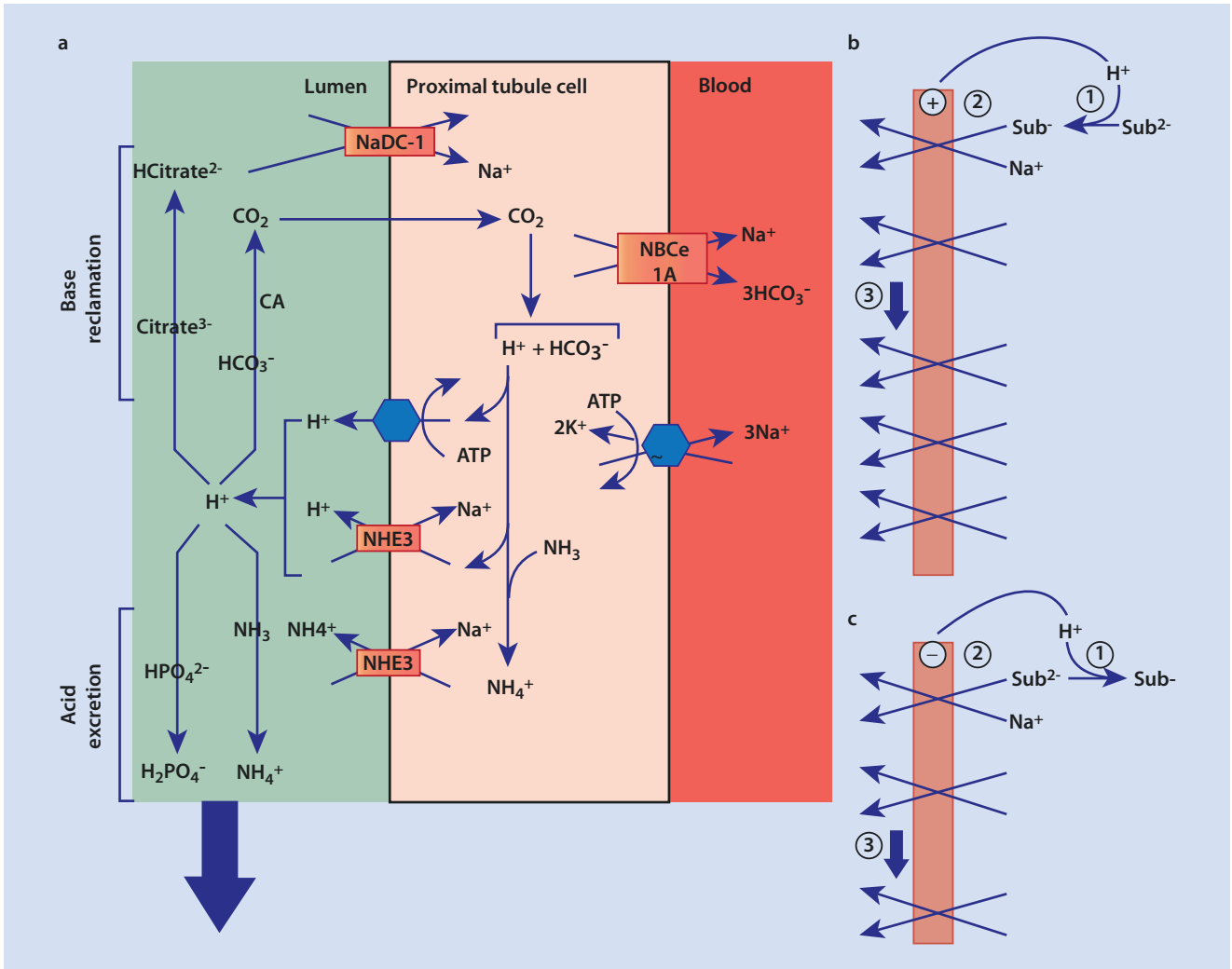
$$HCO_3^- + H^+ \xrightarrow{\text{Carbonic Anhydrase}} H_2O + CO_2$$

Once inside the cell, carbonic anhydrase regenerates both the hydrogen ion and bicarbonate. A transporter protein on the basolateral membrane called NBC1 then transports the bicarbonate into the peritubular capillary along with Na^+, thus reclaiming bicarbonate.

Alternatively, the secreted hydrogen ion can combine with citrate. The change in charge of citrate after its combination with hydrogen makes it compatible with a protein cotransporter called NaDC-1 (Na^+ decarboxylate cotransporter) that imports the citrate along with Na^+ ion into the cell. Citrate is then ultimately metabolized in the proximal tubule cell (in 1 of 2 manners) both resulting in the net generation of bicarbonate, which again can be transported across the basolateral membrane by NBC1. Thus, in summary, the hydrogen ion that was initially transported out of the proximal tubule cell combines with citrate allowing it to be transported from the tubular lumen into and across the proximal tubular cell where it is ultimately reabsorbed into the blood. ◘ Figure 22.5 portrays a nice summarization of these pathways and the transporter proteins involved [7].

Transport of Amino Acids

The majority of amino acids filtered by the glomerulus are neutrally charged, and easily reabsorbed by a specific transporter protein at the apical membrane. Separate transporters exist for acidic and basic amino acids. One important amino



◘ **Fig. 22.5** Illustration of proximal tubule management of filtered bicarbonate. **a** Carbonic anhydrase catalyzes the luminal conversion of bicarbonate into CO_2 and water. The CO_2 diffuses through the cellular membrane where it again is converted by carbonic anhydrase into hydrogen ion and bicarbonate. Hydrogen ion can be secreted back into the tubular lumen by either the H^+-ATPase, or more commonly the Na^+/H^+ exchanger NHE3. NHE3 also has the ability to transport NH_4^+, which is generated intracellularly from NH_3 and H^+. Once hydrogen ion is secreted into the lumen it may be either recycled to participate again in the reabsorption of bicarbonate; may convert citrate to its bivalent form, which is transported into the cell; or may be used to titrate urinary acids, which are excreted. The degree to which the proximal tubule can reabsorb bicarbonate and acidify urine can be either unregulated or down regulated as illustrated in **b** and **c**, respectively. Sub - substrate (Adapted from [7])

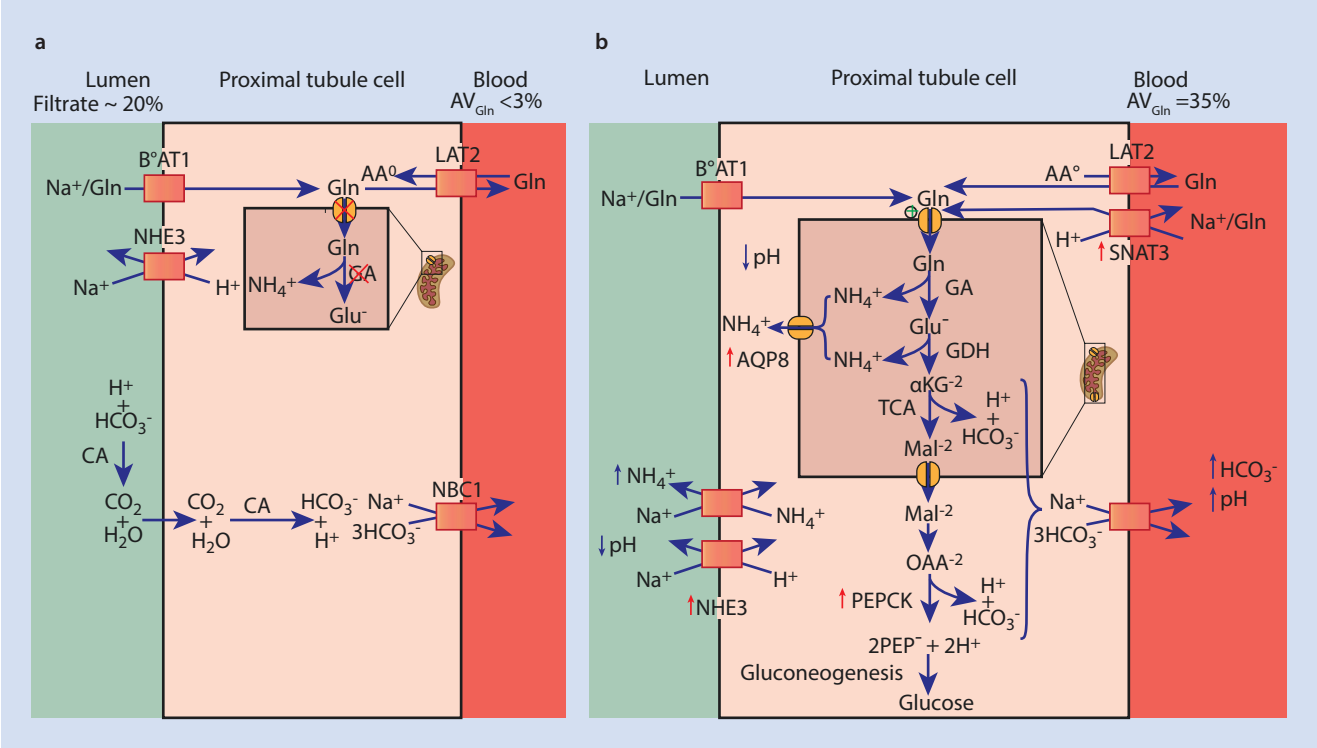


Fig. 22.6 Glutamine metabolism. **a** Represents handling of glutamine in a normal acid – base environment. **b** Illustrates the catabolism of glutamine during conditions of chronic acidosis demonstrating its catabolism to form bicarbonate, which is reabsorbed into the blood, NH4+ which acidifies the urine, and glucose (Adapted from [7])

acid to be familiar with is glutamine due to the role it plays in the renal response to metabolic acidosis [7]. Under normal physiologic conditions only a small amount of glutamine undergoes uptake (approximately 20%) and metabolism (<3%). However, this picture rapidly changes under the acute onset of metabolic acidosis. During the onset of acute metabolic acidosis, the amount of glutamine that the kidneys are exposed to increases as a result of release from muscle tissue. The proximal tubule cell reabsorbs a greater amount of glutamine (roughly 35%) from the tubular lumen and also takes up glutamine from the peritubular capillary blood. This glutamine is then metabolized to generate greater amounts of ammonium cations (which are excreted in the urine), glucose, and bicarbonate (which undergoes reabsorption) (Fig. 22.6) [7]. When metabolic acidosis becomes more chronic, the circulating levels of glutamine drop back to a level that is 70% of normal. However, the percentage of glutamine extracted remains elevated due to continued extraction from both membranes, a process which is facilitated through the increased expression of certain transporter proteins. The enzymes involved in glutamine metabolism have an increased expression resulting in an higher capacity for ammonium cation and bicarbonate production. The expression of proteins such as NHE3 (Na^+/H^+ exchanger protein) and NBC1 (protein that moves bicarbonate across the basolateral membrane of the cell) is increased as well, augmenting the acidification of urine and reabsorption of bicarbonate.

Phosphate

The renal component of phosphate balance is managed almost exclusively by the proximal tubule [7, 9]. Again the electrochemical gradient established by the Na^+-K^+-ATPase serves as the driving force for the apical reabsorption of phosphate via three separate transporters. The mechanism by which phosphate exits the basolateral membrane and is reabsorbed into the blood is not well understood. The amount of phosphate reabsorbed versus lost in the urine will depend on dietary intake as well as the input of signaling factors from parathyroid hormone, dopamine, fibroblast growth factor 23, and a transmembrane protein called klotho.

