Renal Anatomy, Physiology, and Evaluation of Function

CHAPTER 1

GENERAL FUNCTIONS OF THE KIDNEY

The kidneys perform many important functions, including excretion of solute and water, conservation of solute and water, and biosynthesis of hormones. Normal kidney function is vital to the maintenance of a constant internal milieu. The most widely recognized kidney function is to excrete waste products (urea nitrogen, creatinine, nitrogen waste products, phosphorus, symmetric dimethylarginine (SDMA), hemoglobin breakdown products, and hormone metabolites) that are not needed and are potentially dangerous, as they would otherwise accumulate in the body. Waste products are eliminated into urine by various combinations of glomerular filtration and tubular reabsorption and tubular secretion.

Proper kidney function is important for the regulation of water and balance of electrolytes. To maintain homeostasis, the kidneys are able to vary the volume of water excreted into urine, depending on the physiological need of the body. Urine volume can be very small when water is needed to minimize change in the plasma osmolality during times of limited water intake. The combination of glomerular filtration and tubular activity on renal tubular fluid ultimately determines what is excreted and what is conserved from the initial filtrate delivered into Bowman's space.

The kidneys are also responsible for the regulation of arterial pressure, the regulation of acid–base balance, gluconeogenesis, and biosynthesis of erythropoietin, calcitriol, and renin, but discussion of these processes is beyond the scope of this book. The interested reader is referred to detailed reports of renal anatomy and functions elsewhere [1–7].

ANATOMY OF THE KIDNEY

The kidneys are located outside the peritoneal cavity on the dorsal wall of the abdomen. The hilus of the kidney (the indentation on the medial side) is the area in which the renal artery and vein, lymphatics, nerve supply, and ureter enter the kidney. The two major regions of the kidney are the cortex more superficially and more deeply the medulla (Figure 1.1). The cortex contains superficial and juxtamedullary nephrons (glomeruli and tubules) that undergo filtration and tubular processing of filtrate. The medulla contains the long loops of Henle from juxtaglomerular nephrons as well as parts of some loops of Henle from more cortical nephrons, the straight part of the proximal tubule (PT) (S_3) of juxtaglomerular nephrons, connecting tubules, collecting ducts, and vasa rectae. The border of the renal pelvis contains diverticulae that radiate into the deeper medulla. The collecting ducts terminate in the renal pelvis and diverticulae which continues into the upper end of the ureter. Contractile elements in

Urinalysis in the Dog and Cat, First Edition. Dennis J. Chew and Patricia A. Schenck.

the diverticulae, renal pelvis, and ureters propel urine into the urinary bladder where urine is stored until voided [1, 8].

RENAL BLOOD SUPPLY

An elaborate network of renal blood vessels allows for normal glomerular filtration and tubular processing of filtrate that results in urine formation. Once the renal artery enters the kidney, it progressively branches into the interlobar arteries, arcuate arteries, interlobular arteries (radial arteries), afferent arterioles, and finally, glomerular capillaries where fluid is filtered (Figure 1.1). The distal ends of the glomerular capillaries coalesce into the efferent arterioles, which lead to the peritubular capillaries that surround the renal tubules. The peritubular capillaries empty progressively into the interlobular veins, arcuate veins, interlobar veins, and, finally, the renal vein [1, 8].

The glomerular capillaries are unique in that they lie between two arterioles (afferent and efferent arteriole). The hydrostatic pressure of these capillary beds can be altered by changing the resistance of the afferent and efferent arterioles, resulting in a change in the rate of glomerular filtration and tubular reabsorption [2, 7].

ANATOMY OF THE NEPHRON

The nephron is the functional unit of the kidney. Each feline kidney contains approximately 200 000 nephrons, and each canine kidney contains about 400 000 [2, 9–12]. The nephron contains the blood vessels and tubular cellular structures for glomerular ultrafiltration of blood into tubular fluid for further processing. At the core of the nephron is the glomerulus containing the glomerular capillaries, which are covered by visceral epithelial cells. The glomerulus is encased by Bowman's capsule, which includes visceral and parietal epithelial cells (Figure 1.2). Fluid filtered from the glomerulus first passes into Bowman's space and then flows into the PT (Figure 1.3). The PT includes the proximal convoluted tubule and the straight portion distally (also known as S3 or pars recta). The PT starts at the junction with Bowman's space and ends at the junction with Henle's loop. S3 extends into the outer medulla when parent nephrons are juxtaglomerular. The filtered fluid continues to flow into the loop of Henle, which extends into the renal medulla. The loop of Henle consists of three anatomically and functionally different parts. The loop starts from the end of the PT (S3) and ends as it joins the distal tubule. The thin descending limb ends at the hair pin turn, and the thin ascending limb starts at the hair pin turn of the

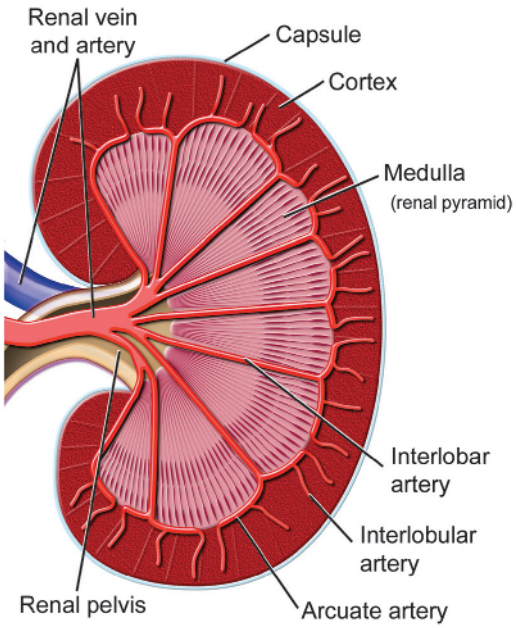

FIGURE 1.1 Sagittal section of the canine kidney. Note vascular architecture from renal artery, to interlobar, arcuate, and interlobular arteries. The afferent arteriole then extends to the glomerular vessels (not shown). Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

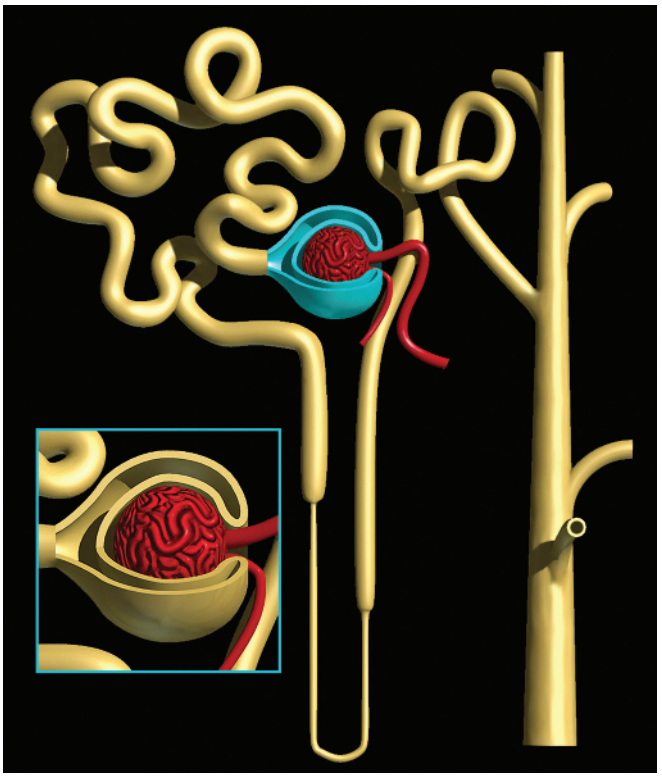

FIGURE 1.2 The renal corpuscle consists of the glomerulus and its covering with visceral and parietal epithelium shown in blue. Bowman's space is the area in between those two layers of epithelium and is where plasma is filtered across the glomerulus to form tubular fluid as it enters the proximal tubule. The insert shows an enlarged view of the renal corpuscle. Note that the afferent arteriole is shown to be larger than the efferent arteriole, since about 30% of the blood volume is filtered across the glomerulus to form the initial tubular fluid. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

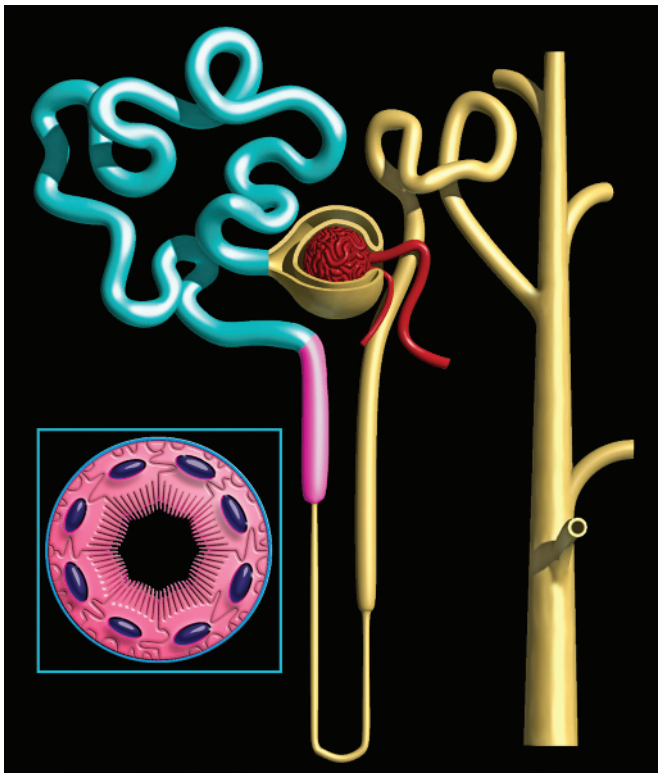

FIGURE 1.3 The proximal tubule (PT) includes the proximal convoluted tubule (blue) and the straight portion distally (pink) (also known as S3 or pars recta). The PT starts at the junction with Bowman's space and ends at the junction with Henle's loop. S3 extends into the outer medulla when parent nephrons are juxtaglomerular. The insert shows the histology of the PT featuring its brush border. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

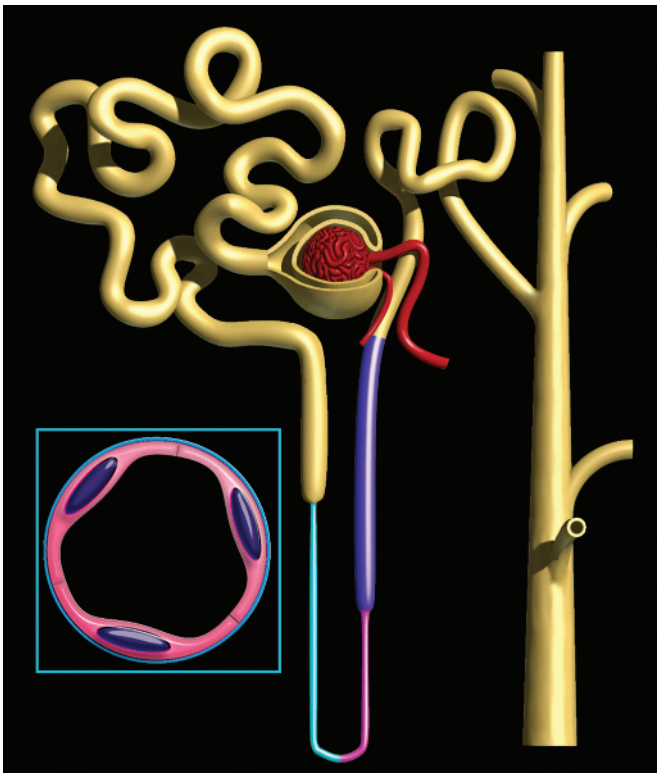

FIGURE 1.4 The loop of Henle has three anatomically and functionally different parts. The loop starts from the end of the proximal tubule (S3) and ends as it joins the distal tubule. The thin descending limb (shown in blue) ends at the hair pin turn and the thin ascending limb (shown in pink) starts at the hair pin turn of the loop. The medullary thick ascending limb (mTAL) (purple) contains considerable metabolic apparatus for energy consuming activities that occur in this region. The insert shows the histology of the thin limbs. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

loop. The descending limb and beginning portion of the ascending limb are very thin, and as the ascending limb passes through the medulla and back into the cortex, the walls become much thicker (medullary thick ascending limb [mTAL]) (Figure 1.4). At the end of the ascending limb, there is a short segment of the initial distal tubule known as the macula densa, containing specialized epithelial cells (Figures 1.5 and 1.6). There is close apposition of the specialized macula densa cells of the distal tubule with the juxtaglomerular cells of the afferent arteriole. Cells of the macula densa are important in sensing solute flowing by this region and then sending signals to the afferent arteriole that control vascular tone of the afferent arteriole, which changes glomerular filtration rate (GFR). Past the macula densa, fluid flows progressively into the later distal tubule (Figure 1.7), connecting tubule, cortical collecting tubule (Figure 1.8), and cortical collecting duct. Collecting ducts merge to form the medullary collecting duct; these collecting ducts merge and empty into the renal pelvis.

Nephron structure and function differs depending on the location within the renal cortex. Nephrons that have glomeruli located in the outer portion of the cortex are called cortical nephrons, and these nephrons only penetrate a short distance into the medulla (Figure 1.9). Some nephrons have short loops of Henle [8, 13, 14]. Glomeruli that are located deeper in the cortex are termed juxtamedullary nephrons. Tubules from these nephrons extend much deeper into the renal medulla from some combination of having longer loops of Henle and closer proximity of parent glomeruli to the medulla. One source notes that all nephrons in both the dog and cat have long loops of Henle and vary by how much of the loop extends into the medulla [2]. The blood supply to the juxtamedullary nephrons is slightly different in that the efferent arterioles are much longer and extend into the outer medulla where they divide into specialized peritubular capillaries (vasa recta). The vasa recta extend into the medulla parallel with the loop of Henle and then return to the cortex to empty into cortical veins. The vasa recta are important in the concentration of urine (described in detail below).

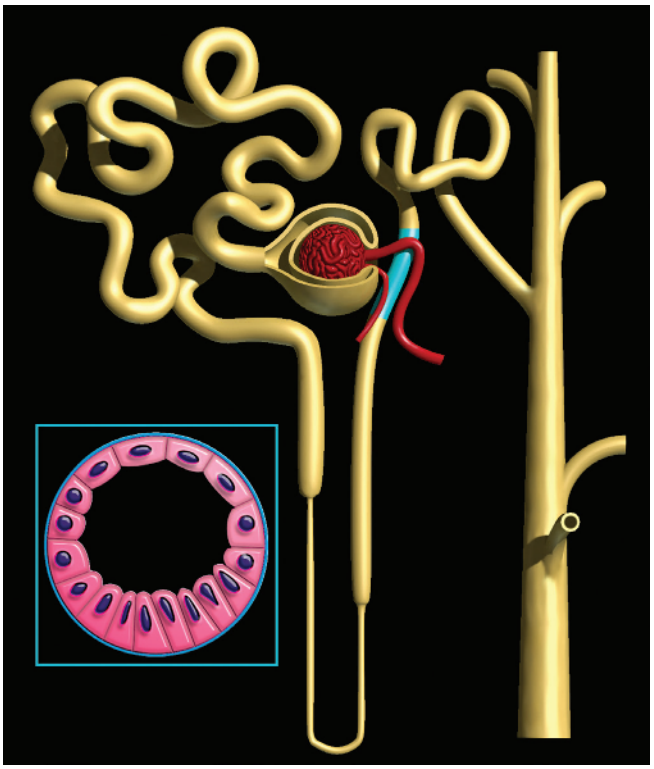

FIGURE 1.5 The first part of the distal tubule is shown in blue to be in close anatomical proximity to the afferent arteriole. This region of the distal tubule contains specialized cells of the distal tubule called the macula densa (shown as the thin long cells in the cut out). Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

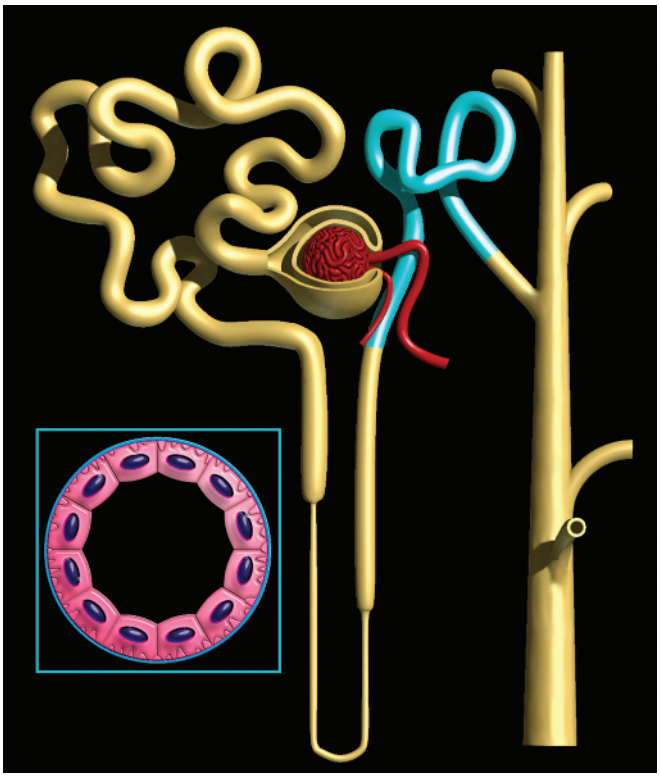

FIGURE 1.7 The distal tubule (DT shown in blue) consists of early (proximal) and late (more distal) portions that have different functions. Some of the DT is convoluted. The DT starts at the end of the mTAL and ends at the junction with the connecting tubule. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