22.1.4 Thin Limbs of the Loop of Henle

Once it exits the proximal tubule, the path of tubular fluid traverses through the descending limb of the loop of Henle (LoH) down into the renal medulla. After traveling through a hairpin curve, it then ascends back up until it reaches the thick ascending limb (TAL) of the loop of Henle. The region from the corticomedullary junction to the papilla is characterized by an increasing osmolarity gradient that plays a critical role in the ability of the body to produce concentrated urine. In fact the osmolarity from the outer renal cortex to the inner renal medulla from values of 300 mOsm to as high as 2700 mOsms. The mechanism by which gradient is established and maintained is not entirely understood, and

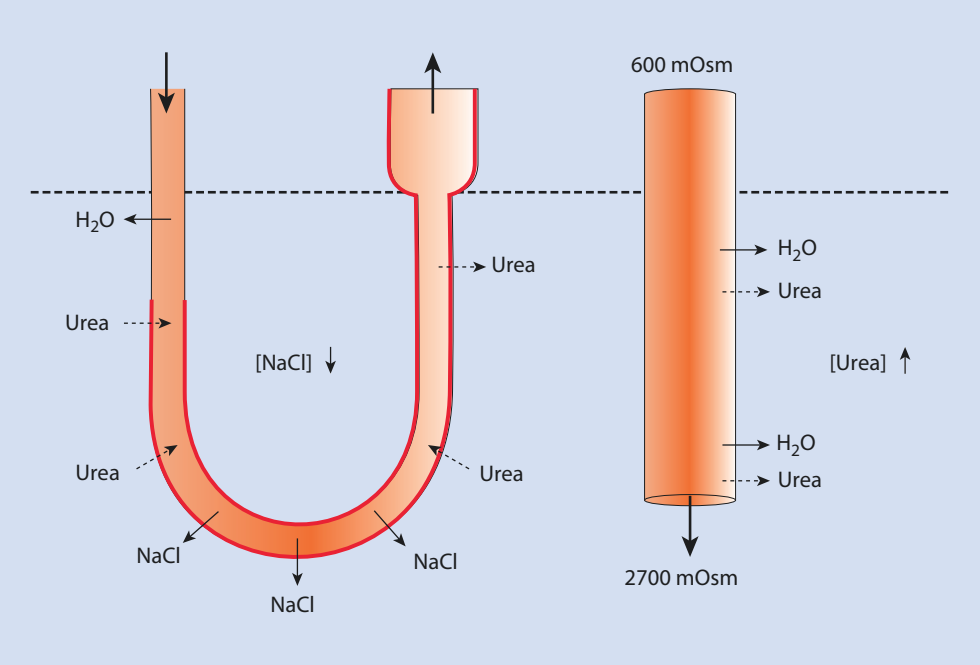


Fig. 22.7 Thin limb of loop of Henle. This figure demonstrates the current understanding of the role of water permeability and urea in creating the hypertonic environment that is responsible for the ability to concentrate urine. The *thick red line* demonstrates the portion of the thin limb that does not express aquaporin and is thus not permeable to water. In contrast, permeability of urea exists along the length of the tubule. Around the bend of the loop, NaCl is passively reabsorbed (Adapted from [10])

older models put forth have not accommodated data measured in experimental models. Thus, the understanding of this process continues to evolve [10].

The best current understanding of how the kidney establishes the osmolar gradient begins with an acknowledgement of the special characteristic of the descending and ascending thin loops themselves (Fig. 22.7) [10]. The first important characteristic relates to water permeability [2]. Contrary to prior models, it has been demonstrated that the descending thin limbs (DTLs) of short looped nephrons fail to express the transmembrane protein aquaporin-1 (AQP1). AQP1, aquaporin, is a water channel that makes the tubule water permeable. For long-looped nephrons, the descending thin limbs only express AQP1 in the initial 40% of their length. Thus, most of the descending thin limbs are actually impermeable to water. Ascending thin limbs (ATLs) are water impermeable as well. While these segments have long stretches of impermeability to water, they do have a high degree of permeability to sodium chloride beginning from just before the hairpin bend and continuing on through the ascending thin limb. Finally, both the descending and ascending loops of Henle have a high permeability to urea along their entire lengths.

As tubular fluid flows down the descending thin limb, water exits the DTL lumen until it reaches the area in which AQP1 expression ceases, at which point water egress ceases. As the DTL dives into the renal medulla, the osmolarity of the tissue surrounding it increases due to increases in interstitial urea concentration. Thus, urea diffuses down its concentration gradient from its high concentration into the tubular fluid. As the bend of the LoH is approached, NaCl passively exits the cell, but due to the lack of water permeability there is no concurrent egress of water. At the bend of the LoH, the tubular fluid is surrounded by the highest osmolar gradients it will see until it again descends back through the inner medulla via the collecting ducts. As the fluid exits the bend and rises back up toward the renal cortex, the concentration of urea begins to decrease the closer the fluid travels to the cortex. Urea will continue to travel down its concentration gradient. Thus, in the areas of the ascending limb that are deep, urea will diffuse into the tubule. However, at some point the fluid in the tubule will have a urea concentration that is greater than the surrounding intersitium, resulting in its diffusion back out into the interstitium. This process is referred to as "counter current multipication" [10].

22.1.5 Thick Ascending Limb of Loop of Henle

The mechanism described previously depends on a high osmolar gradient with a high concentration of urea and low concentration of NaCl in the inner medulla. How does this gradient become established and maintained? In order to understand this process we must continue on to understand the workings of the thick ascending limb (TAL) and collecting ducts (CD) (Fig. 22.8) [2]. The TAL is impermeable to water and travels back up to the renal cortex where the cells of the macula densa abut the afferent and efferent arterioles of the glomerulus. The macula densa represents specialized cells in the TAL that play a role in tubuloglomerular feedback. While movement of NaCl occurs in a passive fashion in the thin limbs, it is actively transported out of the lumen in the TAL. The net result is an increasingly dilute luminal fluid that contributes to counter current multiplication.

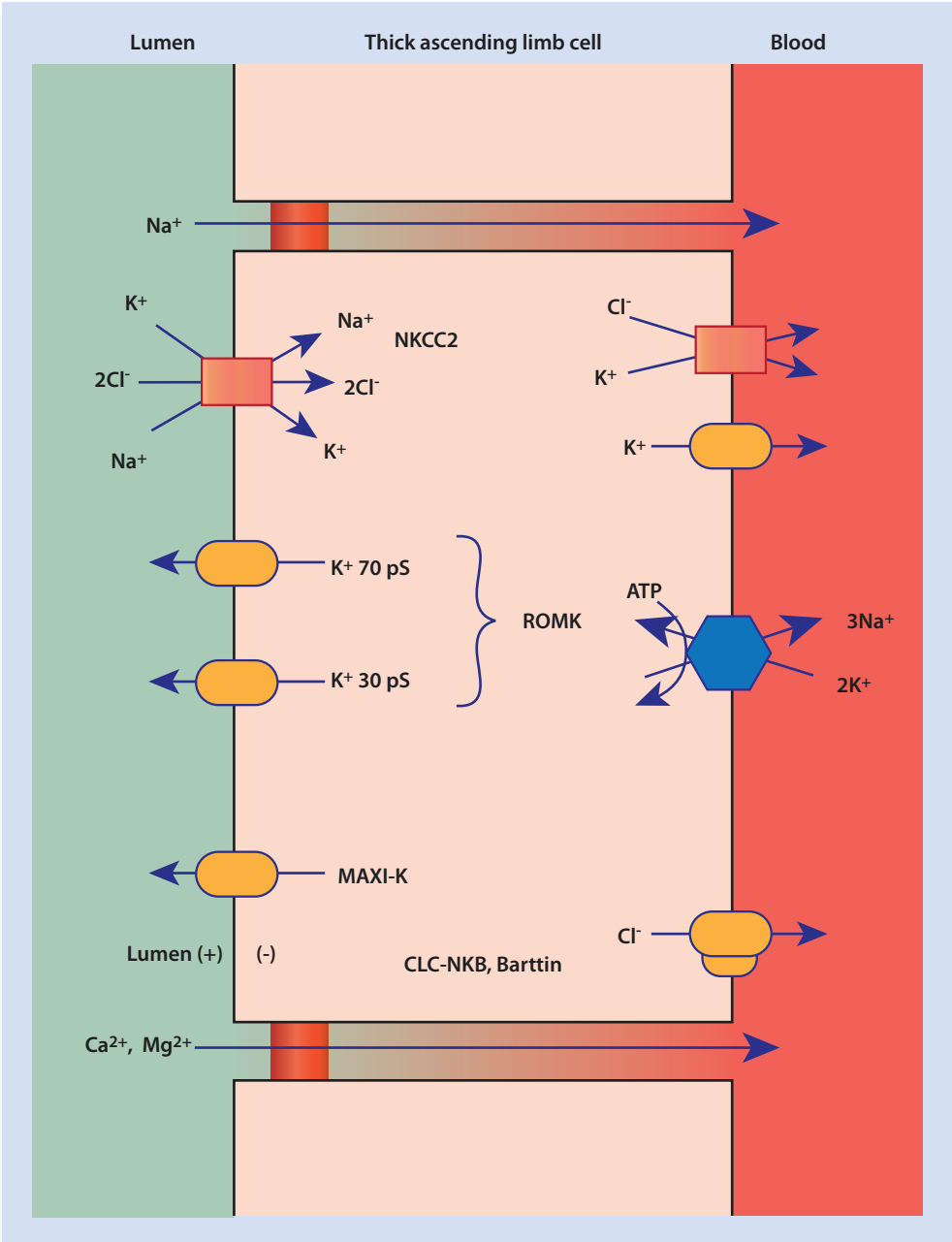


Fig. 22.8 Thick ascending limb of loop of Henle (Adapted from [2])

Transport of NaCl is mediated by the protein complex NKCC2 at the apical membrane [2]. NKCC2 cotransports Na^+, K^+, and $2Cl^-$ to the intracellular space. This transport protein is of clinical importance because it is extremely sensitive to furosemide. Furosemide inhibits the transport of Cl^- across the cell and appears to have effects on the cells of the macula densa, which also contains NKCC2. In the macula densa, Lasix (furosemide) functions to inhibit tubuloglomerular feedback and suppress renin released by chloride in the tubular lumen.

Potassium plays a very important role in the TAL [2]. Its presence is necessary for the NKCC2 (furosemide sensitive, electroneutral Na^+-K^+-$2Cl^-$ cotransporter) transporter to function. Since there is very little potassium in the tubular fluid at this point, in order for continued active transport of NaCl to occur, then the potassium must be recycled back across the apical lumen so that it may be reused. Renal outer medullary potassium channel (ROMK) and maxi-K (also known as BK or "big" K) potassium channels at the membrane fulfill this task. The majority of the potassium transported across the apical membrane is recycled back into the tubular lumen, whereas the NaCl is transported across the basolateral cell membrane courtesy of the Na^+-K^+-ATPase and chloride channels. Thus, the active transport of NaCl in the TAL represents an example of secondary active transport. This process of NaCl reabsorption and K^+ recycling creates a positive potential difference in the tubular lumen.

Other functions of the TAL include reabsorption of more cations via the paracellular pathway [2]. This includes additional sodium, roughly 55% of filtered magnesium, and

around 20% of filtered calcium. Part of the impetus for this movement is found in the positive-lumen potential difference established by furosemide-sensitive, electroneutral Na^+-K^+-$2Cl^-$ cotransporter (NKCC2), and is aided by channels in the tight junctions between cells that have an affinity for cations.

Additionally, the TAL contributes to maintenance of acid-base balance through the reabsorption of approximately 15% of filtered bicarbonate [2]. The mechanism by which this is accomplished involves electroneutral Na^+/H^+ exchanger (NHE3) as described earlier in the proximal tubule. The TAL also is important in the body's management of ammonia. When the ammonia produced by the proximal tubule reaches the TAL, a large percentage of it is reabsorbed back into the systemic circulation. This reabsorbed ammonium anion is transported to the liver where it undergoes metabolism to form urea. The reabsorption of ammonium anion is carried out mechanistically by apical transport of ammonium anion from furosemide-sensitive, electroneutral Na^+-K^+-$2Cl^-$ cotransporter NKCC2 (although NH_4^+ appears to be able to be transported by any transporter protein that handles K^+) and another protein transporter at the basolateral membrane called NHE4 (another electroneutral Na^+/H^+ exchanger). The capacity of the TAL to reabsorb ammonium anion increases under conditions of acidosis. Interestingly, NH_4^+ can be reabsorbed via the paracellular pathway as well. Ultimately, the transport of ammonium anion by the TAL establishes another gradient contributing to counter current multiplication, with the highest amounts of ammonia in the inner medulla and lowest levels located in the renal cortex.

The body's ability to adjust urine production and composition to meet metabolic needs depends on hormonal regulation [2]. Various hormones target the TAL to regulate ion transport. Substances such as vasopressin, parathyroid hormone, glucagon, calcitonin, and B adrenergic activation all increase ion transport through an increase in cyclic adenosine monophosphate (cAMP) levels. As an example, interaction of vasopressin with V2 receptors leads to an increased activity in electroneutral Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) activity as well as an increase in the number of these proteins on the apical membrane. With prolonged exposure to vasopressin, the TAL cells hypertrophy and double their baseline activity of NaCl transport. In contrast, prostaglandin E2, extracellular calcium, and nitric oxide negatively influence ion transport.

22.1.6 Distal Convoluted Tubule

Once tubular fluid passes the macula densa it enters the distal convoluted tubule (DCT) [11]. Tubular fluid is now very dilute as a result of the active transport of NaCl and impermeability of the TAL. The measured transepithelial voltage, or the voltage difference between the DCT cells and the tubular lumen, remains very close to zero at the beginning of the DCT. The continued reabsorption of NaCl (described later) results in the formation of a negative charge in the tubular fluid. This negative voltage in turn helps to drive the secretion of K^+, along with the reabsorption of chloride, calcium, and magnesium.

The DCT is comprised of 2 distinct segments: the early or DCT1 segment, and the late, or DCT2 segment (Fig. 22.9) [11]. These segments differ from one another in a number of ways. The first difference relates to the ability of the mineralocorticoid receptor (MR) to be stimulated by steroid hormones. In the DCT1 region, the MR responds to both glucocorticoids and mineralocorticoids. However, expression of an enzyme called 11-B hydroxysteroid dehydrogenase 2 in the DCT2 section prevents cortisol from exerting its effects at the mineralocorticoid receptor. Thus, the DCT2 segment is much more sensitive to the effects of aldosterone than the early DCT. A second difference relates to the manner in which NaCl reabsorption is executed. In the early DCT, sodium transport at the apical membrane occurs in an electro-neutral manner. The transporter protein NCC (thiazide-sensitive NaCl cotransporter), transports both one Na^+ cation and one Cl^- anion together simultaneously. Activity of NCC also happens to be inhibited by thiazide diuretics. The coupled movement of sodium chloride maintains the zero voltage potential in the tubular lumen. In the late DCT, NCC continues to be expressed and function, but another transmembrane protein called the epithelial sodium channel (ENaC) also contributes to sodium reabsorption. The key difference lies in the electrogenic nature of this transport. Since there is no concomitant transfer of an anion to balance out the movement of the positively charged Na^+ in the epithelial sodium channel (ENaC), a voltage difference begins to occur and the tubular lumen develops a negative potential. The impetus for this influx of Na^+ at the apical membrane can again be traced back to our old friend the Na^+-K^+-ATPase functioning at the basolateral membrane. Remember, 3 Na+ are transported out for every 2K+ carried into the cell creating a negative, low [Na+], high [K+] intracellular environment. The "leak" of potassium back across the basolateral membrane by various potassium channels recycles the potassium and allows this electrochemical gradient to be regenerated and maintained. Basolateral chloride transport is mediated by a chloride-specific channel (ClC-Kb) as well as a KCl transporter protein (KCC4).

The voltage difference generated by the combined effects of an epithelial sodium channel (ENaC) and the Na^+-K^+-ATPase helps drive the movement of other solutes. Paracellular movement of chloride is one example. Other interesting examples are found in the active transport of calcium and magnesium, which will not be expanded upon here. Perhaps the most interesting example lies in the handling of potassium at the apical membrane. When tubular fluid exits the DCT2 it contains more potassium than was originally present due to secretion across the apical membrane [11]. The secretion of potassium increases the further down one travels in the DCT in proportion to the increasingly negative voltage potential. The faster the fluid travels down this path, the more potassium is secreted as well. These effects are mediated by 2 different potassium channels, which

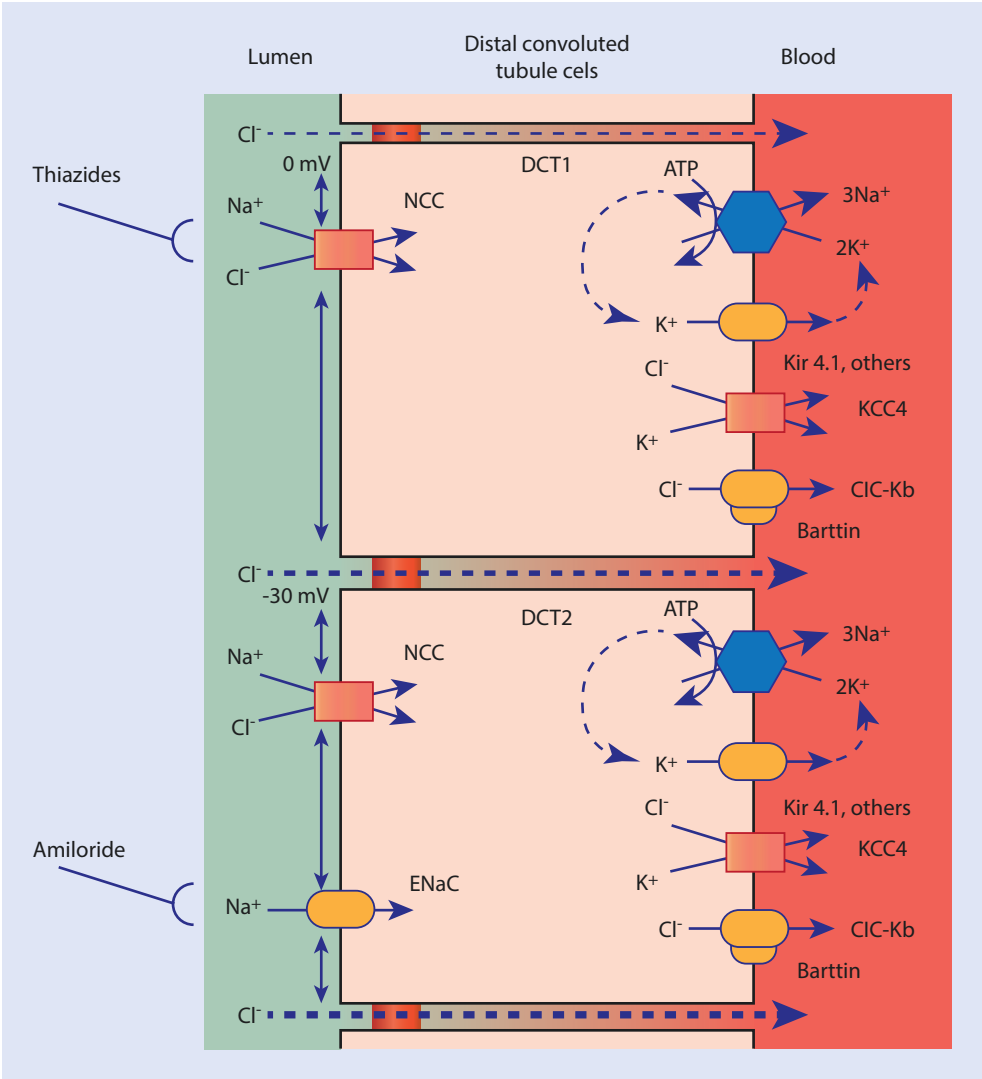


Fig. 22.9 Distal convoluted tubule. Illustration of NaCl reabsorption (Adapted from [11])

we have discussed previously: the renal outer medullary potassium channel (ROMK) and BK ("big" K or maxi K). In the TAL, the function of these proteins at the apical membrane is to recycle potassium back into the tubular lumen to allow furosemide-sensitive, electroneutral Na^+-K^+-$2Cl^-$ cotransporters (NKCC2) to initiate the reabsorption of NaCl. In the DCT, however, their function at the apical membrane is to secrete potassium into the tubular lumen. Renal outer medullary potassium channel (ROMK) is voltage sensitive and it secretes more potassium as the voltage differential becomes more negative. When tubular fluid flows at an increased rate then the BK ("big" K) channel becomes activated due to the sheer stress. Intracellular calcium and nitric oxide are postulated to play a role in this. Thus, high flow rates and a high sodium load will activate both ROMK and BK channels leading to enhanced potassium secretion.

One final interesting fact about ROMK relates to the role that magnesium plays in the regulation of this transporter [11]. When magnesium binds to the renal outer medullary potassium channel (ROMK) on the intracellular side of the transport protein, it blocks the secretion of potassium. Thus, a lack of sufficient magnesium levels will lead to a more "open" ROMK channel and increased loss of potassium through the urine resulting in hypokalemia. This hypokalemia will not be corrected by simply administering exogenous potassium chloride. Instead, until the magnesium deficiency is corrected, the hypokalemic state will persist.

Knowledge of the receptors and mechanisms involved in sodium reabsorption in the nephron may seem overly detailed, but it does have implications on patient care and clinical practice. Diuretics play a very important role in the management of many patient populations, and medications such as furosemide are administered routinely. With continued administration of furosemide its effectiveness wanes. The mechanism behind the phenomenon can be easily understood. NKCC2 (furosemide-sensitive, electroneutral Na^+-K^+-$2Cl^-$ cotransporter) sensitivity to furosemide leads to inhibition of NaCl reabsorption in the TAL, thereby increasing the sodium load delivered to NCC (thiazide-sensitive NaCl cotransporter) transporters in the DCT. Rates of flow in the tubular fluid will increase, as will the electrogenic reabsorption of sodium. As a result of the increasingly negative voltage and flow, ROMK and BK

(both potassium transport channels) will increase potassium secretion. The hypokalemia that is observed with furosemide administration is thus explained [11].

If a patient is on Lasix chronically, the DCT1 segment adapts through cellular hypertrophy in order to increase the capacity to reabsorb sodium [11]. Remember, the distal convoluted tubule 1 segment's ability to reabsorb sodium occurs only as a result of the work of the thiazide-sensitive NaCl cotransporter. Hypertrophy of the cell leads to an increase in the number of the thiazide-sensitive NaCl cotransporters (NCC). This leads to an ability to increase the amount of sodium reabsorbed. This process mitigates the effectiveness of the diuretic with furosemide thereby leading to diuretic resistance. However, since the action of NCC (the thiazide-sensitive NaCl cotransporter) may be inhibited by thiazide diuretics, then their administration may circumvent this adaptive response. In effect, the administration of the thiazide diuretic knocks out the ability of the kidney to reabsorb the increased sodium load that was delivered to the distal convoluted tubule from the administration of furosemide.