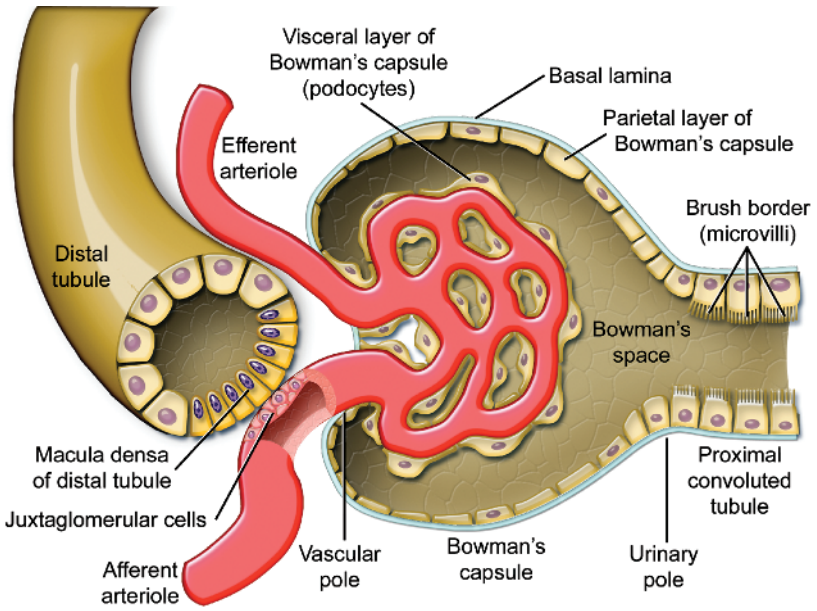

FIGURE 1.6 Juxtaglomerular apparatus. Notice the close apposition of the specialized macula densa cells of the distal tubule with the juxtaglomerular cells of the afferent arteriole. Cells of the macula densa are important in sensing solute flowing by this region and then sending signals to the afferent arteriole that control vascular tone and GFR. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

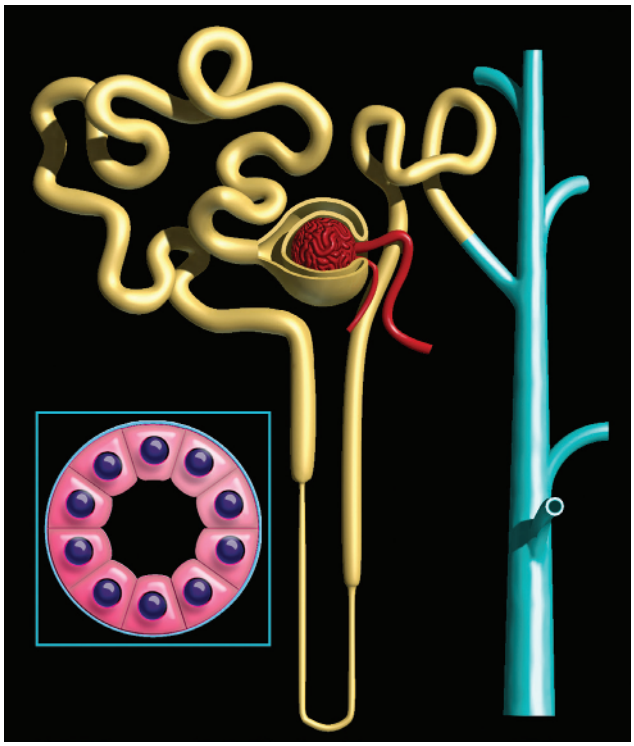

FIGURE 1.8 Collecting tubule (CT). The CT has very different functions depending on whether it is the cortical CT, outer medullary CT, or inner medullary CT. The CT is where most of the final adjustments are made as to how concentrated the urine will be based on water reabsorption under the influence of ADH. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

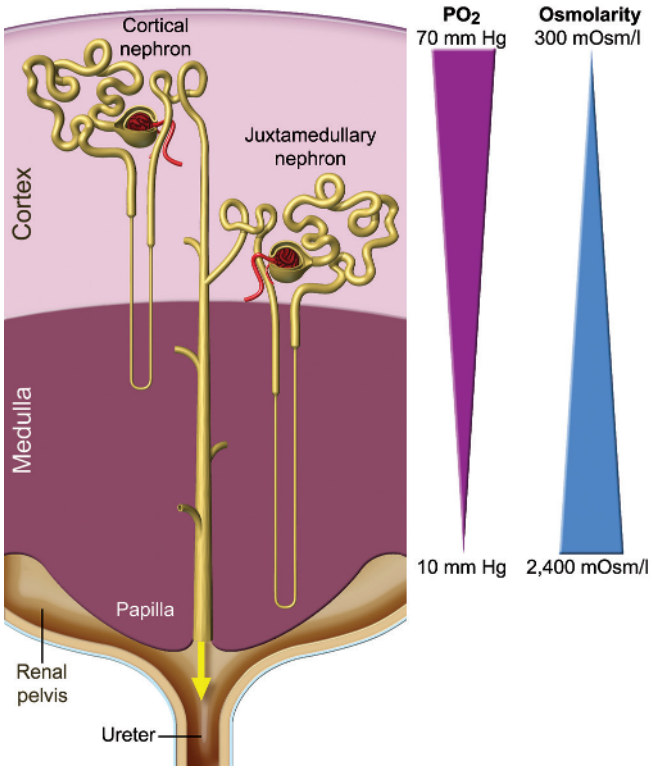

FIGURE 1.9 Cortical and juxtamedullary nephrons. Not all nephrons are identical anatomically. Juxtamedullary nephrons are those that have long loops of Henle that are vital in the generation of medullary hyperosmolality and the ability to maximally concentrate and dilute urine. There are many more cortical nephrons, which explains why renal blood flow is much greater in the cortex than in the medulla. Note that there is a progressive decrease in oxygen saturation from the outer cortex to the inner medulla. This oxygen gradient helps explain the development of certain types of acute kidney injury that happen in regions of relatively low oxygen delivery and high metabolic demand (such as occurs in S3 and the mTAL in juxtamedullary nephrons). Note also the gradient from low to high osmolality in the renal interstitium from outer cortex to inner medulla. Initial interstitial osmolality in the cortex parallels that of plasma (300 mOsm/L) that becomes many times increased over that of plasma in the medulla due to the process of countercurrent multiplication. The maximal osmolality achieved at the turn of Henle's loop parallels the maximal urine concentration that can be elaborated. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

Extensive details describing renal vascular tubular organization and relationships in dogs have been reported. It appears that some postglomerular efferent arterioles provide blood supply to tubules from different nephrons than from the original parent nephron [13–15]. This heterogeneity of vascular tubular relationship is not usually mentioned in traditional renal physiology courses, but it could be important in understanding patchy tubular necrosis that develops in some cases with ischemic acute kidney injury (AKI).

URINE

URINE FORMATION

Ultrafiltration of plasma across the glomerulus into Bowman's space is the first step in the formation of urine. Fluid that enters Bowman's space is quite similar to that of plasma with the exception of protein content. Only small-molecular-weight substances are easily filtered across the glomerulus. Large-molecular-weight proteins in plasma, such as albumin, are excluded from this ultrafiltrate; thus, this fluid is nearly devoid of plasma proteins in healthy individuals (Figure 1.10). The glomerular and tubular handling of filtered proteins is discussed in greater detail in Chapter 7. Tubular fluid is extensively modified in volume and quantity of solute and water as it traverses the tubular lumens along the PT, the loop of Henle, distal tubule, connecting tubule, and the collecting tubules. Isosmotic reabsorption of tubular fluid occurs until the end of the PT [2, 7]. The majority of filtered solutes and water are reabsorbed in the PT. The PT is inherently permeable to water due to the presence of constitutively expressed aquaporins (AQPs) [16] that facilitate transcellular movement of water, as well as some paracellular movement

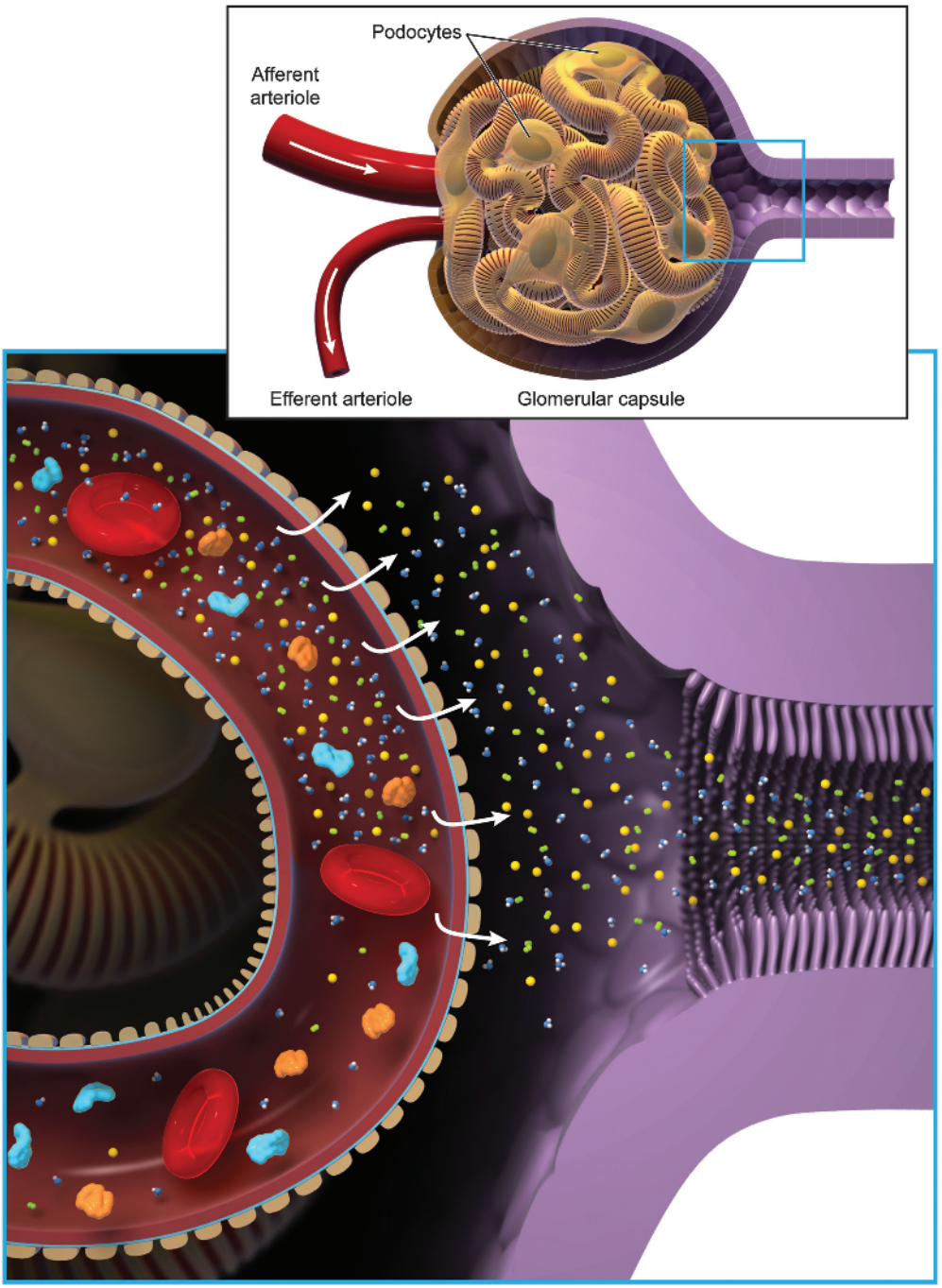

FIGURE 1.10 Glomerular tuft (insert) and view of glomerular filtration process are illustrated. The afferent arteriole, glomerular capillary, efferent arteriole, Bowman's Space and junction with proximal tubule are shown in the insert; podocytes (visceral epithelium) are shown as an outer cover of the glomerular vessel. About 30% of the plasma volume coming in from the afferent delivery is filtered across the glomerulus into Bowman's space. Approximately 2–4 mL/min/kg of fluid traverses the glomerulus into Bowman's space in normal dogs and cats, and this volume is referred to as the glomerular filtration rate (GFR). GFR is largely a function of the algebraic sum of Starling's forces that include glomerular capillary pressure, oncotic pressure within the glomerulus, and tubular pressure in Bowman's space. Glomerular capillary pressure develops as a function of plasma flow into the glomerulus and the resistance to flow of fluid out of the glomerulus. In addition to Starling forces, GFR also depends on the surface area of the glomerular capillaries and their inherent permeability characteristics (Kf). The larger image below shows that only the smaller molecular weight and size molecules are filtered into Bowman's space. Proteins with higher molecular weight and size (shown as larger blue and orange elements within the glomerular capillary) do not cross the glomerulus. This figure also shows that cells (RBC shown) stay within the glomerular capillary and do not cross the glomerulus. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

due to the absence of tight junctions in this region [17]. From 60% to 80% of the glomerular ultrafiltrate undergoes isosmotic reabsorption by the end of the PT, which is important in reduction of fluid volume delivered to more distal nephron segments for further processing. The thin descending limb of Henle's loop is permeable to water due to the presence of AQPs [17], and fluid undergoes reabsorption due to the hyperosmolality of the interstitium that surrounds them. Tubular fluid becomes progressively more concentrated as it travels along the descending loop of Henle (water and solute permeable) and then becomes progressively less concentrated as it travels along the ascending loop of Henle (water impermeable due to the absence of AQPs and presence of extensive tight junctions) [17]. The thin and thick segments of the ascending limb of Henle's loop are often referred to as the "diluting segments" as solute is reabsorbed but water is not, which reduces tubular fluid osmolality. The thick ascending limb is responsible for reabsorption of 20–25% of the filtered load of sodium and chloride, a process that is facilitated by the Na-K-2Cl cotransporter (NKCC2) [18]. Progressive tubular fluid hypotonicity develops in this region, while the degree of interstitial hypertonicity increases at the same time, a development that is important to allow elaboration of urine with maximal concentration. NKCC2 activity in the cells of the macula densa allows a signaling process that controls preglomerular afferent arteriolar tone through tubuloglomerular feedback [18, 19]. Hypotonic fluid (100 mOsm/kg) is presented from Henle's loop to the distal convoluted tubule. The osmolality of tubular fluid within the medullary and cortical thick ascending limb of Henle's loop is low as it joins the distal tubule, regardless of high or low circulating antidiuretic hormone (ADH) status.

Tubular fluid that is delivered from the collecting tubules to the renal pelvis can be either more dilute or more concentrated than the initial (approximately 300 mOsm/kg) glomerular ultrafiltrate, depending on the needs of the animal. The degree of urine concentration is largely determined by the response to ADH, which is discussed in more detail below. Normal urine is markedly different from plasma that was initially filtered across the glomerulus into Bowman's space (waste products, pH, urine concentration). No further modification of fluid occurs after it enters the renal pelvis on its way to the urinary bladder for storage. The interested reader is referred to an excellent veterinary review of basic applied renal physiology and disorders of sodium metabolism for more detail [2, 20, 21]. Failure to conserve water, potassium, glucose, and plasma proteins are processes that can be detected in urine and will be discussed in specific sections of this book that follow. An overview of the functions and physiological processes that occur in each nephron segment is presented in Figure 1.11.

URINARY CONCENTRATION AND DILUTION

See Chapter 4 for clinical details about urine concentration based on urinary specific gravity (USG) and urine osmolality. The ability to concentrate or dilute urine is critical to maintain a healthy plasma osmolality (285–310 mOsm/L population-based reference range for dogs and cats). Normal plasma osmolality is maintained within a very narrow range for an individual animal but can vary according to the volume of fluid intake and solute consumed in the diet, as well as by losses of fluid and electrolytes that alter plasma osmolality. Dehydration from loss of mostly water increases plasma osmolality, which stimulates the release of ADH and elaboration of concentrated urine. More rarely, a decrease in plasma osmolality happens when loss of fluid is replaced by mostly water, and the ability to excrete dilute urine is essential to restore normal plasma osmolality when the serum osmolality was reduced. The kidneys can excrete dilute urine with an osmolality of about one-sixth the osmolality (50 mOsm/L) of normal. The kidneys are also able to regulate solute excretion independently of water excretion, which is important when there is limited fluid intake [22].