22.1.7 Collecting Ducts

Before tubular fluid exits the nephron it must finally pass through the collecting ducts (CD). The cells in the collecting ducts make the final set of fine-tuned adjustments in the areas of water balance, acid base homeostasis, and inorganic solutes such as sodium, chloride, and potassium [12, 13]. Two cells types—principal cells and intercalated cells—carry out the responsibilities of this nephron segment and are interspersed with one another along the length of the epithelium.

The principal cell focuses its efforts in two main areas: sodium chloride and water reabsorption (Fig. 22.10) [13].

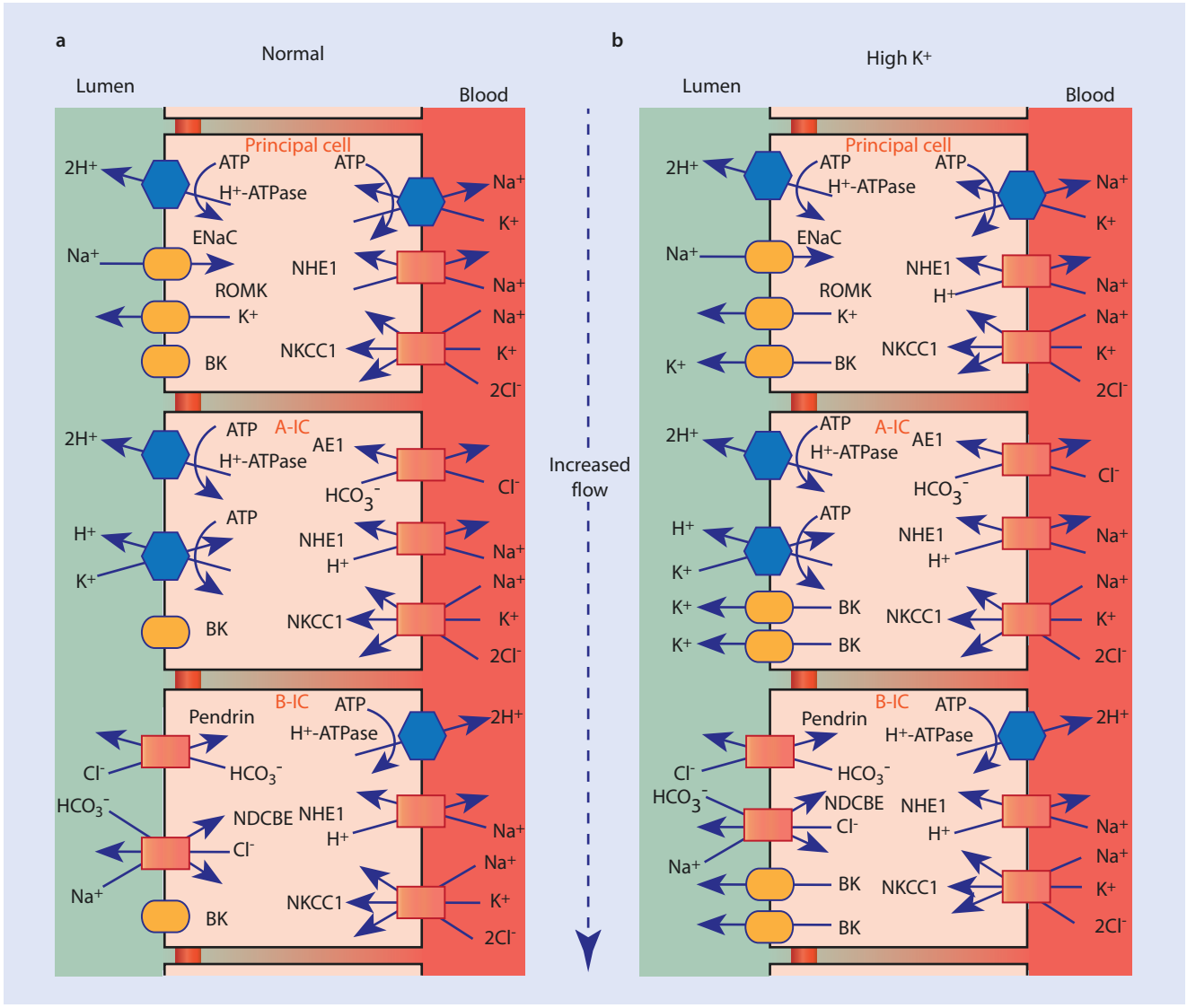


Fig. 22.10 Collecting duct principal cell, type A and type B intercalated cell. Illustration of receptors in each cell type as well as variability of potassium secretion depending on tubular flow. **a** Normal potassium secretion. **b** Potassium secretion as affected by high tubular flow (Adapted from [13])

The electrochemical gradient that acts as the driving force for sodium reabsorption is once again created by the Na^+-K^+-ATPase at the basolateral membrane. However, the ENaC protein channel acts as the vessel through which entrance occurs at the apical membrane [12]. Thus, in the CD, the sodium is not actively transported. The transport is electrogenic in nature, and contributes to the secretion of potassium through ROMK and hydrogen ion in a manner similar to what was described in the late DCT. The ENaC receptor is regulated by a number of hormones including aldosterone, atrial natriuretic peptide, and arginine vasopressin. After stimulating the mineralcorticoid receptor, aldosterone multiplies the number of receptors at the apical membrane, thereby magnifying its effects. Insulin also stimulates ENaC in the principle cell. Conversely, ANP inhibits the ENaC channel in addition to repressing secretion of renin and production of aldosterone.

Principal cells mediate water reabsorption through the aquaporin channels [12]. AQP2 is expressed at the apical membrane while AQP3 and AQP4 are located at the basolateral membrane. By this point in the nephron approximately 90% of the water has already been reclaimed. The amount of fluid reabsorbed at this point is highly dependent on vasopressin levels. Vasopressin is able to modulate the water permeability of the collecting duct though its effects on AQP2. With vasopressin stimulation, AQP2 stores in the cell's interior are transferred to the apical membrane. Additionally, vasopressin can alter protein expression of AQP2 for a longer term effect. Dopamine and prostaglandin E2 antagonize these effects by initiating a mechanism that returns AQP2 from the apical membrane to back to intracellular vesicles.

Intercalated cells (IC) make up the remaining population of the collecting duct [13]. One key difference between intercalated cells and almost every other cell described in this chapter lies in the power source driving transport events. Instead of the Na^+-K^+-ATPase, an H^+-ATPase serves as the generator of the electrochemical gradient in intercalated cells. Similar to cells within the distal convoluted tubule, intercalated cells are very rich in mitochondria and all IC contain the enzyme carbonic anhydrase. Three subtypes of intercalated cells exist: type A, type B, and non-A non-B. Type A intercalated cells help to acidify urine. Type B and non-A non-B cells secrete bicarbonate into the tubular lumen.

Type A intercalated cells position the H^+-ATPase at the apical lumen. In a combined effort with a H^+-K^+-ATPase pump, protons are secreted into the lumen [13]. The loss of the proton drives intracellular carbonic anhydrase to generate bicarbonate, which is reabsorbed by the transporter protein AE1 across the basolateral membrane in exchange for a chloride anion. A clinical example of the importance of this mechanism is found in the inherited forms of distal renal tubular acidosis. In these patient populations, the normal functioning of type A intercalated cells is upset, resulting in a lack of ability to acidify urine. This disruption leads to a state of acidemia, alkalization of urine, and often nephrolithiasis.

Potassium balance can be fine-tuned through the effects of type A IC. The cells can participate in both potassium reabsorption and potassium secretion depending on body conditions. The H^+-K^+-ATPase pump at the apical membrane creates a mechanism for the reabsorption of K^+ from the lumen [13]. However, in response to high luminal flow, a negative voltage gradient, or increased intracellular calcium potassium can be secreted through the BK (maxi-K) channel as has been previously discussed [14].

In type B intercalated cells the H^+-ATPase pump is positioned at the basolateral membrane [13]. The apical membrane contains a transporter protein named pendrin, which excretes a bicarbonate anion into the lumen in exchange for a chloride anion. The presence of an electro-neutral $Na^+/HCO_3^-/Cl^-$ transporter allows these cells to play a role in NaCl reabsorption as well. Thus the two roles of the type B intercalated cells include volume expansion and bicarbonate secretion.

The final topic that needs to be addressed relates to the pivotal role that the collecting duct plays in the generation of the urea gradient necessary to drive counter current multiplication and the body's urine concentrating ability. In the previous discussion of the LoH we discussed the manner in which urea underwent a passive diffusion down its concentration gradient into the tubular lumen from the renal interstitum. What we neglected to mention, however, was genesis of the high concentration gradient in the renal interstitium. A number of urea transporters in the collecting duct establish and help to maintain the high concentration of urea in the inner medullary intersitium [15]. Located at the apical membrane, the urea transporter UT-A1 carries urea from the tubular lumen into the collecting duct cell. The urea is then transferred across the basolateral membrane by the urea transporter UT-A3. The activity and number of these transporters is regulated by vasopressin. In conditions of high osmolarity, or volume depletion, vasopressin increases the number and activity of the urea transporters thereby increasing the concentration of urea in the inner medullary intersitium. The increased osmolarity allows for increased reabsorption of water and further concentration of urine.

What prevents the concentration gradient established in the inner medulla from being diluted by the blood flowing down the vasa recta? Counter current exchange of urea preserves the gradient. The descending vasa recta and red blood cells contain the urea transporter protein UT-B1. This urea transporter allows the red blood cells to absorb urea as they descend into the inner renal medulla and effectively matches the osmolarity of the blood cells with that of the surrounding interstitium. Thus, no water flows out to dilute the surrounding tissue. As the red cells and vasa recta ascend back toward the renal cortex, UT-B1 helps the red cell to quickly eject urea back into the interstitium. This facilitated transport decreases the osmolarity of the red cells, prevents urea from being carried out of the inner medulla, and reinforces the medullary concentration gradient.

22.2 Integrated Processes in the Nephron

The aforementioned journey through all the sections of the nephron has demonstrated some of the cellular mechanisms and processing techniques that the kidney uses in order to

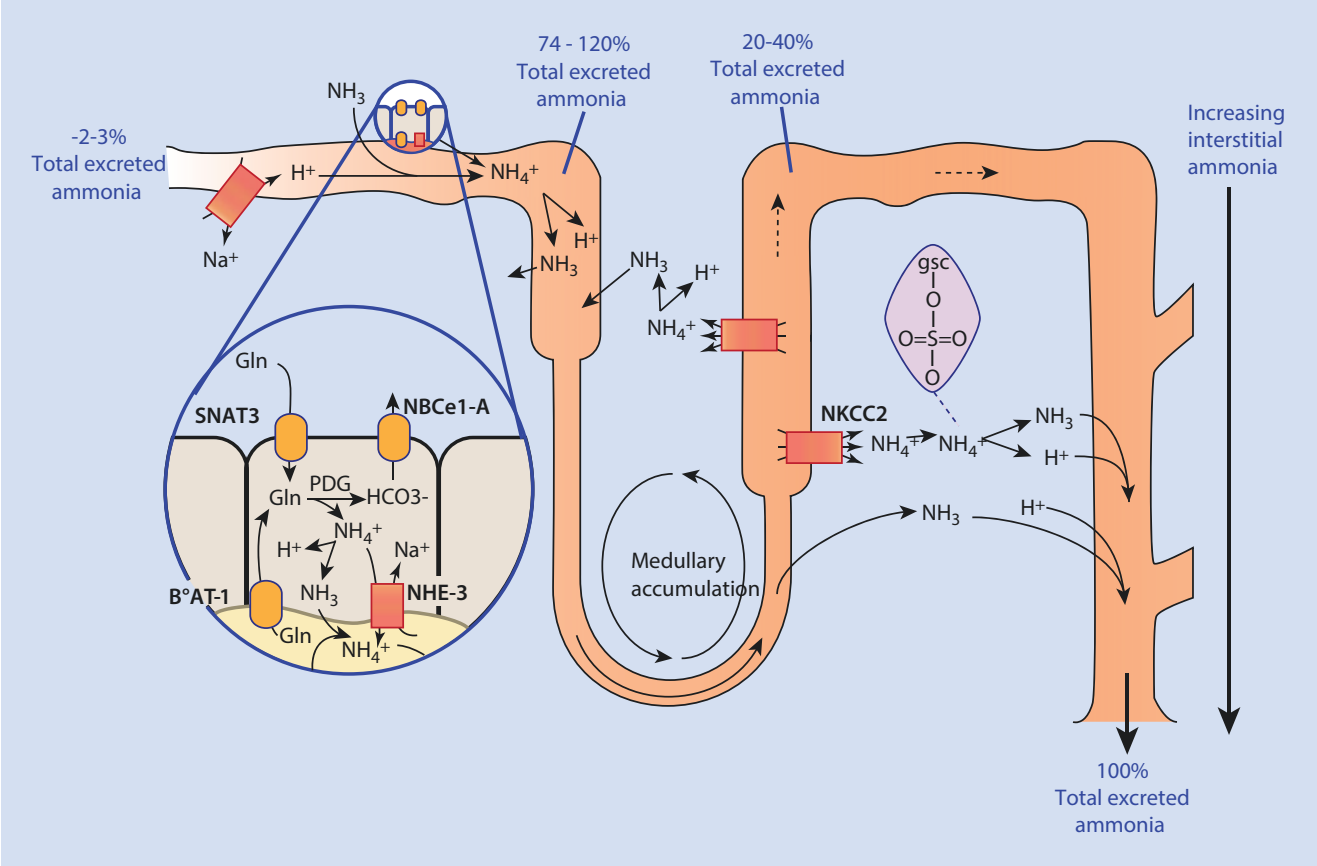


Fig. 22.11 Illustration of ammonia metabolism throughout nephron. See text for details (Adapted from [15])

adapt to the myriad of internal and external changes the body is confronted with (Fig. 22.11) [8, 9, 14–16]. Tables 22.1 and 22.2 summarize key functions, characteristics, and receptors of each nephron segment. Table 22.3 delineates the handling of key electrolytes and solutes in the glomerular filtrate as they pass through the nephron. Now we will use this newfound understanding to broaden our perspective from the microscopic world of the single nephron in order to examine more global processes such as the defense of the body's fluid volume and acid-base status.

22.2.1 Water Homeostasis

Our body's composition consists mainly of water. In fact, water makes up approximately 50–60% of the total body weight [17]. The allocation of water within the body is divided into 2 basic compartments: intracellular and extracellular. Two-thirds of total body water is located in the intracellular compartment with the remaining one-third located in the extracellular space. The extracellular space is further divided into the intravascular space containing 25% of extracellular fluid and the interstitial space comprised of the remaining 75% of fluid.

Water is able to flow freely across most cell membranes. Osmotic forces in the body will cause it to shift from one area into another (Table 22.4). Generally, water flows from areas of low osmolality into areas of higher osmolality. The serum osmolarity of the body is tightly regulated and subjected to hormonal control [17]. Serum osmolarity can be calculated by the following equation:

$$\text{Serum Osmolality} = 2[\text{Na}^+] + \left([\text{BUN}]/2.8\right) + \left([\text{Glucose}]/18\right)$$

As is illustrated by the equation, the serum sodium concentration exerts the largest effect on the serum osmolality. Thus, regulation of the body's fluid status is largely accomplished through its ability to manipulate serum sodium. Osmolarity is monitored through the presence of special receptors in portions of the brain such as the organum vasculosum laminae terminalis (OVLT) and hypothalamus. When an increase in osmolality is detected, the osmoreceptors in these locales depolarize the membrane and lead to an activation of the thirst mechanism and release of vasopressin from the posterior hypothalamus. Binding of vasopressin to V2 receptors in the collecting duct actuates the insertion of the AQP2 channels into the apical membrane and the reabsorption of sodium.

22.2.2 Acid-Base Homeostasis

Normally, the serum pH is tightly regulated over a range of 7.36–7.44 to facilitate the optimal functioning of cellular meta-

Table 22.1 Key functions, characteristics, and receptors of each nephron component

Nephron component	Function	Characteristics	Important component receptors
Glomerulus	Filtering unit of kidney	Capillary bundle situated between efferent and afferent arteriole Structural components: fenestrated endothelium, glomerular basement membrane, podocytes, mesangial cells	
Proximal tubule	Reabsorption of large proportion of water, NaCl, $NaHC0_3$, Glucose, AA, phosphate, and citrate. Hormone production Gluconeogenesis Synthesizes buffers (NH_3/NH_4^+) Excretion of drugs and endogenous toxins	Low-resistance Low transepithelial voltage High ion permeability High water permeability	Na^+-K^+-ATPase NHE3 receptor (Na^+/H^+ transporter) H^+-ATPase SGLT2/SGLT1 (Na^+-glucose transporter) NaDC-1 (Na^+-dependent dicarboxylic acid cotransporter) AQP1 (aquaporin, water channel)
Thin limb loop of Henle	Involved in counter current multiplication to exchange urea and create gradient to concentrate urine	Descending thin limbs (DTL) are permeable to water (upper 40%) Ascending Thin limbs (ATL) impermeable to water High permeability to urea Passive NaCl reabsorption from prebend segment to ATL	AQP1 (DTL)
Thick ascending limb loop of Henle	Extracellular fluid (ECF) volume Concentration of urine Calcium/Magnesium balance Bicarbonate reabsorption/ Ammonium excretion	Water impermeable at apical membrane Actively reabsorbs NaCl leading to a dilute luminal fluid driving countercurrent multiplication Contains the specialized macula densa cells located in close proximity to the arterioles of the glomerulus	NKCC2 (furosemide sensitive, electroneutral Na^+-K^+-$2Cl^-$ cotransporter) ROMK (renal outer medullary potassium channel) BK (K channel) Na^+-K^+-ATPase ClC-KB (chloride channel) KCC4 (K-Cl cotransporter) NHE3/NHE4 (Na^+-H^+ exchangers)
Distal Convoluted Tubule (DCT)	NaCl reabsorption K secretion Calcium and Magnesium balance	Begins downstream of the macula densa Shortest nephron segment Made of 2 functionally different segments: DCT1 and DCT2 Cells very mitochondria-rich	Na^+-K^+-ATPase (BLM) NCC (thiazide-sensitive NaCl cotransporter, AM) ENaC (epithelial sodium channel) (amiloride-sensitive, AM) Kir potassium channels (BLM) KCC4 (K-Cl- transporter, BLM) ClC-KB (chloride channel; BLM) ROMK (renal outer medullary potassium channel, AM) BK (K channel, AM)
Collecting duct	Salt and water transport Control of [K+] Acid/Base regulation through excretion of $NH3/NH_4^+$, reabsorption/generation HCO_3	Two cell types: Principal Cells and Intercalated Cells Aldosterone sensitive part of distal nephron	ENaC (epithelial Na channel) AQP2 (water channel) Na^+-K^+-ATPase (principal cells) ROMK BK H^+-ATPase (Intercalated cells) Pendrin (Cl^-HCO_3 exchanger)

Table 22.2 Key functions of nephron segments and receptors

Hormone	Trigger	Receptor/nephron segment	Effect
Adenosine	Salt loading	Generated by interstitial fibroblasts, which metabolize ATP released by macula densa cells Activates A1 & A2 receptors Inhibition of ENaC in collecting ducts (CD)	Vasodilation of efferent arterioles and Vasoconstriction of afferent arterioles Overall reduction in glomerular filtration rate (GFR) Inhibits renin release
Aldosterone	Hyperkalemia Volume depletion	NCC in distal convoluted tubule (DCT) ROMK (renal outer medullary potassium channel) in DCT Increases # ENaC in DCT and CD Increase pendrin in CD Type B IC	Facilitates K^+ secretion directly Reabsorbs NaCl helping to increase extracellular fluid (ECF) volume Increases K^+ permeability
Angiotensin II	Volume depletion	NCC in DCT Activates ENaC in DCT and CD Increase pendrin and H^+-ATPase in CD Type B IC Inhibits ROMK	Reabsorbs NaCl helping to increase ECF volume Prevents K^+ wasting
Atrial natriuretic peptide	Atrial stretch receptors Angiotensin II Endothelin	Inhibits ENaC in CD Inhibits renin secretion Inhibits aldosterone production in adrenal gland	Inhibits reabsorption of Na^+
Erythropoietin	Decreased tissue oxygen tension	Produced by interstitial renal fibroblasts	Stimulates Erythropoiesis
Insulin	Hyperglycemia	Stimulates NHE3 dependent Na+ in PT Activates NCC in DCT Stimulates ENaC in CD	Stimulates salt reabsorption
Parathyroid	Decreased in plasma concentration of ionized calcium	TRPV5 receptor in DCT Decreases the abundance of phosphate transporter proteins in proximal tubule	Increased renal reabsorption of calcium Stimulates bone resorption Enhanced intestinal calcium and phosphate absorption by promoting the formation of calcitriol in kidney Decreased renal reabsoption of phosphate
Renin	Atrial Natriuretic peptide Angiotensin II Sympathetic Activation (NaCl) in macula densa cells Nitric oxide Prostacyclin/Prostaglandin E2	Cleaves angiotensinogen to angiotensin I	Regulates the renin–angiotensin–aldosterone system (RAAS) Helps to maintain blood pressure and intravascular volume
Vasopressin	Increasing plasma osmolality Circulatory hemodynamics (ie, CV collapse) Low sensed circulating volume NE/ Dopamine Pain Hypoxia Acidosis	NCC in DCT Stimulates ENaC in CD Induced an increase in AQP2 receptors in CD Increases permeability of inner medullary CD to urea by Increasing the number and ability of UT-A1/ UT-A3 transporters	Stimulates salt reabsorption Increased water reabsorption Overall effect: lower Na^+ concentration