Glomerular ultrafiltrate is modified in order to conserve needed solute and water or to excrete excess solutes and wastes and water when needed to maintain the constancy of the internal milieu. Urine volume, in general, is related to the degree of urine concentration in health. A larger urine volume is associated with a lower urine concentration, and a smaller urine volume is associated with a higher urine concentration. Small volumes of highly concentrated urine allow conservation of body water when needed, whereas high volumes of minimally concentrated urine allow excretion of excess water in normal individuals. There is a divergence in this general pattern in patients with severe oligo-anuric AKI, as a small urine volume is associated with minimally concentrated urine.

Urine Concentration

The degree of urine concentration in health is largely determined by plasma osmolality and its association with ADH release. Juxtamedullary nephrons contribute more to urinary concentration than cortical nephrons. There are three main mechanisms within the kidney that control urine concentration. First, the transport of sodium chloride without water from the ascending limb of the loop of Henle creates and maintains interstitial hypertonicity and tubular fluid hypotonicity in this region that is essential to allow the option for the elaboration of either concentrated or diluted urine. Second, the secretion and action of ADH (vasopressin) increases water permeability of the collecting duct to allow concentrated urine to form. Third, urea reabsorption from the medullary collecting tubule under the influence of ADH and urea entry into the descending limb of Henle's loop importantly contribute to interstitial hypertonicity to allow urine that is maximally concentrated to be formed.

The fluid that is filtered by the glomerulus has approximately the same osmolality of plasma (about 300 mOsm/kg). Most of the water and solute that has been filtered by the glomerulus is reabsorbed in the proximal convoluted tubule; yet, the osmolality remains unchanged due to isosmotic reabsorption of

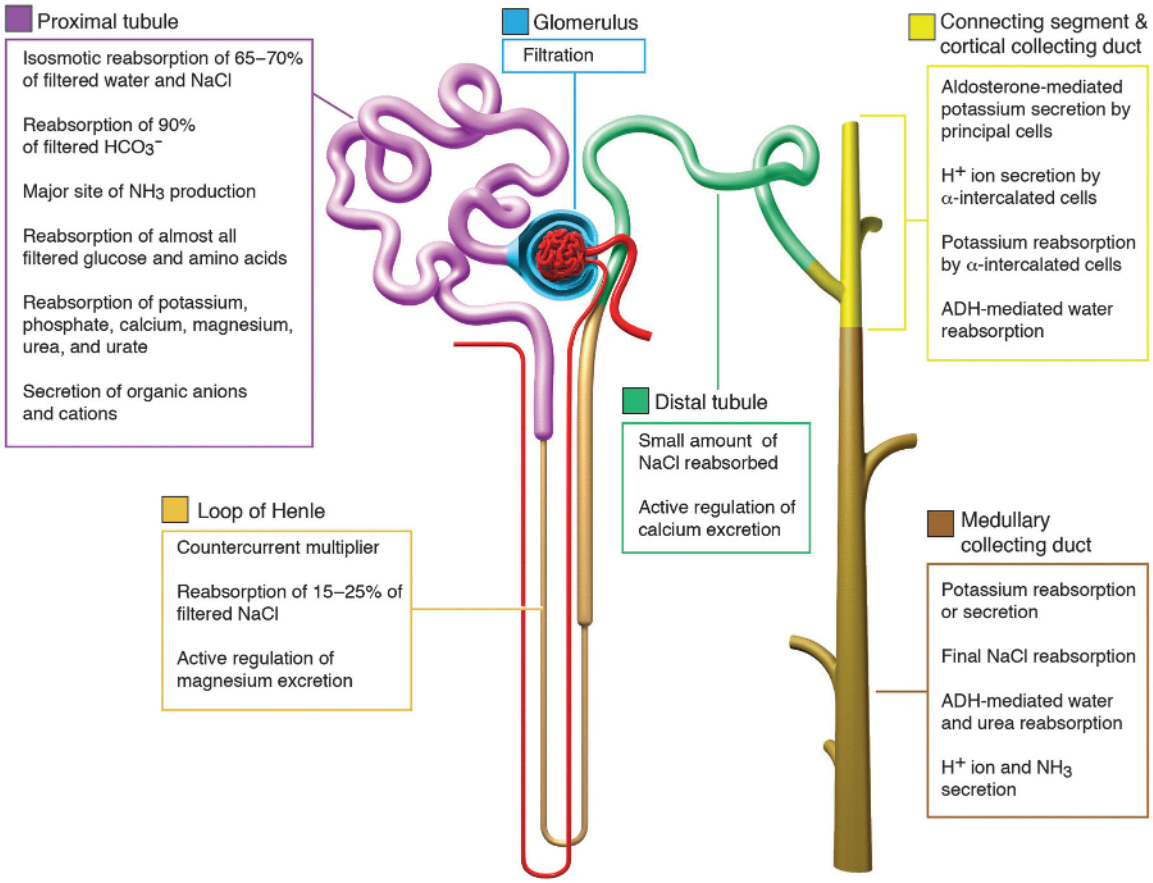

FIGURE 1.11 General overview of functions and physiological processes that occur by nephron segment. See text for more details about GFR, tubular secretion, tubular reabsorption, and urine concentration or dilution. Source: Illustrated by Tim Vojt. Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

water and solute. Approximately 60–80% of filtered fluid is reabsorbed in the proximal convoluted tubule, along with sodium, amino acids, glucose, phosphate, chloride, potassium, and bicarbonate.

Fluid with an osmolality of about 300 mOsm/kg continually enters the descending limb of the loop of Henle from the PT. The descending and ascending loops of Henle have a hairpin turn configuration, which is the basis for countercurrent multiplication, which allows for the multiplication of a single osmotic effect (Figure 1.12). The descending limb of the loop of Henle is permeable to water, but relatively impermeable to electrolytes. The interstitium of the renal medulla, which surrounds the loop of Henle, is maintained at a higher osmolality due to high concentrations of sodium and urea. As fluid travels down the descending limb of the loop of Henle, water exits the descending limb in an effort to equilibrate with the surrounding interstitium. By the time the fluid within the loop reaches the bottom of the descending limb, the osmolality is greatly increased as compared to the original filtrate.

From the descending limb, fluid passes into the ascending limb of the loop of Henle. The ascending limb is impermeable to water, but is permeable to electrolytes, which is opposite to the descending limb. There is active transport of sodium in the thick portion of the ascending limb, which generates an osmotic gradient of approximately 200 mOsm/kg. The activity of the basal membrane Na^+-K^+-ATPase pump of these cells maintains an electrochemical gradient that drives entry of sodium from the tubular fluid into the cell by facilitated diffusion (Figure 1.13). The carrier Na^+-K^+-$2Cl^-$ (NKCC2) binds one sodium ion, one potassium ion, and two chloride ions in the lumen, which then transports these electrolytes into the cell and then into the interstitium [18]. Adequate chloride binding to the carrier is the rate-limiting step in transport. Loop diuretics, such as furosemide, bind to the chloride-binding site within the pocket of the NKCC2 carrier molecule of the mTAL, thereby impairing its function resulting in increased excretion of sodium, chloride, and potassium [18, 24].

The osmotic gradient generated between the tubular fluid and the interstitium is multiplied over the length of the loop of Henle. The magnitude of the gradient from the beginning of the descending limb to the bottommost part where the ascending limb begins is a function of the length of the loop, which is an important component of the countercurrent multiplier concept. The vasa recta are also an important part of the countercurrent system through what is called countercurrent exchange (Figure 1.14). Vasa recta are parallel vessels of the renal medulla that provide much lower blood flow than that in the cortex [17]. The descending vasa recta

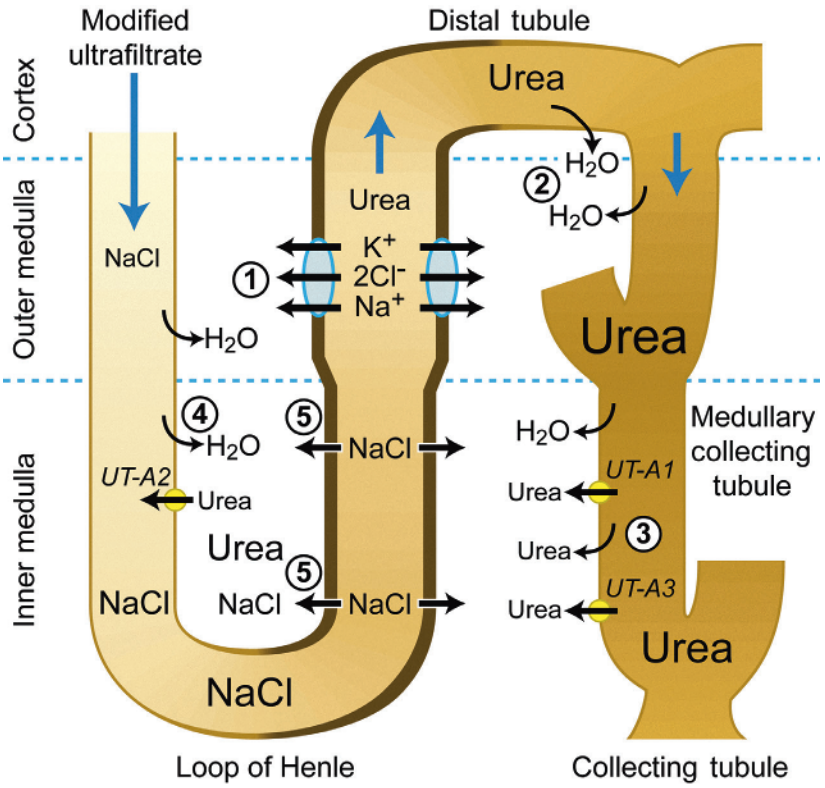

FIGURE 1.12 Factors involved in the elaboration of concentrated or dilute urine. The major factors needed to elaborate maximally concentrated urine are shown in this illustration (excluding the isosmotic reabsorption of solute and water that transpired along the proximal tubule). A critical minimal number of healthy nephrons are needed to make concentrated urine, and the body must be capable of synthesizing and releasing ADH in adequate quantity. The integrity of the collecting tubule V-2 receptor to bind to ADH and initiate intracellular signals for the phosphorylation of aquaporin-2 is essential. The major stimulus for the release of ADH is an increase in plasma osmolality perfusing the hypothalamus; most domestic animals have evolved to produce concentrated urine for much of the day. The ability to concentrate or dilute the urine centers on the ability of the medullary thick ascending limb of the loop of Henle's ability to actively reabsorb sodium and chloride without water. The ascending limb of the loop of Henle is impermeable to water so that the osmolality of tubular fluid progressively decreases during ascent of the loop. At the same time, the osmolality of the interstitium increases as solute is added to this region. ADH increases the permeability of the cortical collecting duct to water but not urea, so the concentration of urea progressively increases as it descends the cortical collecting duct. The increased concentration of urea is represented by the size of the font. The medullary collecting duct is inherently permeable to urea, contributing importantly to the hyperosmolality of this region as urea diffuses into the interstitium. The permeability of the inner medullary collecting duct to urea is enhanced by ADH interaction with specific urea transporters (UT A-1 and UT A-3), which are variably located along the apex, within the tubule cell, and along the basolateral membranes [23] that further movement of urea into the interstitium. The thin descending limb of Henle's loop has a specific urea transporter (UT A-2) that facilitates recycling of urea from the interstitium into the tubular lumen. Step 1: the Na-K-2Cl cotransporter provides the energy that initially generates interstitial hyperosmolality. This process also generates tubular fluid hypoosmolality since this segment of the nephron is not permeable to water. Step 2: in the late distal tubule and cortical collecting tubule, ADH increases water but not urea permeability. Water leaves the tubules from these segments due to the hyperosmolality of the interstitium generated by step 1. Since water but not urea leaves these segments, note that the tubular fluid concentration of urea increases. Step 3: as tubular fluid reaches the medullary collecting tubule, the concentration of urea is quite high. The high concentrations of urea at this location favor diffusion in this region that is permeable to urea. Urea transporters here favor diffusion from tubular fluid into the interstitium, increasing the osmolality of this region. Urea contributes substantially to the solute concentration of this region. Urea transporters also favor the uptake of urea from the interstitium into the thin descending limb of Henle's loop. Step 4: the high concentration of urea and NaCl in the interstitium favors the movement of water out of the descending thin limb of Henle's loop. The thin descending limb is highly permeable to water and not so soluble for sodium and chloride. As a consequence, the concentration of sodium and chloride progressively increases until the point of the hair pin turn of Henle's loop is reached. Step 5: sodium and chloride are shown leaving the thin ascending limb of Henle's loop down their concentration gradient. This occurs because sodium and chloride are relatively permeable in this region and have achieved a high concentration from processes that happened previously in the descending loop (Step 4). The thin ascending limb is impermeant to water, which contributes to the progressive development of hypoosmolality as solute, but not water, is removed from the ascending limb. The sodium and chloride that leave the ascending thin limb contribute to the interstitial hyperosmolality of that region, which further facilitates water movement from the collecting tubule in the presence of ADH activity. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

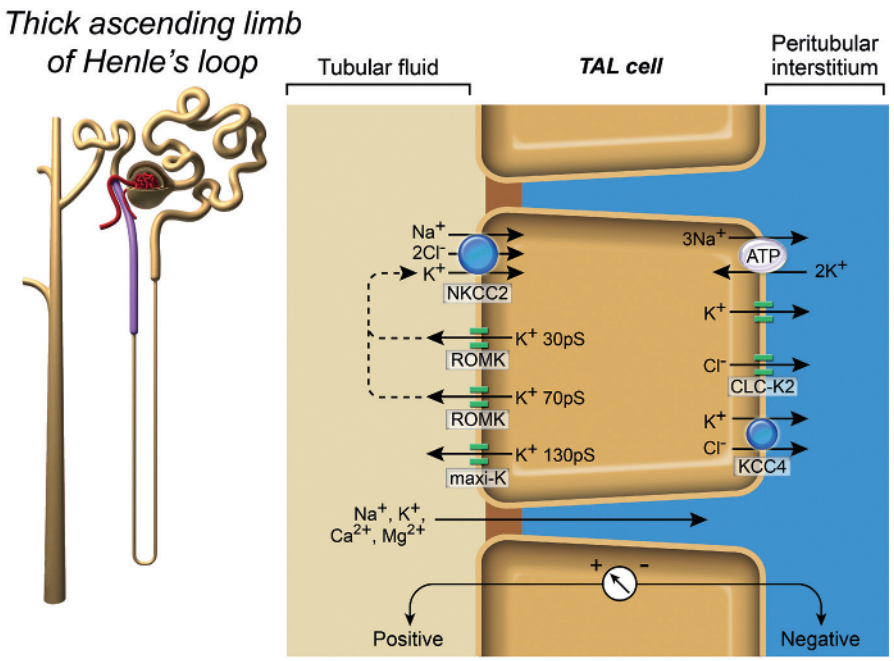

FIGURE 1.13 Medullary thick ascending limb (mTAL) reabsorption of sodium, potassium, and chloride. The apical surface of the mTAL epithelium contains the Na-K-2Cl cotransporter (NKCC2) that is important in the reclamation of 20–25% of the filtered load of sodium and chloride. All four molecules must occupy this cotransporter at the same time in order to allow facilitated uptake of sodium, chloride, and potassium inside the cell. The active sodium–potassium ATPase pump on the basolateral membrane maintains low intracellular sodium concentration, which favors sodium entry into the cell from the apical side. The diuretic furosemide is secreted into tubular fluid and then binds to this transporter in place of chloride, which renders the transporter inoperative. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

originate from the efferent arterioles of juxtamedullary nephrons, supplying blood to capillary plexuses at multiple levels within the medulla. Ascending vasa recta arise from the capillary plexuses and travel parallel to the descending vessels. This unique anatomical arrangement allows countercurrent flow of water and solutes to occur at local levels without dissipating the hypertonicity of the medulla. Sodium, chloride, and urea enter while water leaves descending vessels; the process is reversed in ascending vessels in that sodium, chloride, and urea leave and water enters. This process of countercurrent exchange is facilitated by the presence of AQP-1 water channels and by urea transporter-B urea transporters in the endothelial cells of the descending vasa recta. Without the vasa recta, the hyperosmotic gradient of the interstitium would quickly dissipate. Hypertonicity of the medulla can be disrupted by disorders or conditions that increase blood flow in the vasa recta.

By the time fluid enters the distal convoluted tubule, it is hypoosmotic to plasma (approximately 100 mOsm/kg). The distal convoluted tubule is minimally permeable to water, but sodium and chloride can be reabsorbed from the tubular fluid into the interstitium further lowering tubular fluid osmolality. From the distal tubule, hypoosmotic tubular fluid enters the collecting duct. Even though most water and solute reabsorption occurs in the proximal convoluted tubule and the loop of Henle, the final urine volume is ultimately determined by the collecting ducts. The collecting duct is divided into three segments: the cortical collecting duct, outer medullary collecting duct, and inner medullary collecting duct. In the presence of ADH, the hypoosmotic tubular fluid equilibrates osmotically with the cortical interstitium, and about two-third of the tubular water is removed before delivery to the outer medullary collecting duct. The cortical collecting duct is also permeable to sodium and chloride, and more water can be reabsorbed depending on how much sodium reabsorption occurs in response to aldosterone. The cortical collecting duct is not permeable to urea; thus, fluid with a very high urea concentration is delivered to the medullary collecting duct as water but not urea is reabsorbed from the tubular lumen. The fluid that is delivered to the medullary collecting duct is isosmotic with plasma and greatly reduced in volume.

The action of ADH controls the water permeability of the collecting duct and, therefore, the final concentration of urine. An adequate number of functioning nephrons are necessary for this system to conserve or excrete water. ADH is synthesized by the hypothalamus and secreted by the posterior pituitary [25, 26]. Humans and most mammals secrete arginine-ADH as the predominant form, but pigs and marsupials secrete lysine-ADH as the predominant form [27–29]. The antidiuretic activity of arginine-ADH was greater and lasted longer than that from lysine-ADH in dogs [30]. Increasing plasma osmolality is sensed by osmoreceptors in the hypothalamus that trigger the release of ADH, resulting in water conservation by the kidney. Decreases in plasma osmolality inhibit the release of ADH, which facilitates

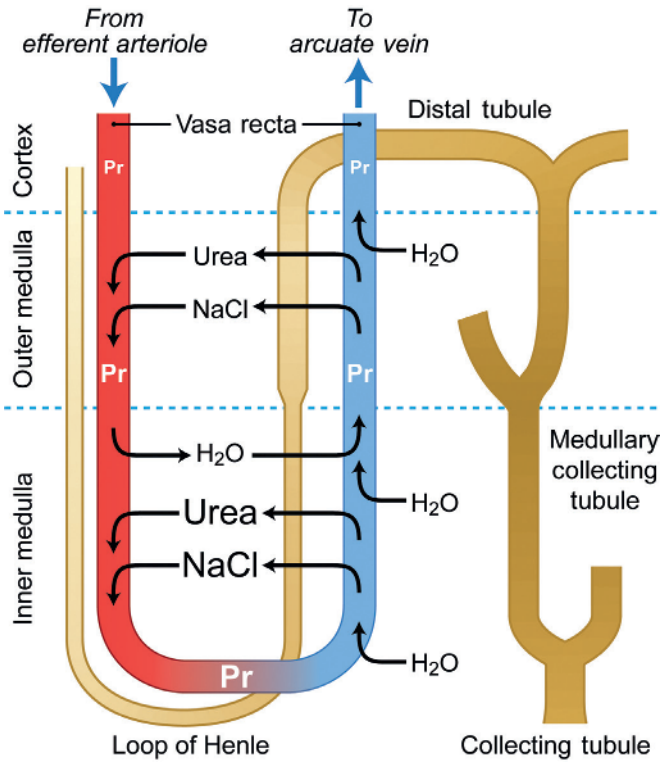

FIGURE 1.14 Vasa recta countercurrent exchanger system. The vasa recta consist of postglomerular efferent arterioles from juxtaglomerular nephrons. This system of vessels (interconnections not shown) runs parallel to the loops of Henle. Solute (NaCl, urea) enters the descending limb and leaves the ascending limb of the vasa recta. Water leaves the descending limb and enters the ascending limb of the vasa recta. The function of this vascular array allows preservation of medullary solute at local levels within the medulla, while still returning extra water to the systemic circulation as needed. Note that the concentration of protein increases within the descending vessel as water leaves and then decreases during ascent as water enters. The size of the font is proportional to the quantity of the compound. This process is sometimes called the Countercurrent Exchanger, and it works in concert with countercurrent multiplication as generated by the loop of Henle. The rate of blood flow through the vasa recta is normally very slow to allow this system to work properly. It is possible for some diseases to increase blood flow through the vasa recta, which dissipates interstitial hyperosmolality, thus reducing the ability to elaborate highly concentrated urine. This process creates one form of medullary washout. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

excretion of excess water by the kidney [17, 31]. An increased concentration of circulating ADH increases the water permeability of principal cells of the late distal tubules, connecting tubules, and collecting ducts via AQPs, which allows water reabsorption and excretion of a more concentrated urine. Increased ADH activity also increases the activity of the Na-K-2Cl pump in the mTAL. This increases sodium, chloride, and potassium absorption across the medullary thick ascending loop of Henle that further increases interstitial solute concentration (osmolality) while lowering tubular fluid osmolality [16].

In the presence of ADH, water is removed from the collecting duct, and the tubular fluid equilibrates with the hyperosmotic medullary interstitium. The maximal urine osmolality can approach 2800 mOsm/kg in dogs and 3000 mOsm/kg in cats. Less than 1% of the fluid filtered across the glomerulus is excreted as the final urine volume under these circumstances.

The role of prostaglandin E2 (PGE2) in urinary concentration is not fully understood and most likely depends on which receptor is activated under varying physiologic conditions. PGE2 has been thought to have negative effects on urine concentration until recently. Water reabsorption and water excretion by the kidney can be facilitated by PGE2, and increased prostaglandin effects have been implicated in some polyuric conditions, especially in hypercalcemia. In some studies, polyuria was decreased by administering anti-prostaglandin medications [32].

Urine Dilution

When there is excess water in the extracellular fluid (ECF), the plasma osmolality decreases. To remove excess water from the body, the glomerular filtrate needs to be diluted as it passes through the nephron. This is accomplished by the reabsorption of solutes to a greater extent than water. The tubular fluid remains isosmotic to plasma as it passes through the proximal convoluted tubule, and fluid is reabsorbed as it passes through the descending limb of the loop of Henle, becoming more concentrated. In the ascending limb of the loop of Henle, sodium, potassium, and chloride are reabsorbed, and the tubular fluid becomes more dilute as it flows to the distal convoluted tubule.

As a result of the decreased plasma osmolality, there is a decrease in the secretion of ADH by the posterior pituitary. In the absence of ADH effect, the collecting duct remains impermeable to water. As the tubular fluid enters the collecting duct, it has an osmolality of approximately 100 mOsm/kg. Sodium chloride continues to be reabsorbed in the cortical and inner medullary collecting duct, without the reabsorption of water. The hypoosmolar fluid becomes even more dilute and can be as low as 50 mOsm/kg (maximally dilute urine) in the absence of ADH.

The Role of Urea

Along with the active transport of sodium and chloride from the thick ascending limb of the loop of Henle and passive transport in the thin ascending limb, urea is also important in establishing and maintaining high interstitial osmolality that is important in the function of the urinary concentrating mechanism [2]. There is a low permeability to urea in most segments of the thin descending limb of the loop of Henle, but there is high permeability to water in this segment. The high concentration of urea in the medullary interstitium causes water to be removed osmotically from the thin descending limb of the loop of Henle, as well as from the medullary collecting tubule in the presence of ADH. Urea increases medullary interstitial osmolality without changing the sodium concentration in this region. In the descending limb, the concentration of sodium of the tubular fluid eventually exceeds medullary inter-

stitial sodium concentration due to the low permeability for sodium. There are select segments of the descending limb that are permeable to urea; these are segments that express the urea transporter UT-A2. This urea transporter is likely important to reclaim urea that entered the medullary interstitium from the medullary collecting duct [16]. The excretion or reabsorption of urea along the tubules is in part dependent on the rate of urine flow. Faster tubular flow rates result in less time for reabsorption of urea along the tubules resulting in more urea excretion. The opposite happens when tubular flow rates are slow (as in dehydration) and there is increased reabsorption of urea.

As the tubular fluid enters the thin ascending limb of the loop of Henle, the sodium in the tubular fluid is reabsorbed passively into the medullary interstitium down a concentration gradient. The entire ascending limb of Henle is impermeable to water, which promotes dilution of tubular fluid as solute is removed but water is not. In the inner medullary collecting duct, the permeability to urea is increased by ADH via urea transporters UT-A1, UT-A3, and possibly UT-A4, which are variably located along the apex, within the tubule cell, and along the basolateral membranes [16, 23]. ADH additionally upregulates the number of urea channels [16]. There is limited permeability to urea in the distal convoluted tubule, cortical collecting duct, and outer medullary collecting duct; thus, the urea concentration of tubular fluid markedly increases in these portions of the nephron.

When the concentration of plasma ADH increases (in times of water conservation), water permeability of the cortical collecting duct increases without an increase in urea permeability, so the concentration of urea progressively increases as it descends the cortical collecting duct. With increased ADH, urea permeability of the inner medullary collecting duct increases via urea transporters, so more urea diffuses into the medullary interstitium. With the entry of urea into the interstitium, maximally concentrated urine can be produced by osmotic equilibration of tubular fluid with the hyperosmotic interstitium in the presence of ADH. In times of water conservation (antidiuresis), urea constitutes more than 40% of the total medullary solute in dogs, but only accounts for about 10% during water diuresis [33].

The Role of Aquaporins

Aquaporins (AQPs) are small membrane proteins that act as semipermeable channels that facilitate water transport. AQPs consist of six alpha helices that span the membrane, containing a central water-transporting pore. Four AQP monomers combine to form a tetramer, which is the functional unit. Water can cross a lipid membrane by diffusion, but the presence of AQP channels greatly increases the water permeability of the membrane [34].

Thirteen AQPs have been identified in mammals (AQP0-12), which are expressed in various tissues. AQPs are highly expressed in renal epithelial cells where they are important in water movement [35]. Eight different AQPs are expressed in the kidney, and five are important in water homeostasis (AQP-1, AQP-2, AQP-3, AQP-4, and AQP-7). AQP-2 is most important, as it is regulated by ADH [34, 36, 37]. AQP-1 is located in the membranes of proximal convoluted tubules, descending limb of the loop of Henle (of long-looped nephrons), and descending vasa recta. AQP-2, AQP-3, and AQP-4 are present in the connecting tubule and collecting duct. AQP-7 is present in PTs. Defects in the expression of any of the AQPs can result in significant impairment of urine concentration.

AQP-2 plays the biggest role in urinary concentration. It is expressed in principal cells of the connecting tubule and collecting ducts and is modulated by the presence of ADH (Figure 1.15) ADH upregulates the expression and half-life of AQP-2 and also promotes phosphorylation of AQP-2, facilitating its migration and insertion into the luminal membrane via exocytosis and inhibition of endocytosis. AQP-2 is endocytosed and degraded in the absence of ADH [16, 17, 40, 41].

ADH binds to the vasopressin type-2 receptor inducing a cascade of events, including G-protein-mediated activation of adenylate cyclase, an increase in intracellular cAMP, activation of protein kinase, and a redistribution of AQP-2 to the apical membranes. Thus, luminal water can enter the cells via AQP-2 and leaves the cells via AQP-3 and AQP-4 in the basolateral membrane. This process results in a concentration of urine. Once ADH levels decrease, AQP-2 is internalized, and the water permeability of the apical membranes return to basal levels. AQP-2 can either be degraded or stored for future use. Without functioning AQP-2, urinary concentrating ability is severely decreased.

A number of conditions characterized by a lack of urinary concentration have been shown to be related to altered function or expression of AQP-2 in humans. In humans, about 10% of congenital nephrogenic diabetes insipidus (NDI) cases are due to mutations in AQP-2 [35]. Acquired causes of NDI that are related in part to altered AQP-2 include hypokalemia, hypercalcemia, urinary tract obstruction, AKI, and lithium treatment [16, 36, 41].

RENAL FUNCTION ASSESSMENT

FRACTIONAL CLEARANCE OF ELECTROLYTES

Evaluation of the concentration of any urinary electrolyte has little meaning by itself. For example, a urinary sodium concentration of 20 or 200 mEq/L could be appropriate depending on the status of the extracellular fluid volume (ECFV), signals for sodium reabsorption, and how much water is excreted or reabsorbed in tubular fluid. A general rule that a urinary sodium of <15 mEq/L indicates prerenal azotemia as used in human medicine appears to be misleading in veterinary medicine.

The fractional clearance of electrolytes is more useful in the evaluation of renal function than the concentration of the electrolyte in urine by itself. The fractional clearance of electrolytes is defined as the ratio of the clearance of the electrolyte in question to that of creatinine [42, 43]. The fractional clearance is calculated by dividing the ratio of the urinary concentration of the electrolyte (U_x) to the plasma concentration of the electrolyte (P_x) by the ratio of urinary

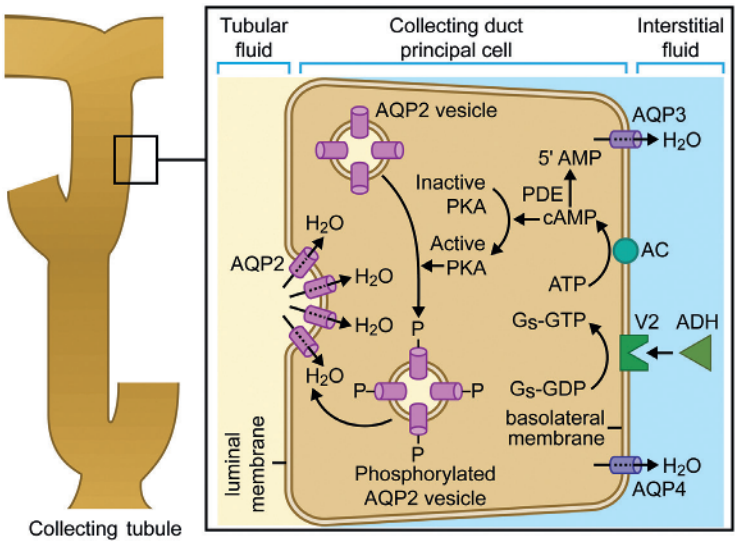

FIGURE 1.15 ADH actions in the principal cells of the collecting tubule. Callout to right is taken from the collecting tubule; the urine side of the collecting tubule (luminal) is on the left and the blood side (basolateral) on the right of this image. Aquaporin-2 (AQ-2) is phosphorylated under the influence of ADH, and then, this vesicle is inserted along the luminal membrane serving as a water channel that facilitates water crossing the collecting tubular lumen into the interstitium [38], [39]. The development and maintenance of a hypertonic interstitium is an overarching requirement to allow water to move out of the descending loop of Henle and out of the collecting duct under the influence of ADH. Circulating ADH binds to a specific receptor (V-2) on the basal side of the collecting duct, which then activates cyclic adenosine monophosphate (cAMP) and a series of intracellular events that lead to phosphorylation of AQ-2. AQ-2 then migrates to the luminal membrane of the collecting duct, where it is inserted allowing it to act as water channel that facilitates water transport across these cells. Under the influence of ADH, urine of low volume and high osmolality is excreted. AC, adenyl cyclase; AQP, aquaporin; Gs, stimulatory guanine nucleotide regulatory protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; PDE, phosphodiesterase; PKA, protein kinase A; V2, vasopressin receptor. Source: Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

concentration of creatinine (U_{Cr}) to the plasma concentration of creatinine (P_{Cr}) × 100. $FC_x = (U_x/P_x)/(U_{Cr}/P_{Cr}) \times 100$). The fractional clearance is expressed as a percentage. Normal values for urinary fractional clearance of electrolytes in dogs and cats are summarized in Table 1.1. However, normal values may be lower in greyhounds for potassium, chloride, calcium, and phosphorus [47]. The advantage of this measurement is that a timed urine collection is not necessary. In normal animals, the fractional clearances of all electrolytes are much less than 100% implying net conservation. Higher values for fractional excretion of potassium and phosphorus exist than for sodium and chloride and can exceed 100% for potassium during some forms of chronic kidney disease (CKD).

Table 1.1 General reference values for fractional electrolyte clearance (%). Values will vary by fasting, dietary intake, and particular study. Several studies provide further details [42–46].

Analyte	Dog	Cat
Sodium	<1	<1
Potassium	<20	<24
Chloride	<1	<1.3
Phosphorus	<39	<73
		<45
Calcium (total calcium)	<1	<1

Source: Adapted from Lefebvre et al. [42], DiBartola et al. [43], Parker et al. [46], Bennett et al. [47], Carr et al. [48].

The fractional clearance of sodium may be useful in the differentiation of prerenal and primary renal azotemia. In animals with prerenal azotemia and volume depletion, sodium conservation should be avid and the fractional clearance of sodium very low (<1%). Higher numbers suggest the presence of primary kidney disease in this setting. A significantly increased urinary fractional excretion of electrolytes (sodium, chloride, potassium, calcium, magnesium, and phosphorus) allowed early identification of dogs with intrinsic AKI compared to volume responsive AKI. Increased urinary fractional excretion of electrolytes was also associated with nonsurvival in this study [49].