Table 22.3 Key electrolytes and solutes in the glomerular filtrate

Solute	Percent	Loop of Henle	Distal convoluted tubule (DCT)	Collecting ducts (CD)
Na+	60–70% reabsorbed	25–30% reabsorbed: descending thin limb (DTL) and ascending thin limb (ATL) - passive reabsorption Thick ascending limb (TAL) - active reabsorption via NKCC2 and NH3	Reabsorbed via NCC and ENaC DCT and CD together only reabsorb 5–10% filtered Na^+	Reabsorbed via ENaC
Cl-	60–70% reabsorbed	DTL and ATL – passive reabsorption TAL - active reabsorption	Reabsorbed by NCC and paracellular	Transport via: 1. paracellular transport
Bicarbonate	70–90% reabsorbed/ generated	TAL- 15% reabsorbed via apical Na^+/H^+ exchanger		New generation in IC
K+	Majority of filtered potassium is reabsorbed through paracellular pathway. Only ~10% reaches distal nephron.	TAL - Paracellular reclamation plus transcellular transport via NKCC2 transporter	Secreted into tubular lumen via ROMK (renal outer medullary potassium channel) and BK (K channel)	Secreted into tubular lumen via ROMK in PC May be secreted by BK receptor in Type A IC depending on conditions.
Ca^{2+}	60–70% reabsorbed mainly by paracellular transport. Small amount actively transported.	20% reabsorbed in TAL mainly via paracellular transport.	7–10% actively reabsorbed	5% reabsorbed
Mg^{2+}	10–30% reabsorbed via paracellular pathway	40–70% reabsorbed in TAL via paracellular pathway	5–10% actively reabsorbed	
Phosphate	85% reabsorbed			
NH_4^+	Generates NH_4^+ from glutamine and secreted into lumen	Approximately 50% reabsorbed in TAL into peritubular space with formation of medullary gradient		Secretion of ammonium into lumen by intercalated cells
Urea	Concentration increases as salt and water are reabsorbed	Countercurrent multiplication, (urea) increases in thin limbs due to secretion but is reabsorbed as the thin limbs ascend; develops an equilibrium with medullary interstitium at TAL	Reabsorption of some of urea. Concentration changes from approx 110% filtered load to roughly 70%	Excretion of urea into interstitium. Tubular fluid (urea) reduced to 50% filtered load
Water	60–70% reabsorbed	Some reabsorption in DTL ATL, TAL water impermeable	Impermeable	Reabsorbed via AQP proteins
Glucose	>99% reabsorbed			

bolic processes. When physiologic derangements occur to drive the body's acid-base status outside of this range, compensatory responses are initiated in an effort to mitigate the perturbation from normal values [18]. Responses to alterations in pH are managed in a number of ways including the use of buffers, regulation of PCO_2, and manipulation of plasma HCO_3. Proteins and the bone act as important physiologic buffers. However, the bicarbonate/carbon dioxide buffer system is perhaps the most important buffering system in the body.

The Henderson-Hasselbach equation illustrates the relationship between pH, plasma bicarbonate, and PCO_2:

$$\mathrm{pH} = 6.1 + \log \mathrm{HCO_3^-} / \left(0.03 \times \mathrm{PCO_2}\right).$$

Thus, in order to compensate for a specific change in pH, the body must manipulate values of either HCO_3 or PCO_2. These values can be independently regulated by the kidneys and the lungs respectively.

Through changes in ventilation, the lung is able to exert it effects on pH. The key to this effect lies in the interaction of carbon dioxide and water. When the two are combined, carbonic acid is formed, which then dissociates into hydrogen ion and bicarbonate. These forms of water and carbon dioxide exist in an equilibrium and are thus affected when the level of carbon dioxide changes:

$$\mathrm{CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-}$$

Table 22.4 Mechanisms of action in homeostasis

Type of diuretic	Mechanism of action	Result	Example
Osmotic diuretic	Filtered at glomerulus with minimal reabsorption in proximal tubule. Offsets the passive reabsorption of water in the proximal tubule.	Excretion of water	Mannitol
Loop diuretic	Inhibit Na and Cl reabsorption in the TAL by inhibiting the electroneutral Na^+-K^+-$2Cl^-$ cotransporter (NKCC2)	Increases excretion of filtered Na load. Increased Calcium and Magnesium excretion in urine. Increases K+ & H+ excretion at DCT & CT	Furosemide Bumetanide Torsemide
Thiazides/ Thiazide like diuretics	Inhibit sodium reabsorption through inhibition of the luminal NaCl cotransporter protein.(NCC) Augment Ca+ reabsorption in distal tubule.	Increase Na+ excretion by a small amount. Increased Na+ excretion results in enhanced secretion of K+ &H+	Hydrochlorothiazide Metolazone Indaptamide
Potassium sparing diuretic	Collecting tubule: Inhibit Na+reabsorption and K+ secretion by blocking epithelial sodium channel (ENaC). May inhibit Na+-K+-ATPase activity as well	May result in hyperkalemia and metabolic acidosis	Amiloride
Aldosterone Antagoinists	Antagonize aldosterone receptors in CT	Na+ reabsorption and K+ secretion is inhibited. Can lead to hyperkalemia and metabolic acidosis	Spironolactone
Carbonic anhydrase inhibitors	Proximal tubule: inhibit carbonic anhydrase. Inhibits renal acidification (H^+ secretion and Na^+ / HCO_3^- reabsorption)	Weak diuretic due to ability of distal segments of nephron to compensate for the effects on Na^+ and HCO_3^-. End result is a mild hyperchloremic metabolic acidosis	Acetazolamide

Hypoventilation results in an increase in PCO_2. This elevation in PCO_2 will drive the formation of carbonic acid, and thus hydrogen ion and bicarbonate. Changes in ventilation are triggered by specialized chemoreceptor cells within the medulla oblongata responding to alterations in pH and PCO_2. In the example of hypoventilation, the decrease in cerebral intrastitial pH causes the chemoreceptors to stimulate ventilation. As a result of this activation, PCO_2 decreases. Initially, ventilatory changes are most sensitive to changes in PCO_2. The maximal ventilatory response to changes in pH takes approximately 12–24 h. While PCO_2 is easily diffusible, the blood-brain barrier acts as hurdle slowing the response to nonvolatile acids and alterations in plasma bicarbonate. Generally speaking, there is a limit to the effect that ventilation can exert upon the pH via PCO_2, with decreases greater than 12 mm Hg unlikely.

The contribution that the kidney makes in regulating acid-base balance is accomplished through its handling of bicarbonate. Remember that bicarbonate is freely filtered by the glomerulus. The proximal tubule then reabsorbs about 80% of this filtered bicarbonate with the remaining amounts reclaimed in the thick ascending limb of the loop of Henle. The mechanism by which this reabsorption occurs was discussed in detail earlier [7], but essentially relies on the production of hydrogen ion and bicarbonate intracellularly through the work of carbonic anhydrase. The bicarbonate is then reabsorbed into the blood. Meanwhile the hydrogen ion gets secreted into the luminal fluid. Here the hydrogen ion either gets bound as a titratable acid excreted in the urine or is recycled back into the proximal tubular cell as carbon dioxide (thanks to carbonic anhydrase again) or a form of citrate (remember that citrate can be converted into bicarbonate in the liver). The extent to which these processes occur in the proximal tubule will depend on the body's acid-base and volume status.

Final refinement of the body's acid-base balance is achieved in the distal tubule through the work of the Type A and Type B intercalated cells as previously mentioned. The ultimate effect on the urine, either acidification by type A intercalated cells, or bicarbonate secretion by type B intercalated cells will be influenced by a variety of factors [18]. For example, in type A intercalated cells vesicles full of H^+-ATPase pump proteins lie beneath the cellular membrane awaiting the pH induced signal to move to the apical membrane and insert themselves increasing the productivity of the cell. In a sense these vesicles act as a type of SWAT team immediately responding to pH conditions sensed in the tubular fluid. While these vesicles act as a type of temporary workforce, if conditions persist more chronically, the cell begins to synthesize more transporter proteins, in a sense hiring a larger more permanent workforce. Factors such as potassium depletion, increased sodium reabsorption, and hormonal effects—such as those caused by mineralcorticoids, aldosterone, and angiotensin II—will all lead to increased tubular hydrogen ion secretion.

As illustrated, the kidney has a variety of tools that it can employ in order to compensate for different acid-base disturbances (Table 22.4). As with any compensatory response,

the effects are not able to completely correct for the original acid-base disturbance and take some time to fully develop. Chronic respiratory acidosis results in increased reabsorption of bicarbonate by the proximal tubule and its increased production in the distal tubule. Conversely, chronic hypocapnea decreases the reabsorption of bicarbonate by the proximal tubule and results in hydrogen ion secretion in the distal tubule. Large metabolic acid loads that overwhelm the kidney's ability to generate bicarbonate engenders a different set of responses. The plasma bicarbonate will fall despite increased reabsorption of bicarbonate by the proximal tubule. Concurrently, the distal tubule will increase both hydrogen ion secretion as well as secretion of NH_4^+. These responses will take approximately 3–5 days to become fully realized. In addition, a compensatory respiratory response to increase ventilation thereby decreasing PCO_2 will be stimulated. This respiratory response will fully manifest over a 12–24-h period, and again will not be able to fully correct for the metabolic derangement [7].

The response to metabolic alkalosis will depend on a variety of factors [7]. A pure alkali load, such as when a patient is given a bicarbonate infusion, is relatively easily dealt with by the kidney. Decreased proximal tubule reabsorption will lead to loss of bicarbonate in the urine and thus correct the derangement. From a respiratory standpoint, the compensatory response will be to decrease ventilation slightly leading to retention of PCO_2. However, when a metabolic alkalosis is caused by loss of acid such as with vomiting, the kidney's response may actually maintain the metabolic alkalosis until other factors such as hypokalemia, hypochloremia, and hypovolemia are corrected. Chronic hypokalemia results in secretion of hydrogen ion. In patients who are volume contracted, the effects of hormones (such as angiotensin II, aldosterone, adrenergic agonists) will result in the increased reabsorption of sodium and bicarbonate. Until the volume depletion is corrected, the metabolic alkalosis may be maintained.

22.3 Renal Function Tests

To this point we have focused on the function of a single nephron. This microscopic level is difficult for clinicians to measure, and considering that there are millions of glomeruli, knowledge of a single nephron's activity is not clinically relevant. As clinicians we are more focused on the productivity of the kidneys as a whole unit. Unlike the cardiac myocytes, which leak troponin when injured and inflamed, we do not have any similar easily measurable, widely available, validated compounds that provide an early warning of injury to nephrons. This area is currently being actively researched and in the future may unearth a biomarker useful in practical day to day decision making.

At present, the glomerular filtration rate (GFR), which represents the cumulative functioning of all of the millions of nephrons in the kidney, is the parameter referred to as an indicator of global renal function. This value represents to the amount of plasma that is filtered through all the glomeruli per unit of time, usually minutes. A normal value for GFR ranges from 120–130 ml/min/1.73m^2. This value will vary from person to person depending on factors such as age, sex, and race. Patients with Stage 5 kidney disease have end stage renal disease requiring dialysis and have a GFR of less than 15 ml/min/1.73m^2.

A few challenges surround the use of the GFR by clinicians. First and foremost is the fact that the GFR cannot be measured directly. Instead we must rely on surrogates that act as representative markers of filtration [19–25]. These markers can either be produced endogenously by the body or be introduced exogenously. In order to portray an accurate picture of renal function the concentration of these markers must not be altered by the kidney; i.e., they must not be metabolized, reabsorbed from, or secreted into the tubular lumen. Second, although we use GFR as a measure of renal function, the degree of injury to nephrons does not translate to a proportional decrement in the GFR. For example, a 10% loss of functioning nephrons does not result in a reduction of GFR by 10%. The kidney can be thought of as a stoic organ in the sense that early injuries to it are not easily visible. When nephrons are lost, the remaining units adapt by a "hyperfiltration" of the higher amounts of solutes and water. In this manner nephron damage can occur without any perceptible change in GFR until this "functional reserve" has been exhausted.

Is it necessary to exactly measure GFR? Knowledge of this marker of kidney function allows us to accurately dose medications thereby reducing toxicity. While it may be imperfect, the GFR helps clinicians follow the progression of loss of renal function. Knowledge of the GFR may aid with issues in prognostication regarding need for renal replacement therapy or listing for renal transplant as well. Given this utility, how then do we reconcile the practical needs of this value with the fact that we cannot directly measure it? Fortunately, in many cases an estimate of the GFR (eGFR) sufficiently answers many clinical queries [19–25]. For the instances in which a measured value is needed due to concerns of inaccuracies with estimated values, use of exogenous markers can be employed.