The fractional clearance of potassium may be useful in the evaluation of hypokalemic patients to determine if the kidneys are contributing to the hypokalemia. The fractional excretion of potassium should be low during states of hypokalemia if the kidneys are functioning normally; a high value incriminates the kidneys as contributing to the hypokalemia. The fractional excretion of phosphorus is often high in CKD due to the effects of increased parathyroid hormone and fibroblast growth factor-23 that decrease proximal tubular reabsorption of phosphate. An increased fractional excretion of phosphorus may delay the onset of hyperphosphatemia in early CKD as a compensatory mechanism [44]. A decrease in the fractional excretion of phosphorus can sometimes be used

to document the effectiveness of dietary and intestinal phosphate binding treatment during CKD [45].

The fractional clearance of calcium based on serum total or ionized calcium and the use of urinary-calcium-to-urinary-creatine ratio may be useful for investigation of risk factors or diagnostic indices in dogs with calcium containing urinary stones [48, 50, 51], dogs with CKD [46], in cats with idiopathic hypercalcemia [52], and in obscure cases of hypercalcemia.

GLOMERULAR FILTRATION

Glomerular filtration is a passive process within the kidneys that largely depends on systemic blood pressure and blood volume that perfuse the kidneys. GFR represents the sum of all the single-nephron glomerular filtration rates (SNGFRs) of all nephrons in both kidneys. The SNGFR varies among nephron populations; thus, an average SNGFR value is typically evaluated. Glomerular capillaries are impermeable to large-molecular-weight plasma proteins and cells, so the normal glomerular filtrate is almost protein-free and devoid of cellular elements.

The kidneys receive about 25% of the cardiac output [3]. GFR is about 20–30% of the renal plasma flow (RPF) (GFR/RPF or filtration fraction) [2]. This high rate of glomerular filtration depends on a high blood flow to the kidney and on properties of the glomerular capillary membranes. Glomerular capillaries are thicker than most other capillaries, but are more porous to water and small molecules, enabling a high fluid filtration rate (mL/minute). GFR is determined by the hydrostatic and colloid osmotic forces across the glomerular membrane and the glomerular capillary filtration coefficient, which is the product of the permeability and filtering surface area of the capillaries. Forces favoring filtration include glomerular hydrostatic pressure and Bowman's capsule colloid osmotic pressure, and forces opposing filtration include Bowman's capsule hydrostatic pressure and glomerular capillary colloid osmotic pressure. Changes in these forces impact GFR [53].

Since the glomerulus does not normally filter protein, the colloid osmotic pressure of fluid within Bowman's capsule is essentially zero and does not contribute to GFR. Alterations in the glomerular capillary filtration coefficient can increase or decrease GFR, but this is not a primary mechanism for day-to-day regulation of GFR. Chronic hypertension, diabetes mellitus, and progressive CKD can increase the thickness of the glomerular capillary basement membrane, thereby decreasing the capillary filtration coefficient. Changes in the hydrostatic pressure of Bowman's capsule can increase or decrease GFR in disease, but this is also not a primary mechanism for day-to-day regulation. Increased hydrostatic pressure of Bowman's capsule occurs with urinary tract obstruction or with renal edema that develops during AKI, which decreases GFR.

Glomerular capillary colloid osmotic pressure importantly impacts GFR. As blood passes from the afferent arteriole to the efferent arteriole, the plasma protein concentration progressively increases since water is being filtered by the glomerulus, but protein is not. Thus, there is loss of fluid, increasing the concentration of protein as the plasma flows through the glomerulus. This increase in protein increases the capillary colloid osmotic pressure, progressively decreasing GFR from the highest near the afferent arteriole to the lowest near the efferent arteriole. The increased oncotic pressure in blood leaving the glomerulus is important in regulating reabsorption of tubular fluid and solutes into the peritubular capillaries.

A change in capillary hydrostatic pressure is the primary mechanism for the regulation of GFR. An increase in this pressure will increase GFR, and GFR is decreased with a decrease in capillary hydrostatic pressure. The glomerular capillary hydrostatic pressure is controlled by arterial pressure and the resistance of both the afferent and efferent arterioles. Increased systemic blood pressure will raise the glomerular hydrostatic pressure, which can increase GFR. However, there are autoregulation mechanisms in place to maintain a relatively constant glomerular pressure as arterial pressure fluctuates. Autoregulation in healthy individuals ensures that GFR and RBF remain relatively constant over a wide range of systemic arterial blood pressures from 80 to 180 mmHg [2, 7].

A decrease in afferent arteriole (preglomerular) resistance leads to an increase in both renal blood flow (RBF) and GFR. An increase in afferent arteriole resistance leads to a decrease in both RBF and GFR. When the resistance decreases in the efferent arterioles (postglomerular), RBF increases, but GFR decreases. Conversely, when the resistance increases in efferent arterioles, RBF decreases and GFR increases. These changes in arteriolar resistance allow for rapid alterations in glomerular blood flow, minimizing changes in GFR [2, 7].

The resistance of the afferent and efferent arterioles is regulated by the autonomic nervous system and vasoactive mediators. Vasoconstrictors of both afferent and efferent arterioles include norepinephrine, angiotensin II, endothelin, and thromboxane. ADH (vasopressin) also causes constriction of the efferent arteriole but not the afferent arteriole. Norepinephrine is released by stimulation of the sympathetic nervous system, causing constriction of both the afferent and efferent arterioles; however, constriction of efferent arterioles predominates, decreasing RBF while minimizing changes in GFR. Angiotensin II also causes more vasoconstriction in the efferent arterioles than in the afferent vessels. Vasodilators of both afferent and efferent arterioles include acetylcholine, nitric oxide, dopamine, bradykinin, prostacyclin, and prostaglandin I_2 (PgI_2). PGE2 causes relaxation in afferent arterioles, but not efferent arterioles. The release of norepinephrine, angiotensin II, and ADH causes vasoconstriction and, at the same time, promotes the production of prostaglandins that promote vasodilation. The production of PGE2 and PgI_2 is important in maintaining RBF when norepinephrine and angiotensin II concentrations are increased (hypovolemic states). The balance between afferent and efferent arteriolar tone (vasodilation or vasoconstriction) determines the effective transglomerular hydrostatic pressure available to drive glomerular filtration [2, 7].

Approximation of Glomerular Filtration Rate

GFR is directly related to functional renal mass in health and in those with acute loss of renal mass (AKI). Measurement of GFR (mL/min, mL/min/kg, mL/min/m^2) is the gold standard for the assessment of renal function and the detection of renal disease progression. It is important to recognize that there is discordance between the percentage of renal mass that is initially lost and the percentage of decrease in measured GFR, due to renal hypertrophy and increases in SNGFR that occur as adaptations over time in chronic disease [54]. Consequently, the percentage loss of renal mass is greater than the percent decrease in GFR during CKD. Determination of RBF also can be useful in detecting progression of renal disease but is less commonly evaluated than GFR.

Sedatives and anesthetics have little effect on GFR. In one study, GFR was similar in dogs sedated with butorphanol and diazepam, acepromazine and butorphanol, and diazepam and ketamine. GFR in sedated dogs was not significantly different from that of awake dogs [55]. Ketamine and acepromazine have minimal effects on GFR in cats [56]. A combination of medetomidine, butorphanol, and atropine has been evaluated in dogs using technetium-labeled diethylenetriaminepentaacetic acid (99 m) renal scintigraphy as an estimate of GFR and was found to have effects on GFR, similar to those observed after saline alone [57].

GFR is not routinely measured in the evaluation of renal function due to technical difficulties and costs. Instead, surrogates for GFR such as blood urea nitrogen (BUN), serum creatinine, and SDMA concentrations, are used because they are more easily determined than is GFR. The simultaneous evaluation of various combinations of circulating surrogates for GFR (including creatinine, cystatin C, galectin-3, and SDMA) improves the accuracy for the calculation of estimated GFR in humans [58, 59].This is likely to also be true for veterinary medicine, though equations that accurately predict GFR have yet to be successfully developed. An attempt to increase the accuracy for prediction of measured GFR based on serum creatinine and an estimation of muscle mass failed to adequately perform in one study of cats [60].

An ideal substance for estimation of GFR should be excreted from the body entirely by the kidneys, produced at a constant rate in the body, have little binding to plasma proteins, be freely filtered by the glomerulus, and undergo no tubular reabsorption or secretion. It also should not alter renal function if injected, should be well distributed and restricted to the ECF, and should not be metabolized by the kidney.

GFR and serum analytes used to estimate GFR often have wide reference ranges for the general population, which limits their usefulness to detect early changes in renal function in individuals. General reference ranges for clinical tests related to glomerular function are provided in Table 1.2. Individual animals have far less variability in these measurements. Due to large variation in veterinary patient size, GFR is usually normalized to body weight or surface area. The use of age- and breed-specific reference ranges can increase the utility of these measurements to detect early renal disease. Trending for increases in the concentration of molecules used to estimate GFR can also provide meaningful evidence for progressive renal disease, even when their concentrations are still within the reference range. Analytic variability of a particular analyte should also be considered during the evaluation of changes in reported values from serial samples [54].

Table 1.2 General reference ranges for clinical tests related to glomerular function. The reference range provided by a specific laboratory should be used.

Test (Units)	Dog	Cat
Blood urea nitrogen (mg/dL)	8–25	15–35
Serum creatinine (mg/dL)	0.3–1.3	0.8–1.8
Serum cystatin C (mg/dL)	0.5–1.5	0.6–2.0 [61]
SDMA (μg/dL)	<14	<14
	<18 IRIS	<18 IRIS
Endogenous creatinine clearance (mL/min/kg)	2–5	2–5
Exogenous creatinine clearance (mL/min/kg)	3–5	2–4
Iohexol clearance (mL/min/kg)	1.7–4.1	1.3–4.2
24-hour urine protein excretion (mg/kg/day)	<30	<20
U_{Pr}/U_{Cr}	<0.5	<0.4
	<0.2 for most	<0.2 for most
Microalbuminuria (mg/dL)	<1	<1
	<2.5	<2.5

GFR was measured by scintigraphy in one study of client-owned dogs with either a diagnosis of CKD or those likely to have CKD. GFR was compared to serum creatinine, SDMA, and cystatin C. The sensitivity for the finding of a serum creatinine >1.3 mg/dL or SDMA >14 μg/dL to predict a low GFR was 90%, whereas the specificity was 90% for creatinine and 87% for SDMA. Overall performance of SDMA or serum creatinine to predict a low GFR was similar in this study, but cystatin C was inferior to both creatinine and SDMA [62].

The relationship between GFR measured by iohexol, SDMA, and serum creatinine was reported in one study of client-owned nonazotemic dogs. Serum creatinine and SDMA were only moderately correlated to GFR and to each other. An SDMA cutoff of >14 μg/dL was sensitive at 90% for the detection of dogs with a ≥40% decreased in GFR, but specificity was low at 50%. More than half of the dogs with an increased SDMA >14 μg/dL had GFR values that were either increased or decreased by <20% of the expected normal GFR. Sensitivity was maintained at 90%, but specificity increased to 83% when the SDMA cutoff was increased to >18 μg/dL for the detection of those with ≥40% decreased GFR [63].

RENAL CLEARANCE

The renal clearance of a substance is that volume of plasma that would have to be filtered by the glomeruli each minute to account for the amount of that substance appearing in the urine each minute. The renal clearance of a substance (x) that is neither reabsorbed nor secreted by the tubules is equal to the GFR. Thus, GFR equals the concentration of the substance in the urine (U_x mg/dL) times the volume of urine per total minutes (V^o mL/min), divided by the concentration of the substance in the serum or plasma (P_x mg/dL) times the weight of the animal (W kg) ($GFR = U_xV^o/P_xW$). This method for precise GFR is mostly restricted to the research environment. Nuclear scintigraphy can also be used to determine GFR at tertiary referral centers [64].

GFR is classically measured by physiologists and researchers using serum/plasma along with urine samples that have been accurately collected by volume over time. Inulin clearance is the gold standard, but it is not easily measured and not available at commercial laboratories. Bolus and continuous infusion of inulin or exogenous creatinine are usually the molecules employed for the most precise results. Endogenous creatinine clearance can be used to calculate GFR as a less accurate alternative than exogenous creatinine clearance [65–67]. Single-injection plasma clearance methods using inulin, iohexol, or creatinine have been used to estimate GFR. Plasma clearance of the substance is calculated using the area under the plasma concentration-versus-time curve. These methods do not require urine collection, but accuracy depends on the number of plasma samples and the timing of their collection [64, 68, 69].

Iohexol is readily available for clinical use to estimate GFR [70, 71], but a laboratory with special equipment must be available to measure it. Very few veterinary commercial laboratories offer iohexol determination. Iohexol is given IV and plasma samples collected after administration. It was recommended in one report to collect samples at 5 and 120 minutes following injection for dogs and at 20 and 180 minutes for cats when using the two-sample method. For the single-sample method, sampling at 120 minutes in dogs and 80 minutes in cats was recommended [72, 73]. Iohexol clearance may be useful in further investigation of renal function to determine if occult renal disease is present or not when routine serum surrogates for GFR (BUN, creatinine, SDMA) are normal. Although not specifically reported in dogs and cats, hypersensitivity to iohexol is possible. Because 1 mL/kg of iohexol is given IV relatively rapidly, caution should be used in those with marginal cardiac function and should not be used if the patient is overhydrated.

GFR determined by iohexol clearance (mL/min and mL/per/kg) was significantly higher for normal dogs in the lowest quartile of body weight (1.9-12.4 kg) of one study [74] similar to that found in another study of healthy dogs [72]. Age did not exert a significant effect on GFR in this study when all dogs were considered. A weak trend for increasing age associated with a decrease in GFR was found only in dogs of the lowest quartile body weight. It was suggested that a separate reference range for GFR of dogs with low body weight should be used [74].

Creatinine is produced endogenously from muscles (see details below) and excreted by glomerular filtration. Thus, its clearance can be used to estimate GFR. For endogenous creatinine clearance determination in clinical cases, collect all urine for 12 or 24 hours and record the volume [43]. Failure to collect all urine produced will decrease the calculated clearance value. The animal's body weight should be recorded, and the serum and urine creatinine concentrations should be determined. Normal endogenous creatinine clearance is approximately 2–5 mL/min/kg in the dog and cat [2]. However, endogenous creatinine clearance measurements are higher than adult reference ranges in puppies from 9 to 21 weeks of age [75]. The main indication for determination of endogenous creatinine clearance in clinical practice is the suspicion of renal disease in a patient with polyuria and polydipsia that has a normal BUN and serum creatinine concentrations.

For exogenous creatinine clearance, administer creatinine (100 mg/kg) SQ or IV to increase the serum creatinine concentration approximately 10-fold. Approximately 40 minutes later, collect at least one timed urine sample using an indwelling urinary catheter (e.g. all urine produced in 20 minutes). The average of three 20-minute collection periods is recommended to minimize collection errors. Determine the animal's body weight and serum and urine creatinine concentrations. Exogenous creatinine clearance exceeds endogenous creatinine clearance and approximates inulin clearance (the gold standard for determination of GFR) in the dog [64, 66]. In cats, exogenous creatinine clearance may be slightly lower than inulin clearance [64].

Renal excretion of urea occurs by glomerular filtration, and BUN concentrations are inversely proportional to GFR. However, urea clearance is not a consistently reliable estimate of GFR, and in the face of volume depletion, decreased urea clearance may occur without a decrease in GFR due to increased tubular reabsorption of urea.

BLOOD UREA NITROGEN/SERUM UREA NITROGEN

More than 90% of urea is excreted by the kidney, with minor excretion in the gastrointestinal (GI) tract and skin [76]. The production and excretion of urea are not constant depending on dietary protein intake, degree of tissue catabolism, liver function, and renal tubular fluid flow rate. Urea is produced primarily from ammonia in the liver via the urea cycle (also known as the ornithine cycle or Krebs–Henseleit cycle), which effectively removes excess ammonia from the circulation. A small amount of urea is also generated in brain tissue [77]. The urea cycle disposes of approximately 90% of circulating nitrogen [78]. The rate of urea production depends on the amount of ammonia produced from protein catabolism from dietary sources and endogenous protein, primarily derived from muscle [76]. Ammonia

enters the urea cycle either directly from the blood or from the breakdown of glutamine. Ammonia and bicarbonate form carbamoyl phosphate, which combines with ornithine to form citrulline. Citrulline and aspartate combine to form arginosuccinate, resulting in the release of fumarate and the production of arginine. With the action of arginase, urea is released and ornithine forms, completing the cycle [79]. Carnivores are unique in that they are unable to synthesize ornithine from proline and glutamine; thus, ornithine is produced exclusively from arginine, which is required in the diet [79]. After urea is produced, urea distributes evenly in the total body water since it is able to diffuse through cell membranes.

Circulating levels of urea nitrogen have long been used to assess renal function and still have value today when close attention is paid to nonrenal factors involved in its interpretation. The term "blood urea nitrogen" is often used, but since the measurement is rarely performed on whole blood, the term "serum urea nitrogen" (SUN) should be used to reflect the measurement utilizing serum.

Urea is measured primarily by enzymatic methods. Urea in the sample is hydrolyzed with urease to generate ammonia, and the ammonia is then quantified. The analysis actually measures urea and is converted to a urea nitrogen measurement for reporting as BUN or SUN. In the USA, BUN/SUN in mg/dL is the typical reported value and is NOT equivalent to the true measurement of urea. In most parts of the world, the reporting of urea concentration in mmol/L is preferable. Urea contains two nitrogen atoms (each with a mass of 14), and the mass of urea is 60. Thus, the concentration of urea in mg/dL is 60/28 (2.14) times the urea nitrogen concentration in mg/dL. To convert to molar units, 1 mg/dL BUN (or SUN) = $10 \times 2.14/60 = 0.357$ mmol/L urea. Multiply the BUN concentration in mg/dL by 0.357 to get the urea concentration in mmol/L. [76]

Reagent test strips have also been used for rapidly estimating BUN concentration. These strips group results into 4 categories: category 1, 5-15 mg/dL, category 2, 15-26 mg/dL, category 3, 30-40 mg/dL, and category 4, 50-80 mg/dL. For dogs in one study, category 1 and 2 results were considered nonazotemic and categories 3 and 4 were considered azotemic. In cats, category 1-3 results were considered nonazotemic and category 4 results were azotemic. Results from the test strips were compared to an automated analyzer measurement of SUN and were found to have high sensitivity and specificity in both dogs and cats. Since the test strips only give a semiquantitative measurement, BUN/SUN should be verified by quantitative measurement [80].

The age of the patient can have a significant impact on the urea nitrogen concentration [81]. In a study of 68 puppies, BUN was higher in puppies as compared to adults until 28 days of age. From days 28 through 84 (end of study), BUN levels were lower in the puppies than in adult dogs [82]. In Borzoi and beagle puppies, plasma urea concentrations were higher than that for the adult reference range from birth to one week old [83]. Reference intervals based on adult dogs should not be used for puppies. A mechanism for the higher BUN in puppies was not obvious. Gender did not appear to significantly affect BUN concentration in 896 dogs less than one year of age [81]. Yorkshire terriers have been anecdotally noted to have an increased BUN independent of increased serum creatinine as a breed predisposition, and some have also been noted with renal proteinuria. Most are not clinically ill for long periods of time [84, 85].

Both postprandial and diurnal effects were found for urea concentrations in one study from cats. Mean plasma concentrations of urea were 15% higher when measured at 8 p.m. compared to 8 a.m. [86]. The circulating urea concentration can be increased in any condition characterized by increased (endogenous tissue or exogenous dietary intake) protein catabolism [87]. The effect of some drugs (e.g. long-term glucocorticosteroids, azathioprine, or tetracyclines) on BUN has not been well studied, but it appears to be minimal. The feeding of higher protein diets to dogs with CKD preferentially increases BUN over creatinine. BUN will decrease during the feeding of lower protein content diets due to less generation of BUN despite decreased GFR during CKD. Lower protein dietary intake may be associated with a slight increase in serum creatinine concentration due to the decreased GFR during CKD. During the feeding of higher protein diets, serum creatinine may decrease some due to increased GFR.

Comparing preprandial and four-hour postprandial BUN concentrations in dogs, BUN increased from 13.7 to 16.0 mg/dL in dogs receiving a 5% protein (as-fed) diet, and increased from 16.0 to 26.8 mg/dL in dogs receiving an 8.5% protein (as-fed) diet [88]. In general, the BUN concentration increased significantly within three hours of a meal and peaked at about six to nine hours. This elevation lasted up to 18 hours [89]. Therefore, an 18-hour fast has been recommended prior to the measurement of BUN [90]. The feeding of small amounts of diet tends to decrease the magnitude of the increase in BUN [89, 91]; thus, feeding multiple small meals during the day may be better to maintain BUN at a lower level. It is likely that increases in BUN following feeding will be more pronounced in those that have underlying renal disease and decreased GFR.

Low BUN is detected less frequently than increased BUN. Polyuria is often considered as a potential cause for a low BUN since tubular reabsorption of urea is heavily influenced by the tubular fluid flow rate. Higher tubular flow rates are associated with lower BUN due to less reabsorption of urea, an effect that does not occur with creatinine [92].

When protein is limited in the diet in the presence of adequate nonprotein calories, the BUN decreases because most of the ingested protein is used for protein synthesis with little waste left over for excretion [90]. However, in a study of 152 very underweight or emaciated dogs with chronic disease (starvation), BUN was increased. BUN was increased more frequently than serum creatinine, and the BUN/Cr ratio was elevated due to some combination of accelerated catabolism and loss of muscle mass [93].

SERUM CREATININE

For a more detailed veterinary review of creatinine, refer to the articles by Braun [94], Kovarikova [95], and Finco [96]. Measurement of serum creatinine concentration is the most commonly used surrogate to estimate GFR in clinics. Serum creatinine is generally preferred over BUN for evaluation of renal function since creatinine has fewer nonrenal variables [94, 97, 98]. Creatine is taken up by muscle from the circulation after its synthesis by kidney, liver, and pancreas. Creatine is enzymatically phosphorylated to phosphocreatine inside muscle cells. Both creatine and phosphocreatine undergo spontaneous conversion to creatinine, which is released into the circulation at a relatively steady rate for each individual animal [76]. The daily rate for creatinine production is determined by age, sex, and muscle mass of the individual. The small-molecular-weight nonprotein-bound creatinine undergoes glomerular filtration but negligible tubular secretion or reabsorption in the dog and cat [94]. Increased concentration of circulating creatinine can lead to less creatinine generation by several postulated mechanisms including negative feedback for creatine synthesis [76].

Since creatinine arises from muscles, serum creatinine concentration is lower than it would otherwise be in patients that have lost substantial amounts of lean muscle mass regardless of the cause. In these instances, serum creatinine overestimates the degree of excretory renal function in that animal. During the progression of CKD, loss of lean muscle mass can parallel loss of renal function, which results in little or no change in serum creatinine concentration. This phenomenon reduces the ability for serum creatinine to detect ongoing CKD progression early, even when tracking serial changes in serum creatinine.

An increased serum creatinine is not associated with a decreased GFR, however, in some instances [99]. Healthy dogs and cats with large lean muscle mass can have higher serum creatinine concentrations than those with less muscle mass [98, 100, 101]. Increased production of creatinine by heavily muscled normal dogs can increase serum creatinine concentration to some degree, as can release of preformed creatinine from muscle into the circulation during rhabdomyolysis.

Greyhounds have higher reference range serum creatinine than the general reference range for all breed dogs [102–104], despite also having higher GFR than average for other breeds [105]. Whippet, Afghan hound, and Saluki dogs have also been described with a higher reference range for serum creatinine [106]. Birman cats had higher serum creatinine compared to Abyssinian, Norwegian Forest, and Siberian cats of one study [107], a finding that was confirmed in another study using mostly domestic short-hair cats as the control group [108]. Physiologically higher serum creatinine concentrations may also occur in Siberian, Siamese, and Somali cats [108, 109].

Serum creatinine concentrations are often lower in puppies and kittens than those encountered in adults [100, 101, 110, 111]. Serum creatinine concentrations slowly rise as they age [75]. Normal client-owned puppies at 8 and 16 weeks of age from two different large-breed litters had either a 0.4 or 0.5 mg/dL serum creatinine (Chew and Meuten 1982 unpublished observations). Five-month-old Beagle puppies had a mean serum creatinine of 0.5 mg/dL [112], and mean serum creatinine was 0.46–0.54 mg/dL in healthy mongrel puppies at 10, 20, and 30 days of age in another study [113]. In normal beagles, mean creatinine was 0.4 mg/dL at four and six weeks, which increased and stabilized to 0.9 mg/dL at six months of age [114]. Creatinine concentrations in Borzoi and beagle puppies up to eight weeks of age were lower than the adult reference range, varying from 24 to 51 μmol/L (0.27–0.58 mg/dL) [83]. Compared to the adult reference range, lower creatinine values were also found in puppies from a variety of breeds between 16 and 60 days old. Median creatinine concentration in these puppies was from 41 to 50 μmol/L (0.46–0.57 mg/dL) [115]. In a study of healthy dogs of various breeds, sex, body size, and age, median circulating creatinine and urea concentrations were lower in puppies than in adults. Median creatinine was 0.45 mg/dL in four-to-eight-week-old puppies and the creatinine values progressively increased in older puppies until a median creatinine of 1.05 mg/dL was reached in dogs >52 weeks of age. Similarly, urea was a median of 20 mg/dL in four-to-eight-week-old puppies and progressively increased to a median urea of 34 mg/dL in dogs >52 weeks of age. Sex and body size did not impact creatinine or urea values in this study [116]. An increase in serum creatinine may not be readily recognized in this population when using an adult reference range. The same concerns exist during the measurement of serum creatinine in small-breed and geriatric dogs. A reference interval for serum creatinine was suggested to be used for specific breeds that differed from those used for mixed breeds. Serum creatinine was significantly lower in all dogs <1 year of age and serum creatinine values were lower in small breed than that found in larger breeds of this study [117].

In contrast, mean serum creatinine progressively declined in normal beagles from 10 to 14 years of age in one study, possibly attributed to loss of lean muscle mass in this age group. The mean serum creatinine was 0.57 mg/dL at 14 years old compared to 0.88 mg/dL at one year of age [118]. In another study of normal beagles, the median creatinine decreased from 0.9 mg/dL at three years old to 0.6 mg/dL at nine years old [119].

Creatinine concentrations are not constant throughout the day, with higher concentrations observed in the afternoon or in the evening [86, 95]. In one study of normal cats, plasma creatinine concentrations were lower during feeding than following fasting, possibly an effect of feeding that increases GFR. This finding was different than that encountered in the dog in which increased circulating creatinine occurred after feeding. The composition of the diet and the amount eaten also factor into these effects [86]. A circadian rhythm has been shown for creatinine in both the dog [120] and cat [86]. Mean serum creatinine was 18% higher when measured at 8 p.m. compared to 8 a.m. in one study of normal cats [86]. The magnitude of disparity in

serum creatinine concentrations from these different methods of collection (preanalytical) is not likely to be of clinical importance unless the serum creatinine values are slightly above or below the upper reference range or near levels used by the International Renal Interest Society (IRIS) for staging.

Creatinine can be measured in plasma or serum, but creatinine was 5–10 μmol/L (0.06–0.1 mg/dL) higher in serum compared to plasma from dogs [121]. Plasma creatinine values were higher in most samples when blood was collected from the jugular vein compared to the cephalic vein in dogs, with a maximal difference of 15 μmol/L (0.17 mg/dL) [122].

The concentration of serum creatinine concentration is generally considered to change little over weeks, months, and years in individual healthy dogs and cats [54]. However, in 20 normal dogs studied at nine time points, even though all values were within the normal reference range for serum creatinine, there was a high interindividual variability [123]. Analytic variability in the measurement of serum creatinine in the same sample is minimal in some labs, but differences in serum creatinine have been reported to be as high as 0.45 mg/dL when measured by the same laboratory and up to 0.57 mg/dL when measured by different laboratories. The variability in measurement of serum creatinine was greater in the same laboratory and between laboratories in the face of moderate-to-severe azotemia. It appears that an increase in serum creatinine of ≥0.3 mg/dL is a clinically relevant indicator of decreased renal function even when the creatinine value is still within the reference range if measurements are made with the same analyzer and laboratory [54].

The median upper reference limit for creatinine and BUN varied by breed of cats in one study between Holy Birman, Chartreaux, Maine Coon, and Persian cats, with Birman having the highest median values [109]. The median and range reference values for creatinine and BUN were significantly different, but the degree of difference was small in four large-breed dogs [124]. The reference interval for circulating creatinine concentrations in various breeds of small dogs (<12 kg) was lower than for the general population, 45–90 μmol/L (0.51–1.0 mg/dL) in comparison to 54–144 μmol/L (0.61–1.63 mg/dL) in one study. It has been suggested that the use of breed-specific reference intervals is important in order to allow early diagnosis of renal impairment in small-breed dogs based on creatinine [125, 126]. A narrower reference range for serum creatinine was established for the dog breed Dogue de Bordeaux compared to the general population [127].

The type and volume of meat consumed as well as the timing of the blood sample have the potential to influence serum creatinine [76]. Cooking of meat favors the conversion of creatine to creatinine, which increased serum creatinine by as much as 20% in one study of dogs consuming a pelleted food [128]. No change in serum creatinine occurred after the feeding of dry, semimoist, or canned food to dogs in another study; BUN increased after the feeding of these diets in all dogs with the greatest increases observed in dogs consuming canned food [129]. Fasting prior to collection of blood is recommended to limit the degree of dietary impact on the measurement of serum creatinine [128].

Most laboratories use automated methods to measure serum and urine creatinine by the Jaffe colorimetric method, which employs alkaline picrate to bind with creatinine to form a reddish complex [130, 131]. The basic Jaffe picric acid reaction overestimates the amount of true creatinine since some noncreatinine molecules contribute to the color that develops. The original Jaffe method was subject to measurement of noncreatinine chromogens in addition to true creatinine that can slightly increase the creatinine value reported [65, 128, 131, 132]. In a study of multiple domestic species, the kinetic Jaffe reaction was positively influenced by acetone and glucose, whereas there was negative bias from acetoacetic acid, bilirubin, and lipid. The enzymatic method to measure creatinine was not affected by acetoacetic acid, acetone, or glucose, but was negatively affected by bilirubin and lipid [131]. The overestimation of true circulating creatinine is most noticeable in healthy animals with low total creatinine values. Total creatinine chromogens measured by the original Jaffe method were compared to that following extraction with Lloyd's reagent to determine true creatinine. True creatinine accounted up to 55% and pseudocreatinine up to 45% of the total circulating creatinine in normal dogs of this study [133]. A pseudocreatinine chromogen correction of −0.3 mg/dL is arbitrarily applied by some automated analyzers to generate the reported circulating value [134].

A concern is that measurement of noncreatinine chromogens in addition to true creatinine could falsely increase total creatinine to a higher value within or above the reference range [128]. An increase in creatinine from noncreatinine chromogens becomes less clinically relevant in animals with azotemic kidney disease since most of the increase in creatinine is now from true creatinine [94, 133]. Interfering noncreatinine chromogens in urine do not occur as frequently as those in serum [76], so the use of the Jaffe method on urine does not overestimate urine creatinine. The kinetic method currently used is an improvement as this decreases the detection of interfering pseudocreatinine compounds [131]. Some interfering molecules in the circulation can also lower the amount of creatinine that is detected [131].

Deproteination of the sample before creatinine measurement improves the accuracy but does not remove all interfering substances. Methods to modify the sample to an alkaline pH allow only true creatinine to combine with picric acid, allowing a more accurate measurement of creatinine. Many different modifications of the basic Jaffe method are used by commercial laboratories that are designed to remove or account for interfering substances. Consequently, it is difficult to accurately compare serum creatinine results generated between different commercial laboratories or results from different in-house analyzers on the same patient. It has been recommended that all methods to measure creatinine be standardized to that using the method of stable isotope dilution tandem mass spectrometry in order to allow comparison of results between laboratories [135].

Standardization in the measurement of serum creatinine is common in human medicine laboratories, but there is no such standardization in veterinary laboratories. Repeatability

for the measurement of serum creatinine (intralaboratory precision) among 10 veterinary laboratories was high using the same analyzer in the same laboratory, but there was quite a bit of variation between results reported by some laboratories (interlaboratory imprecision) [136, 137].

Enzymatic methods to measure creatinine result in lower values than those generated using the Jaffe method [94, 131]. Use of enzymatic methods to measure serum creatinine allowed endogenous creatinine clearance to approximate that of inulin clearance in one study of dogs [65]. Automated enzymatic methods to measure creatinine are more costly, and these reagents have a shorter shelf life than that needed for Jaffe measurements. Some authors have advocated to measure creatinine only by enzymatic methods in order to improve the quality of data for clinical decision-making, especially at low levels of circulating creatinine [130].

In a study of over 300 healthy dogs from 32 breeds, the mean fasting plasma creatinine determined with an enzymatic method was 0.93 ± 0.24 mg/dL with a range of 0.45–1.40 mg/dL for the general population. Mean serum creatinine varied by body weight category in this study. Serum creatinine magnitude was the lowest in dogs with a body weight of 0–10 kg (0.70 ± 0.12, range 0.48–1.02) and progressively increased in dogs 11–25 kg (0.85 ± 0.16, range: 0.55–1.24), 26–45 kg (1.01 ± 0.26, range: 0.60–2.01), and dogs >45 kg (1.19 ± 0.24, range: 0.88–1.82). Males had a higher creatinine (1.00 ± 0.25 mg/dL) compared to females (0.90 ± 0.24 mg/dL) in this study [126]. Dogs with a body weight of 1–10 kg had a mean serum creatinine of 0.79 mg/dL, 0.91 mg/dL when 11–25 kg, and 1.08 mg/dL when >25 kg in another study using the Jaffe methodology [138]. Creatinine was lower in adult small-sized dogs of seven breeds at 45–90 μmol/L (0.51–1.0 mg/dL) compared to the population-based reference range of 54–144 μmol/L (0.61–1.62 mg/dL) [125]. The median creatinine for Greyhound puppies was 0.8 mg/dL, similar to that for other breeds but lower than that for adult Greyhounds [139].