22.3.1 Exogenous Markers of Renal Function

Inulin clearance remains the gold standard for determination of GFR. Inulin is a polysaccharide, which is not protein bound, that is solely excreted by glomerular filtration and is neither secreted nor absorbed. The GFR is determined by the exogenous administration of inulin either by a bolus or an infusion. The specifics of how the calculation is determined depends on the method used, but does require multiple samples of blood, and may require a timed urine collection as well. Thus, the method is invasive, expensive, and time consuming [19–25].

The use of different radioisotope compounds largely replaced the use of inulin, but again were difficult to

administer, not as accurate, expensive, and not safe in certain patient populations. The use of iohexol or iothalamate provide an alternative nonradioactive method. These compounds are radiocontrast agents. Iohexol is administered as a intravenous bolus injection and calculation of its plasma clearance provides a measurement of GFR. This spares the bedside clinician of having to perform a timed urine collection and deal with all of the special requirements of radioactive isotopes. Iohexol has been shown to underestimate GFR slightly compared to inulin. Renal clearance of iothalamate has also been demonstrated as an accurate method to measure GFR [19–25].

22.3.2 Endogenous Markers of Renal Func tion

The convenience associated with the use of serum creatinine has led to its widespread use as an indicator of renal function for many years. Creatinine is a product of skeletal muscle breakdown and is freely filtered by the kidney without being reabsorbed. Its serum levels correlate inversely with GFR. However, unlike inulin, creatinine undergoes secretion by the proximal tubule in varying amounts depending on body conditions. Thus it is not an ideal renal biomarker to assess glomerular filtration rates. While this secretion can be blunted by the administration of cimetidine, the use of plasma creatinine is problematic for other reasons as well. Evidence exists of external elimination of creatinine thus adding to inaccuracies. Also the generation of serum creatinine will depend on factors such as muscle mass, obesity, and dietary intake. For example, a creatinine level of 1.2 may not have the same clinical implications in a body builder taking dietary supplements compared to a frail, wheelchair bound 80-year-old woman. Patients who have a high volume of distribution from edema can have a "diluted" serum creatinine concentration which again may create a false impression of renal function. Nevertheless use of creatinine is widely favored because of its ease of use, and cost effectiveness [19–25].

The creatinine clearance can be easily calculated by:

$$\mathrm{Cr\,Cl}\left(\mathrm{mg/min}\right)=\frac{\left[\text{urine creatinine}\left(\mathrm{mg/mL}\right)\right]\times 24\,\text{hour urine volume}\left(\mathrm{mL}\right)}{\left[\text{plasma creatinine}\left(\frac{\mathrm{mg}}{\mathrm{mL}}\right)\right]\times 24\times 60\,\mathrm{min}}$$

Performing a 24-h urine collection can be difficult, so in order to minimize inaccuracies, the equation can be modified for a shorter time period of urine collection. Unfortunately, the improvement in convenience and cost effectiveness comes at the price of decreased accuracy in the information obtained. Studies that have been performed comparing the accuracy of serum creatinine in relation to other renal markers have demonstrated that creatinine clearance consistently overestimates the GFR. Additionally, the amount of overestimation increases when the GFR is low.

In most instances, creatinine clearance is not calculated based on plasma and urinary creatinine levels but rather an estimated GFR, which is calculated with equations that incorporate laboratory results with demographic data. Various equations have been developed and evaluated for accuracy compared to measured GFR values. The familiar Cockcroft-Gault equation takes age, weight, and sex into account to calculate eGFR:

$$\mathrm{CrCl}=\left(140-\text{Years Age}\right)\times\left(\text{Kg bodyweight}\right)\times\left(0.85\text{ if female}\right)/\left(72\times\text{sCr in mg/dL}\right)$$

Numerous other equations have been developed over time including the Modification of Diet in Renal Disease (MDRD) study equation, and the CKD-EPI (CKD epidemiology collaboration) equation. These equations have their strengths and weaknesses regarding their accuracy in predicting eGFR accurately and will vary depending on the patient population in question, cultural dietary factors, genetic factors, and level of kidney function.

The issues with serum creatinine described earlier have led to a search for other endogenous renal markers that do not suffer from the limitations described above. A lot of interest has surrounded cystatin C [20, 21]. This compound is generated by nucleated cells and released into the blood. Serum levels also vary inversely with GFR. In the kidney it is freely filtered by the glomerulus and then reabsorbed and metabolized by the proximal tubules. As such it is not possible to calculate a cystatin C clearance in the way that we calculate one for creatinine. While cystatin C levels are not impacted by factors such as diet and muscle mass in the manner serum creatinine levels are, they can be affected by conditions affecting cell turnover rate such as high doses of steroids and thyroid dysfunction. There is also some evidence from animal studies of extrarenal elimination that may overestimate true GFR. As with serum creatinine, various equations have been developed to estimate GFR based on cystatin C levels. Additionally, equations have been developed that combine the use of both serum creatinine and cystatin C levels in an attempt to determine an equation that accurately reflects GFR and without needing modification due to race. In addition, cystatin C may help to better define and risk stratify patients with chronic kidney disease when compared to use of eGFR based on creatinine alone [20, 21]. Research into the best use and role of cystatin C is dynamic, and recent improvement such as the standardization of assays and increased availability suggest an increasing role for this renal biomarker in the future.

22.4 Acute Kidney Injury and the Role of Emerging Biomarkers

The difficulties inherent in determining global renal function make it challenging to identify when the kidney has sustained an injury or is under stress. The clinical relevance of this reality is obvious. If one cannot identify that an injury has taken place, it is difficult to institute therapies or guide clinical decisions that reverse or arrest the pathologic process.

In the past, a lack of consensus regarding the definition of acute renal injury has further complicated the clinical picture. Prior research has utilized many different definitions and endpoints making it difficult to interpret research findings [26–32]. Fortunately, this issue is being addressed. A standardized criteria for the definition of acute kidney injury was proposed in 2004 by the Acute Dialysis Quality Initiative and is commonly referred to as the RIFLE criteria (Risk Injury Failure Loss End-stage renal disease) [26]. This definition has been refined over the years and the current consensus definition has been described by the Kidney Disease Improving Global Outcomes (KDIGO) group [27]. The classification system according to the KDIGO criteria describes 3 stages of acute renal injury. These stages are generally differentiated from one another based on 2 variables: increases in serum creatinine and changes in urine output. The KDIGO criteria is summarized in Table 22.5. The differentiation between the stages is relatively straightforward when one considers the urine output parameters. With a Foley catheter in place it is not difficult to determine how much urine a patient has produced over the specified time period. On the other hand, the criteria for changes in serum creatinine are more difficult to interpret. Oftentimes, clinician do not have the luxury of knowing what a patient's baseline creatinine is. This makes it difficult to interpret a given elevated creatinine level. A creatinine level that seems normal for the average individual may actually be pathologic in an older patient since they tend to produce less creatinine as a result of lower levels of muscle mass. Thus, an elevation in creatinine may either: (1) go completely unrecognized missing the acute renal injury completely, or (2) have a renal injury recognized but be underestimated in terms of its severity.

Table 22.5 The Kidney Disease Improving Global Outcomes (KDIGO) criteria [27]

KDIGO stage of renal dysfunction	Serum creatinine	Urine output
1	Increase 1.5–1.9 × baseline *or* >0.3 mg/dL increase within 48 h	<0.5 mL/kg/h for 6–12 h
2	Increase 2.0 to 2.9 × baseline	<0.5 mL/kg/h for >12 h
3	Increase 3.0 × baseline *or* Rise serum creatinine to > or equal to 4.0 *or* Initiation of renal replacement therapy (RRT) *or* Decrease eGFR to <35 mL/min per 1.73 m2 in patients <18 years old	<0.3 mL/kg/h for > or equal to 24 h *or* Anuria for > or equal to 12 h

eGFR estimated glomerular filtration rate

As mentioned previously, the desire to improve our clinical ability to both diagnose and improve prognostic prediction in acute kidney injury has led to a very active search to identify renal biomarkers. A number of different biomarkers have shown promise in helping to identify both acute kidney injury and kidney stress. Here we will focus on a few of the compounds that have thus far shown promise.

Neutrophil gelatinase-associated lipocalin or NGAL is a compound that is released from neutrophils in response to systemic inflammation. This protein is expressed in the lung, liver, and kidney. Its utility as a biomarker has been investigated in many different clinical settings and appears to be a good predictor of acute renal injury. A limitation of the use of this biomarker is the fact that elevations are not limited to just renal dysfunction, but other inflammatory conditions such as cancer, preeclampsia, and sepsis. In addition, its clinical utility is not as robust in patients with preexisting renal dysfunction.

Two compounds that have shown a lot of promise recently include insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases-2 (TIMP-2). Both of these biomarkers are classified as G1 cell cycle arrest proteins. Epithelial cells in the body express IGFBP7 while TIMP-2 is expressed in tubular epithelial cells. Epithelial cells have developed a protective mechanism that they employ in times of stress; they enter cell cycle arrest. Because of this protective mechanism, elevations in TIMP-2 and IGFBP7 may provide the opportunity to identify risk for renal injury before any damage actually occurs. In truth, these two biomarkers have shown the best results in identifying acute renal dysfunction than any others studied thus far. In addition, they do not appear to become elevated in patients with sepsis or chronic renal dysfunction in the way that NGAL does. With time, the role of these two biomarkers will become better elucidated.

22.5 Renal Drug Excretion

Stop and think for a moment of the myriad of compounds that the kidney must handle over the course of a person's life. In addition to managing levels of electrolytes, amino acids, and glucose, etc. that we addressed earlier, nephrons also manage the excretion of metabolic byproducts, both endogenous and exogenous toxins, as well as drugs and vitamins. The manner in which this impressive logistical feat is accomplished is not all that well understood and is being actively investigated. A discussion regarding the manner of disposal by the kidney of all the compounds of interest to the anesthesiologist is beyond the scope of this discussion. Instead we will focus on developing an overall understanding of how drugs and

endogenous toxins are manipulated by the kidney as well as the complexity of the interactions involved [33–35].

As discussed, the glomerulus is the site of initial filtration of most compounds with the exception of anions and large molecules. When the glomerulus is damaged, it may lose its ability to prevent these compounds from being filtered, leading to a loss of albumin and a subsequent alteration in binding and free fraction of protein bound drugs.

The proximal tubule segment shares a significant portion of the responsibility for drug and endogenous toxin excretion as well, according to our current understanding of renal drug elimination. It manages those compounds that are either too large or too protein bound to be effectively filtered by the glomerulus. The sheer number of different compounds makes it impractical for a specific transporter protein to be created for every type of compound to which the body is exposed. Instead the kidney appears to rely on specialized drug transporter proteins to process compounds depending on characteristics such as size and electrical charge. Compounds can be classified as positively charged organic cations, negatively charged organic anions, or containing both positive and negative charges.