Population-based reference ranges are broader than the reference range for an individual animal. For this reason, population-based reference ranges are less sensitive than an individual's reference range [140] for the diagnosis of CKD using surrogates of GFR such as creatinine or SDMA. An individual's reference range includes inherent random biological variation for the concentration of an analyte around its homeostatic set point [140].

The utility of serum creatinine to accurately detect renal disease can be increased when population-based reference intervals for creatinine are adjusted to account for small-, medium-, and large-breed dogs (based on body weight) to interpret results. Interpretation of serum creatinine should also take into account different reference ranges for animals that are very young and old. When available, specific breed reference ranges should be used instead of population-based reference ranges [95]. Small changes in serum creatinine can indicate clinically relevant changes in GFR as long as the conditions of blood collection and specific analyzer used are kept the same.

For a discussion of urinary creatinine measurement, refer to Chapter 7.

SYMMETRIC DIMETHYLARGININE

Although SDMA was discovered decades ago, there is renewed interest in this molecule as a surrogate to improve estimated GFR in humans [59] and as another surrogate for GFR in veterinary medicine. SDMA is largely eliminated by the kidneys [54, 141] and changes in circulating SMDA parallel that for GFR in the dog [142] and the cat [143]. SDMA results from methylation of arginine that occurs in all nucleated cells and then enters the circulation. Dietary intake does not appear to affect SDMA concentrations regardless of amino acid intake [54]. SDMA is excreted almost entirely into urine by glomerular filtration without further tubular processing and, consequently, increases in blood as GFR decreases. Urinary concentration of SDMA has not been used to study renal function in clinical practice to date. The measurement of SMDA offers an advantage over the measurement of serum creatinine in some patients, as SDMA is not influenced by lean muscle mass in cats [144] or dogs [100]. Consequently, SDMA concentration can be increased when serum creatinine concentration is still within the reference range in those with low lean muscle mass and reduced kidney function.

Liquid chromatography tandem mass spectrometry (LC/MS/MS) methodology has been validated as precise and accurate to measure SDMA concentration in dogs and cats [142, 144]. An automated proprietary clinical immunoassay that correlates with the LC/MS/MS method [145] is offered by one commercial veterinary laboratory (IDEXX); an in-house test for veterinarians to measure circulating SMDA concentration is also available from the same laboratory [146]. A competing veterinary laboratory (Antech) now measures SDMA by a different methodology as of late 2019, but comparisons of measured SDMA between these two methods have not yet been published. For IDEXX, SDMA in the dog and cat ≤14 μg/dL is normal, 15–19 μg/dL is mildly increased, 20–24 μg/dL is moderately increased, and ≥25 μg/dL is severely increased [147]. For Antech, normal SDMA is <14 μg/dL for the dog and <15 μg/dL for the cat. A mild increase is 14–16 μg/dL for the dog and 15–20 μg/dL for the cat. A high increase is >16 μg/dL for the dog and >20 μg/dL for the cat [148].

There was wide dispersion of SDMA results measured by a commercial immunoassay method in cats of one study. Dispersion includes variability in a measured analyte based on both analytical imprecision and biological variability. Biological and analyzer variability were considered important factors to consider for proper clinical interpretation, especially values that are at the edges of the reference range or medical decision threshold (such as IRIS CKD staging). Without this nuanced interpretation, it is possible for clinicians to ascribe too much clinical relevance to small changes in SDMA [149].

There was wide dispersion in SDMA results when analyzed by both a proprietary commercial laboratory method using high-throughput immunoassay and a proprietary point-

of-care analyzer method in another study of cats. Results were not comparable in 20–50% of SDMA values generated by these two methods. A measured SDMA of 14 μg/dL could represent values from 8 to 20 μg/dL based on results from this study. Interpretation of SDMA values near the reference limit or threshold for medical decisions (slightly above or below) is difficult to make with certainty due to analytical and biological variability. Consequently, reference intervals based on specific analyzers were recommended [150].

SDMA measured with IDEXX SDMA using high-throughput competitive immunoassay, SDMA measured with the DLD SDMA ELISA on microtiter plates, and the gold standard of SDMA measured by liquid chromatography–mass spectrometry were compared. IDEXX SDMA exhibited less bias and less variation in the measurement of low and high concentrations of SDMA than when measured by the DLD SDMA ELISA method and was considered more suitable for veterinary patients in one study [151].

In general, the upper limit reference range for SDMA is <14 μg/dL for both adult dogs and cats, though the concentration of SDMA occasionally reaches or exceeds the 14 μg/dL reference limit in juvenile dogs [54]. The IDEXX reference range for puppies is currently 0–16 μg/dL; the reference range for kittens is the same as for adults. The average SDMA concentration was higher in nonracing Greyhounds than for the general population; the upper reference range was near 20 μg/dL [152]. SDMA and serum creatinine concentrations were evaluated in pretraining Greyhound puppies (three to eight months old) in one study. The median SDMA of 14 μg/dL (11–19 μg/dL) for puppies was similar to that for previously reported adults (median 14 μg/dL; 9–20 μg/dL). It was suggested that a reference range of ≤20 μg/dL for SDMA could be used for both age populations of Greyhounds. SDMA concentration also was higher in normal Birman cats than in cats of other breeds, though it was less commonly increased than serum creatinine concentration [108]. An SDMA <18 μg/dL is used as one criterion in the assignment of IRIS CKD stage 1, and an SDMA persistently >14 μg/dL can be used to diagnose CKD based on IRIS 2019 recommendations [153]. An SDMA >18 μg/dL was considered optimal for detection of ≥40% decrease in GFR in dogs of one study [63].

It is important to consider total variability in the interpretation of serum creatinine and SDMA when comparing multiple measurements over time. Total variability includes biological and analytical variability. It appears that total variability for serum creatinine is about ±0.2 mg/dL with the analytical variability at ±0.1 mg/dL. Similarly, for SDMA, total variability is about ±2 μg/dL and analytic variability is about ±1 μg/dL. More analytic variability is expected during repeated measurements of the same sample at the extreme upper limits of measurement, but variability in those measurements is not usually important since it is unlikely to result in a level that would substantially change clinical decision-making. For a hypothetical example, it is possible that the serum creatinine was 20 mg/dL on the first run through the analyzer and 15 mg/dL on a second run immediately thereafter on the same sample. In this instance, the interpretation of high-magnitude azotemia will not change. If the creatinine was first measured as 20 mg/dL and the creatinine repeated on the same sample was 5 mg/dL, there is an error in the measurement of one or both samples. Analytical variability can be much higher when comparing results of the same samples on different analyzers, as often occurs for measurements determined in-house compared to those sent to a referral laboratory (Mack-Gertig personal communication October 2020). Excellent analytic performance for SDMA measurement was shown in one study of dogs; SDMA was highly stable in serum and plasma [142]. The interested reader is referred to an extensive recent review of SDMA and circulating creatinine for the evaluation of excretory renal function in the dog and cat [136].

The biological variability for serum creatinine and SDMA was determined over nine time points of varying intervals in 20 normal dogs [123]. There was considerable variability in the values for these analytes so that values exceeded the upper reference range at times in some dogs. In order to be considered different values on sequential measurement, SDMA had to change by 1.34 μg/dL [123]. In a study of week-to-week variability in dogs with hereditary nephritis, serum creatinine and SDMA had to change by 21–24% in order to be confident for a true increase or decrease from baseline values [154]. SDMA and serum creatinine were measured once weekly for six weeks to determine biological variability in a study of healthy cats. The degree of variability in the magnitude of these analytes was higher than generally appreciated, but similar to findings in dogs [155]. Findings from these studies support the recommendation to evaluate SDMA and serum creatinine on sequential samples and not a single sample, especially for values that are near the reference range limits or near values used in IRIS staging.

It appears that, on average, SDMA concentration increases when there has been about a 40% decrease in GFR. The concentration of SDMA can increase in CKD patients when there is as little as 25% loss of renal mass and GFR at times [142, 156]. Concentrations of SDMA have been shown to increase above the reference range before serum creatinine increased in multiple studies of dogs and cats eventually diagnosed with azotemic CKD [101, 141, 142, 144, 157–159]. The concentration of SDMA increased many months before serum creatinine concentration when relatively high values for the upper limit of creatinine reference (above 2.0 mg/dL) ranges were used to diagnose the onset of CKD. When lower creatinine values were used for the upper limit as the comparator, SDMA concentration still increased before creatinine but by a shorter time. The concentration of SDMA was increased much more frequently than serum creatinine concentration in both dogs and cats when measured on the same sample (diagnostic discordance) [160], but it is not clear how many of these patients will develop progressive azotemic CKD.

A creatinine at >1.9 mg/dL and SDMA >18 μg/dL were used as surrogates for decreased GFR in a study of dogs. Decreased GFR using this definition was common in older dogs of most breeds, especially dogs >10 years of age. Using both

creatinine and SDMA identified more dogs with decreased GFR than with either test alone. Fourteen breeds were identified to be at increased risk to discover an increased creatinine or SDMA. Geriatric and senior Shetland sheepdogs, Yorkshire terriers, and Pomeranians were significantly overrepresented with increased creatinine or SDMA. Boxers were also identified with significant increases in creatinine or SDMA up to 10 years of age [161].

CKD was diagnosed by a variety of criteria in 51.2% of initially healthy senior dogs when followed for over the next four years. Increased serum SDMA ≥14 μg/dL occurred in 8 of the 22 dogs diagnosed with CKD, compared with only 2 of 22 dogs with increased serum creatinine >1.8 mg/dL. A persistent increase in SDMA was documented in all dogs diagnosed with CKD. Based on these results, it was recommended to include SDMA as part of routine screening of elderly dogs [159].

A first-time mild increase in SDMA (15–19 μg/dL) was found to persist (>14 μg/dL) in the subsequent measurement in many dogs and cats of one study. This was observed in 33% of cats that had an initial SDMA of 15 μg/dL and in 62% of cats with an initial SDMA of 19 μg/dL. Increased SDMA persisted similarly in dogs, in 44% with an initial SDMA of 15 μg/dL and in 68% of dogs with an SDMA of 19 μg/dL [162].

The concordance between increased SDMA and serum creatinine was assessed in one large study in dogs and cats. When SDMA was increased, serum creatinine was increased in 30% of cats and in 28% of dogs, a finding that increased to 57% in cats and 54% of dogs one year later. When serum creatinine was increased, SDMA was increased in 75% of cats and in 69% of dogs, a finding that increased to 93% of cats and 87% of dogs one year later [163].

SDMA and serum creatinine were significantly correlated to each other and to GFR measured with iohexol clearance in one study of azotemic and nonazotemic cats. Plasma SDMA and serum creatinine were similar in sensitivity for the detection of reduced excretory renal function, but creatinine had higher specificity. Increased SDMA identified cats with decreased GFR in this study, but the superiority of SDMA over serum creatinine as a value-added test for early detection, as highly touted for detection of decreased GFR in other reports, was not demonstrated in this study. Thirty-nine of 49 cats had concordant results between serum creatinine and plasma SDMA. Discordant results were observed in 10 of 49 cats. SDMA was increased in eight cats, while serum creatinine was within the reference range; six of these eight cats, however, had normal GFR indicating a false positive test result for SDMA. SDMA was within the reference range in two cats, in which the serum creatinine was increased and GFR was reduced, indicating a false negative result for SDMA. Using a cutoff of 14 μg/dL was associated with many false positives for SDMA; increasing the cutoff to 18 μg/dL resulted in a more optimal sensitivity and specificity for SDMA [164].

Elevated SDMA with normal renal function has been identified on occasion in some reports [165, 166], a finding that has also been anecdotally noted by others [136] and also observed by this author. SDMA was reported to be lower in cats with diabetes mellitus than in control cats of one study [165]. An increased SDMA was considered to be falsely positive in 32% of dogs evaluated for the presence of kidney disease in dogs, whereas there were no false positives for increased serum creatinine at the same time; the gold standard of reduced GFR measured by iohexol clearance was used to define the presence of kidney disease [166].

There is sometimes discordance between the finding of a very high IDEXX SDMA value compared to a minimal or no increase in serum creatinine at the same time in animals with cancer [167]. Reductions in GFR to account for this magnitude of increase in SDMA were considered unlikely, as these patients had minimal clinical signs that would typically be expected in those with CKD. One proposed theory is that cancer cells infiltrating the kidney change the filtration barrier in some way that inhibits filtration of cationic SDMA but does not inhibit the filtration of nonpolar creatinine, allowing SDMA to increase, sometimes greatly, without an accompanying increase, or minimal increase, in serum creatinine [167]. Neoplastic infiltration of the kidney was shown to occur in all animals studied with a variety of cancers that had an increased SDMA with normal serum creatinine in one study [168]. Some dogs with lymphoma of another report had a disproportionate increase in SDMA at a time of reference range serum creatinine; SDMA declined when the lymphoma went into remission. Whether the increased SDMA reflected a reduced GFR was not determined. A large burden of tumor cells that were synthesizing SDMA was also considered as an alternative explanation for the preferential increase in SDMA [169].

CYSTATIN C

Serum cystatin C concentration appears to be a useful marker of GFR though its use has not entered routine clinical practice because measurement of this molecule is not yet available from veterinary referral laboratories, and a clear superiority for use of cystatin C has not been demonstrated. Measurement of serum and urine concentrations of cystatin C has been validated for use in the dog and cat using methods employed in human medicine [170].

Cystatin C is a protease inhibitor freely filtered by the glomeruli, does not undergo tubular secretion, and is almost completely reabsorbed by the proximal tubular cells [95]. Cystatin C is produced at a constant rate in all nucleated cells, and its excretion is not dependent on age, sex, or diet. Cystatin C has been extensively reviewed for its possible use in dogs and cats [61, 95, 170–172]. Normal cystatin C concentration is approximately 1 mg/L in dogs and 0.6–2.0 mg/L in cats [95, 171, 173]. Serum cystatin C concentration may also be increased by the presence of inflammation [174] or some types of neoplasia [175]. There are many reports with no consensus for the use of serum cystatin C in dogs and cats to evaluate renal function. Cystatin C appears to be less useful as a surrogate for GFR in cats than in dogs. There is no convincing evidence that cystatin C is superior to the use of serum creatinine in the evaluation of renal disease in the dog or cat [95].

INTERPRETATION OF RENAL FUNCTION TESTS

INTERPRETATION OF RENAL FUNCTION TESTS IN RENAL DISEASE

Serum creatinine concentration is the most commonly used serum biochemical indicator of renal function in the clinical setting. The relationship between GFR and serum creatinine is described as curvilinear, exponential, or hyperbolic [94, 96]. There is an exponential relationship between decreasing GFR and increasing serum creatinine (Figure 1.16). Notice that despite a substantial decrease in GFR, there is a minimal increase in serum creatinine concentration until a pivot point is reached at the exponential rise part of the curve. At that point, each subsequent further decrease in GFR is associated with a much larger increase in serum creatinine concentration. The slope of the curve is small when GFR is mildly or moderately decreased but large when GFR is severely reduced. Thus, large changes in GFR early in the course of renal disease cause small increases in BUN or serum creatinine concentration. Small changes in GFR in advanced renal disease cause large changes in BUN or serum creatinine concentration.

Neither BUN nor serum creatinine concentrations are specific enough for an early diagnosis of renal disease or clear identification of patients with normal renal function. BUN and serum creatinine are reported to increase at nearly the same time during the diagnosis of CKD in dogs and cats, neither being more sensitive than the other, but BUN concentrations are impacted by many more nonrenal factors [90, 176].

In a patient with primary renal disease, a serum creatinine concentration above the population reference range (>2.0 mg/dL) is often interpreted to be associated with a loss of greater than 75% of functional renal mass and GFR [54, 94, 177, 178]. When a lower value for the upper limit of serum creatinine such as 1.4, 1.5, or 1.6 mg/dL is used, an increased serum creatinine concentration is associated with a loss of about 50% renal mass and GFR [54]. Thus, the diagnostic utility of serum creatinine to detect decreased renal function as a surrogate for GFR can be enhanced when the upper limit of the reference range is lowered (<1.4 in dogs and <1.6 mg/dL in cats), when seemingly small increases in serum creatine ≥0.3 mg/dL are interpreted to be important, when breed- and age-specific dynamics for serum creatinine are brought into consideration, and when the same analyzer and conditions are used during the measurement of serum creatinine. An individual patient's serum creatinine concentration will increase over time with progression of renal disease, and trending patterns of increasing serum creatinine concentrations should not be ignored even if the results are still within the reference range. This is due to a high interindividual variation in serum creatinine values. Trends for increasing serum creatinine and BUN concentrations in individual animals are helpful for earlier detection of CKD [54, 179].