Those drugs not undergoing glomerular filtration reach the proximal tubule through peritubular capillaries. Multispecific drug transporters then import the compounds into the peritubular cell. Two general types of transporters exist and are differentiated from one another by the way that they fund the energy expenditure necessary for their actions. The first type, solute carriers or SLC carriers, either take advantage of a favorable concentration gradient or make use of secondary, or even tertiary active transport. (This again underscores the importance of the Na^+-K^+-ATPase.) The 2 most important members of this family of transporters are the organic anion transporters (OAT) and the organic cation transporters (OCT). The second general class are the ATP-binding cassette (ABC) transporters. These transporters make use of ATP hydrolysis to generate the energy needed for the transport process. Once the compounds have been imported across the basolateral (capillary-cell interface) membrane then they travel across the cell to the apical membrane where they are shuttled across into the tubular lumen.

Different transporters are involved in the handling of organic anions versus organic cations. For organic cations, OCT2 mediates transport on the basolateral membrane while proteins such as MATEs are involved in the apical transport. Transport of organic anions occurs via OAT1 and OAT3 on the basolateral membrane with OAT4 and ABC transporters such as MRP2 functioning at the apical interface.

The key here is not to get caught up in this veritable alphabet soup of transporters, but to have an awareness of the pathway drugs and toxins take in order to undergo secretion and elimination. This knowledge also helps us to understand the reasons for different drug-drug interactions and the variability of responses to medications in certain patients. With the many different substrates available to these transporter proteins it should not be surprising that they can become saturated. Fortunately, many of these compounds can be managed by more than 1 type of renal transporter. Still, the addition of a new substrate may alter the excretion of another one through its occupation of the renal transporter binding site. An example of this type of competitive inhibition was mentioned earlier when we discussed the ability of cimetidine to inhibit secretion of creatinine. Creatinine and cimetidine are both substrates of OCT2. Another example involves the interaction between methotrexate and nonsteroidal anti-inflammatory drugs (NSAIDs). The use of NSAIDs in preoperative care is very common. Many patients with Crohn's disease or other autoimmune diseases have been prescribed methotrexate. The elimination of methotrexate is mediated by OAT transporters, which can be inhibited by NSAIDs. Thus, the coadministration of these medications can result in methotrexate toxicity and bone marrow suppression.

Variability in the clinical effects of drugs in different individuals may be partially explained by different variations or polymorphisms of these transporter proteins. Changes in the makeup of these proteins may lead to differences in rates of transport, which may affect drug efficacy and or toxicity. Metformin is an example of a drug whose variable effectiveness may be explained by this mechanism.

Finally, the above discussion has mainly focused on drug elimination in the health kidney. Chronic kidney disease results in many changes in drug elimination and excretion. These changes result from the accumulation of uremic toxins and have effects outside of the kidney itself. They can alter bioavailability of drugs, the expression and activity of cytochrome P450 3A enzymes in the liver and drug transporter proteins in the liver. The transporter proteins in the kidney itself can also be affected leading to reduced excretion of drugs. Alterations in albumin levels affect free drug levels, and uremic toxins can affect the ability of the remaining albumin to bind to acidic drugs. Fortunately, it appears that the removal of uremic toxins by hemodialysis can reverse some of these effects.

22.6 Questions and Answers

Questions (choose the most appropriate answer)

1. Which of the following correctly pairs the highest degree of reabsorption of the indicated substance with the nephron segment?
   A. Proximal tubule: albumin
   B. Collecting duct: glucose
   C. Ascending thin limb loop of Henle: water
   D. Magnesium: loop of Henle
   E. Collecting duct: ammonium ion
2. The sodium potassium ATPase pump creates the electrochemical gradient responsible for cellular activities in all of the following cell types EXCEPT:
   A. Proximal tubule cell
   B. Descending thin limb loop of cell
   C. Thick ascending limb cell
   D. Principal cell in collecting duct
   E. Intercalated cell in collecting duct

3. Which of the following compounds/solutes is NOT reabsorbed in the proximal tubule?
   A. NH_4^+
   B. Amino acids
   C. Bicarbonate
   D. Water
   E. Sodium
4. Which of the following statements regarding renal anatomy is INCORRECT?
   A. The left kidney has a more cephalad position in the body compared to the right kidney.
   B. The body contains approximately 2 million nephrons.
   C. The kidney receives approximately 15% of total cardiac output.
   D. The majority of nephrons in the renal cortex are cortical rather than juxtamedullary.
   E. The left renal vein is longer than the right renal vein.
5. Which is the TRUE statement regarding the loop of Henle?
   A. The thin descending and ascending limbs are permeable to water.
   B. The thin descending and ascending limbs have limited permeability to urea.
   C. The area from the hairpin loop to the thick ascending limb of the loop of Henle is permeable to NaCl.
   D. Reabsorption of NaCl in the thick ascending limb occurs mainly via the paracellular pathway.
   E. More calcium than magnesium is reabsorbed in the thick ascending limb.
6. Which of the following statements about the distal convoluted tubule (DCT) is TRUE?
   A. Of the 2 segments of the DCT, the DCT2 segment is the most sensitive to the effects of aldosterone.
   B. Sodium reabsorption in the early DCT is electrogenic in nature, but electro-neutral in the late DCT.
   C. The late DCT is an important mediator of potassium reabsorption in the nephron.
   D. Magnesium plays an important role in the mechanism through which potassium reabsorption is achieve in the DCT.
   E. None of the above
7. Which of the following patients would be expected to have a normal serum osmolality?
   A. An 18-year-old patient with type 1 diabetes admitted with a blood sugar of 480.
   B. A 55-year-old patient who has been taking salt substitutes (KCl).
   C. An 81-year-old patient with end-stage renal disease (ESRD) prior to institution of dialysis.
   D. A 75-year-old patient with syndrome of inappropriate antidiuretic hormone secretion (SIADH).
   E. A 56-year-old patient treated with hypertonic saline in the neuro ICU secondary to elevated intracranial pressure (ICP).
8. Which of the following is a FALSE statement regarding inulin?
   A. Measurements of its clearance is considered to represent the "gold standard" for determination of renal function.
   B. Inulin is neither secreted, excreted, or reabsorbed by the kidney.
   C. Measurement of GFR using inulin clearance is costly and effort intensive.
   D. Inulin clearance has been replaced by other methods in order to measure renal function.
   E. Inulin is an endogenous compound and is a polysaccharide.
9. Which of the following regarding endogenous markers of renal function is TRUE?
   A. Serum creatinine gives an accurate representation of renal function because it is not secreted or reabsorbed by the kidney.
   B. A serum creatinine level of 1.0 would be considered to be representative of normal renal function in all patients.
   C. Renal clearance of cystatin C acts as an alternative marker of renal function in a manner similar to creatinine clearance.
   D. Both cystatin C and creatinine levels vary inversely with GFR.
   E. Cystatin C levels are influenced by dietary factors in a manner similar to that of serum creatinine.
10. Regarding the handling of drugs and toxins by the kidney, which of the following represents an accurate pairing:
    A. Glomerulus: filtration of anions
    B. Proximal tubule: small, non-protein bound drugs
    C. SLC carriers: make use of ATP hydrolysis to generate the energy needed for the transport process.
    D. Peritubular capillaries: Mechanism for transportation of large protein-bound compounds to the proximal tubule.
    E. ABC transporters: Depend on a favorable concentration gradient, secondary, or even tertiary active transport to drive the energy expenditure necessary for the transport of drugs.

Answers

1. **D.** The highest rate of reabsorption of magnesium occurs via the paracellular pathway in the loop of Henle. Under normal physiologic conditions, albumin is not filtered by the glomerulus and thus does not undergo reabsorption. Glucose is reabsorbed almost completely by the proximal tubule. The thin ascending limb of the loop of Henle is impermeable to water. The collecting duct secretes ammonium ion rather than reabsorbing it.
2. **E.** Instead of the Na^+-K^+-ATPase, a H^+-ATPase serves as the generator of the electrochemical gradient in intercalated cells.

3. **A**. All of the listed compounds/solutes undergo reabsorption by the proximal tubule with the exception of NH_4^+. Ammonium is actually *produced* by the proximal tubule not reabsorbed. Ammonium ion can be created in a variety of ways. Hydrogen ion secreted into the proximal tubule lumen can combine with NH_3 to form ammonium anion thus functioning as a titratable acid. Under conditions of acidosis, glutamine undergoes metabolism in the proximal tubular cell. The mitochondrial enzyme glutaminase interacts with glutamine to form glutamate and an ammonium ion. The glutamate then undergoes enzymatic conversion into alpha-ketoglutarate and another ammonium ion. The 2 ammonium ions can either be secreted into the lumen by the NH_3 transporter, or can undergo dissociation into a hydrogen ion and NH_3 with subsequent transport into the lumen. In summary, metabolism of glutamine under conditions of acidosis leads to the formation of 2 ammonium ions, and alpha-ketoglutarate. The ammonium is secreted into the tubular lumen and flows through subsequent nephron segments.
4. **C**. The kidney receives approximately 20–25% of cardiac output. The other statements are all correct.
5. **C**. The area from just prior to the hairpin loop to the thick ascending limb (TAL) is permeable to NaCl, which is passively reabsorbed. The ascending thin limb and the majority of the descending thin limb are impermeable to water due to a lack of expression of the aquaporin channel. The thin limbs of the loop of Henle are highly permeable to urea, which diffuses into the luminal fluid as the descending thin limb dives into the renal medulla. The urea then diffuses back out of the lumen as the tubule ascends back toward the renal cortex. In the TAL, the transport of NaCl occurs actively. While there may be a small amount reabsorbed from the tubular lumen via the paracellular pathway, the majority occurs as a result of secondary active transport. The paracellular pathway plays an important role in the reabsorption of both magnesium and calcium. However, 55% of filtered magnesium is reabsorbed by the TAL compared to only 20% of calcium.
6. **A**. It is true that of the 2 segments of the DCT, the DCT2 segment is the most sensitive to the effects of aldosterone.
7. **B**. Serum osmolarity can be calculated by the following equation:

$$\text{Serum Osmolality} = 2\left[\text{Na}^+\right] + \left(\left[\text{BUN}\right]/2.8\right) + \left(\left[\text{Glucose}\right]/18\right)$$

8. **E**. All of the answers are true with the exception of the fact that inulin is an exogenous compound administered to a patient in an effort to measure renal function.
9. **D**. Creatinine is not an ideal marker of renal function because it is secreted by the proximal tubule. Also the generation of serum creatinine will depend on factors such as muscle mass, obesity, and dietary intake. For example, a creatinine level of 1.2 may not have the same clinical implications in a body builder taking dietary supplements compared to a frail, wheelchair-bound 80-year-old woman. Patients who have a high volume of distribution from edema can have a "diluted" serum creatinine concentration, which again may create a false impression of renal function. Cystatin C is generated by nucleated cells and released into the blood. In the kidney it is freely filtered by the glomerulus and then reabsorbed and metabolized by the proximal tubules. As such it is not possible to calculate a cystatin C clearance in the way that we calculate one for creatinine. Cystatin C levels are not impacted by factors such as diet and muscle mass in the manner serum creatinine levels are affected. Both creatinine and cystitis C serum levels vary inversely with GFR.
10. **D**. The glomerulus is the site of initial filtration of most compounds with the exception of anions and large molecules. The proximal tubule manages those compounds that are either too large or protein bound to be effectively filtered by the glomerulus. Those drugs not undergoing glomerular filtration reach the proximal tubule through peritubular capillaries. Multi-specific drug transporters then import the compounds into the peritubular cell. Two general types of transporters exist and are differentiated by the way that they fund the energy expenditure necessary for their actions. The first type, solute carriers or SLC carriers, either take advantage of a favorable concentration gradient or make use of secondary, or even tertiary active transport. The second general class are the ATP-binding cassette (ABC) transporters. These transporters make use of ATP hydrolysis to generate the energy needed for the transport process.

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