It has been recommended to adjust reference ranges for an individual animal's serum creatinine results to improve the interpretation. The animal's breed, body weight, and muscle/body condition scores can be factored into the assessment as one way to increase the utility of serum creatinine to diagnosis CKD. Similarly, trends for an increasing serum creatinine within the general population reference range can increase

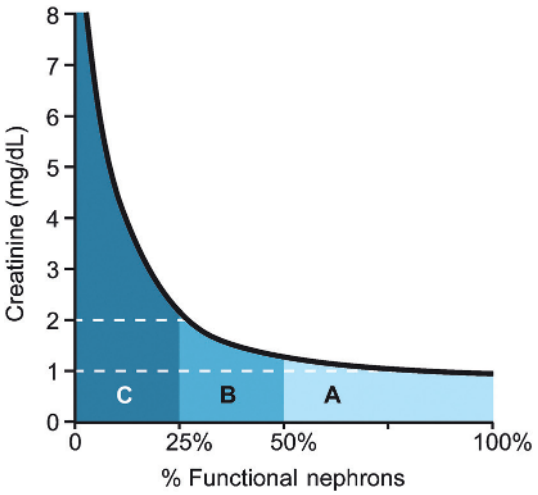

FIGURE 1.16 Serum creatinine concentration compared to the percent of functional nephrons (GFR). Note that the relationship of serum creatinine concentrations to GFR is exponential and described as a rectangular hyperbola. The slope of the curve is relatively flat when GFR is mildly or moderately decreased, but steep when GFR is severely reduced. Thus, decreases in GFR early in the course of renal disease cause only small increases in serum creatinine. With further decrease in GFR, a larger magnitude of increased serum creatinine occurs while on the exponential rise part of the curve (to the left). Small increases or decreases in GFR along the exponential steep rise part of the curve can create large numerical change in serum creatinine. Classically, a 75% decrease in GFR must occur before serum creatinine increases above the reference range, but this depends on what value for the upper end of the reference range for creatinine is used. When lower values for the upper reference range of serum creatinine are used, a decrease in GFR of about 50% can be detected when serum creatinine is found to be increased. In CKD, the area up to 50% decrease in GFR is referred to as that of decreased renal reserve, from 50% to 75% loss is referred to renal insufficiency, and 75% loss is renal failure associated with azotemia. Area A represents diminished renal reserve with advancing chronic renal disease (International Renal Interest Society [IRIS] Stage 1). Area B represents renal insufficiency (late IRIS Stage 1 and early IRIS Stage 2); submaximally concentrated urine is often documented in dogs but less commonly in cats in this stage. Area C represents overt azotemic renal failure (IRIS Stages 3 and 4). The percent of nephron mass loss and the decrease in GFR are parallel when there is an acute loss of nephron mass. In those with CKD, adaptive hypertrophy of the surviving remnant nephrons (anatomically and physiologically) over weeks to months limits the decrease in GFR, so that the percentage loss of GFR is less than compared to the loss of nephron mass. Illustration by Tim Vojt, reproduced with permission of The Ohio State University.

the sensitivity for the diagnosis of CKD over a period of months or over days for the diagnosis of AKI. The use of a previously established baseline creatinine for individual patients allows values that escalate within the reference range to be detected early [54, 95].

The loss of renal mass is proportional to the decrease in GFR during an acute insult, but there is discordance between the percentage loss of renal mass and decrease in GFR in chronic disease. In CKD, GFR gradually increases over weeks to months following an initial loss of renal mass due to adaptive mechanisms of renal hypertrophy and increases in SNGFR [54, 180, 181].

The magnitude of a single high BUN, serum creatinine, or SDMA concentration cannot be used to predict whether azotemia is prerenal, primary renal, or postrenal in origin and cannot be used to distinguish between acute and chronic, reversible and irreversible, or progressive and nonprogressive processes. In addition, the finding of a reference range BUN, serum creatinine, or SDMA concentration does not exclude the possibility of renal disease. A reference range BUN, serum creatinine, or SDMA concentration implies that at least 25–40% of renal mass is functional, but how much more renal mass is functional cannot be determined by these tests. In some situations, either the BUN or serum creatinine is increased, but not both at the same time (discussed previously). It is not always possible to explain discordant results between BUN and serum creatinine concentrations (Table 1.3).

Table 1.3 Discordant results between blood urea nitrogen (BUN) and serum creatinine (SCr) concentrations.

↑ BUN-to-SCr ratio (high BUN, low creatinine, combinations)	↓ BUN-to-SCr ratio (low BUN, high creatinine, combinations)
Dehydration and volume depletion-early oliguria	Polyuria
High-protein-content meal	Low-protein-content meal
Gastrointestinal hemorrhage	Anorexia/hyporexia
Loss of lean muscle mass	Highly muscled individual
Emaciated animal – chronic disease	Sighthounds
Hyperthyroidism	Acute muscle injury
Physiological low muscle mass – young animal	Anabolic steroids
Geriatric – normal aging low muscle mass	Liver disease
Uroabdomen (early)	
Congestive heart failure	

Discordance between serum creatinine and SDMA is common, with increases in SDMA being reported more commonly than increases in creatinine in both dogs and cats [160]. SDMA is reported to increase earlier than serum creatinine in both dogs and cats, and in dogs that are eventually diagnosed with azotemic CKD. SDMA and creatinine concentrations both varied within the same normal dog when blood was sampled at multiple time points in one study. Both of these analytes are limited as individual screening tests because of this individual variation. Serial monitoring and trend detection were recommended to detect early renal dysfunction before concentrations escalate above the population reference range. SDMA had less biological and analytical variability than serum creatinine in this study suggesting SDMA to be a superior early biomarker for detection of kidney dysfunction [123]. In another study, median SDMA concentrations were increased to the same magnitude in dogs with CKD or AKI, but serum creatinine concentrations were significantly higher in dogs with AKI compared to dogs with CKD. The ratio of SDMA (μg/dL) to serum creatinine (mg/dL) was significantly higher in dogs with CKD (median 10.0) than in dogs with AKI (median 6.5). Unfortunately, substantial overlap in this ratio limits its usefulness to distinguish CKD from AKI [182]. An SDMA-to-creatinine ratio >10 was associated with a higher risk of death in dogs and cats with CKD in one report. Most dogs and cats with CKD have an SDMA-to-creatinine ratio of <10. The use of this ratio for prognosis should not be used for dogs and cats with values within the reference range as they can have ratios >10 that would be very misleading [183]. The prognostic value for this ratio in dogs with CKD was not confirmed in another study [182].

Serum creatinine and/or SDMA are used to assign an IRIS CKD stage [153]. Serum creatinine and urine output are the mainstays for assigning an IRIS AKI grade, though SDMA can be used to provide evidence for Grade 1 AKI [184]. Staging is performed AFTER CKD has been diagnosed in patients that have stable CKD. The CKD staging system uses an upper reference range for creatinine of <1.6 mg/dL for cats and <1.4 mg/dL for dogs and an SDMA <18 μg/dL for both dogs and cats. CKD stages from 1 to 4 are assigned based on escalating magnitude of serum creatinine concentration and/or SDMA. Substages for CKD are assigned based on blood pressure and magnitude of proteinuria measured by urinary-protein-to-urinary-creatinine ratio (UPC) (discussed later in Chapter 7). An acute increase of ≥0.3 mg/dL serum creatinine is used to help assign IRIS AKI stage 1.

SDMA concentration was added to the IRIS CKD staging and treatment recommendations in 2019 (Table 1.4). Adding SDMA is helpful to further characterize CKD and the degree of renal dysfunction in patients in which serum creatinine concentration underestimates the decline in GFR. The finding of a serum creatinine concentration <1.6 mg/dL for the cat and <1.4 mg/dL for the dog, and an SDMA concentration that is

Table 1.4 IRIS CKD staging guidelines (updated 2019).

		Dog	Cat	Units	Comments
Stage 1	Creatinine	<1.4	<1.6	mg/dL	Other abnormalities are found that support primary kidney disease (USG, renal imaging, UPC, others)
		<125	<140	μmol/L	
	SDMA	<18	<18	μg/dL	SDMA persistently >14 μg/dL may be used to diagnose CKD
Stage 2	Creatinine	1.4–2.8	1.6–2.8	mg/dL	Clinical signs minimal or absent
		125–250	140–250	μmol/L	
	SDMA	18–35	18–25	μg/dL	
Stage 3	Creatinine	2.9–5.0	2.9–5.0	mg/dL	Early and late-stage 3 based on severity of clinical signs
		251–440	251–440	μmol/L	
	SDMA	36–54	26–38	μg/dL	
Stage 4	Creatinine	>5.0	>5.0	mg/dL	Increasing risk for systemic signs and uremic crisis
		>440	>440	μmol/L	
	SDMA	>54	>38	μg/dL	

<18 μg/dL, provides one entry point into IRIS Stage 1 CKD in the presence of other abnormalities that suggest primary kidney disease (urinalysis with low USG, high systemic blood pressure, increased UPC, renal imaging changes) [153].

The same IRIS CKD stage will be assigned in most patients based on both SDMA and serum creatinine concentrations. Guidelines for the use of SDMA concentration in combination with serum creatinine concentration continue to be evaluated due to concerns that serum creatinine may underperform in its estimation of declining GFR, especially in patients with low lean muscle mass. During times of discordant staging between creatinine and SDMA, the higher stage is chosen based on the highest value of either of these analytes [153]. Most discordant results will be associated with a creatinine suggesting a lower stage and SDMA suggesting a higher stage, likely due to loss of lean muscle mass that generates a lower serum creatinine concentration. An SDMA >18 μg/dL in a dog with a serum creatinine <1.4 mg/dL, or <1.6 mg/dL in a cat, is upstaged to Stage 2 rather than Stage 1 when based on creatinine alone. Patients designated as Stage 2 by serum creatinine should be restaged to Stage 3 if the SDMA is >35 μg/dL in dogs or if the SDMA is >25 μg/dL in cats. Similarly, an SDMA >54 μg/dL in the dog or >38 μg/dL in the cat would result in an assignment of Stage 4 in those originally assigned to stage 3 based on creatinine [153].

The BUN/serum creatinine ratio has also been used to evaluate renal disease. The BUN/creatinine ratio often is 15:1 to 30:1 in mature healthy dogs and cats. In one study, the median BUN/serum creatinine ratio in healthy dogs was 14.4 [185]. This ratio may be increased in prerenal and postrenal azotemia as a result of increased tubular reabsorption of urea at lower tubular flow rates or easier absorption of urea than creatinine across peritoneal membranes in animals with uroabdomen. Hyperthyroid cats may also have an increased ratio due to increased GFR and loss of lean muscle mass. It may be increased during cachexia as a result of lower serum creatinine concentration due to loss of lean muscle mass. The BUN/serum creatinine ratio may be decreased after fluid therapy as a result of increased tubular flow and decreased tubular reabsorption of urea, rather than as a result of a change in GFR.

It is important to remember that any analyte serving as a surrogate for GFR will be influenced by prerenal, primary renal, and postrenal factors. In addition, the finding of normal serum creatinine, BUN, and/or SDMA concentrations does NOT exclude the presence of primary renal disease, as loss of substantial renal mass and renal function must occur before the concentrations of these molecules increase above the reference range. Discordant test results between serum creatinine, SDMA, and BUN occur at times, so it is important to not rely solely on the results of just one test of renal function. Close attention to results of urinalysis (especially USG and proteinuria, as well as cylindruria and renal epithelial cyturia) can suggest the presence of ongoing renal disease when renal parameters on routine blood testing are normal.

Thyroid Status and Renal Function Evaluation

Functional thyroid hormone status should be considered during evaluation of renal function parameters since thyroid status influences GFR and creatinine synthesis. High levels of circulating thyroid hormones increase RBF and GFR [186, 187], whereas low levels decrease them [188, 189]. Extremes of thyroid functional status (hypothyroid or hyperthyroid) can

be of clinical significance for an effect on GFR especially for evaluation of patients with underlying CKD. Dogs with naturally occurring hypothyroidism were reported with slightly higher serum creatinine concentrations than euthyroid dogs in one study, but they were rarely above the reference range [190]. In dogs with experimental hypothyroidism, decreased GFR was documented, but no change was observed in serum creatinine. Decreased synthesis of creatinine accounted for the failure of serum creatinine to increase in the face of decreased GFR in these hypothyroid dogs [189]. Reduced GFR was documented in dogs with clinical hypothyroidism, and GFR was restored to higher levels following thyroxine supplementation [188].

Hyperthyroidism is associated with increased RPF and GFR following some combination of increased cardiac output, increased blood volume, renal vasodilatation, and activation of the renin–angiotensin–aldosterone system [187, 191–193]. Around 11–41% of cats with hyperthyroidism have been reported with azotemia prior to treatment [187, 194–198]. There was discordance in the frequency of increased BUN (11%) compared to increased creatinine (6%) in hyperthyroid cats prior to treatment in one report [191]. An increased BUN-to-creatinine ratio in hyperthyroidism can occur due to some combination of increased BUN and decreased creatinine. Increased production of urea from increased body protein turnover can increase the BUN, and decreased muscle mass can result in less creatinine synthesis and lower circulating creatinine concentrations [191, 192]. SDMA was more frequently increased in a large series of hyperthyroid cats (20%) than was creatinine (3.5%) in one report [199]. Higher total thyroxine (T4) concentrations were associated with lower serum creatinine concentrations in cats diagnosed with hyperthyroidism, but SDMA was not affected by the level of T4 in one study [200].

In patients with underlying CKD, increased GFR associated with hyperthyroidism can "mask" its detection based on BUN, serum creatinine, and SDMA that are lower than they would otherwise be during euthyroidism [186, 192, 198, 201–204]. Some of the lowering of serum creatinine concentrations can also be associated with the loss of muscle mass associated with hyperthyroidism [201].

Azotemia emerges as a new finding or increases in magnitude from baseline in some cats following treatment for hyperthyroidism. Around 10–51% of cats have been reported to develop "overt" renal failure following various treatments for hyperthyroidism that resulted in euthyroidism or hypothyroidism [191, 194, 197, 201, 203–207] [187]. The process of conversion from nonazotemia to a diagnosis of azotemic CKD is often referred to as "unmasking" of subclinical CKD that was not apparent during the "masked" hyperthyroid state associated with increased GFR [201, 206]. Post-treatment increases in circulating creatinine are attributed to decreases in GFR associated with a return to euthyroidism or iatrogenic hypothyroidism. GFR was significantly higher in cats with hyperthyroidism compared to control cats and significantly decreased when euthyroidism was achieved during methimazole treatment in one study. Mean BUN and serum creatinine did not significantly increase after treatment, but overt azotemia developed in 2 of 12 cats [203].

Cats with hyperthyroidism were studied after treatment with bilateral thyroidectomy, I-131, or methimazole treatment. Mean values for T4, BUN, and serum creatinine were not different between treatment groups at baseline, 30, or 90 days after treatment. Mean serum creatinine and BUN at 30 and 90 days were significantly higher and T4 significantly lower than before treatment in all treatment groups. Mean increases of up to 1.0 mg/dL for serum creatinine and up to 9 mg/dL for BUN over baseline developed following treatment for hyperthyroidism in these cats. Though mean BUN and serum creatinine were within the reference range before treatment, up to 27% of these cats had BUN or serum creatinine above the reference range [198].

In another study, the mean GFR decreased by 44% at 30 days following bilateral thyroidectomy in 13 cats with hyperthyroidism [204]. At the same time, mean serum creatinine (1.26 versus 2.05 mg/dL) and BUN (26.6 versus 34.9 mg/dL) were significantly increased over baseline and T4 was significantly decreased. Seven of 13 cats had post-treatment T4 levels below the reference range, but none developed azotemia. Two of 13 cats in this study developed overt renal failure following treatment and both had normal reference range T4 [204].

Following I-131 treatment of hyperthyroid cats, T4 declined at six days, but there were no significant changes in GFR, BUN, or creatinine at this time in one study. Significant increases in BUN and serum creatinine were observed at 30 days along with further decreases in T4. Nine of 22 cats were in renal failure (serum creatinine >1.8 mg/dL) prior to treatment and 13 cats were in renal failure 30 days following treatment [194].

The decrease in GFR following restoration of euthyroidism stabilizes within one month of treatment [208] and is of the same magnitude following thyroidectomy, methimazole or carbimazole, or I-131 treatments [187]. Some increase in BUN, creatinine, and SDMA is expected at this time, but creatinine can continue to increase for up to six months [187] from a combination of decreased GFR and increased muscle mass [187, 191, 197, 201, 205, 206]. GFR was above the reference range before treatment of hyperthyroid cats in another study of hyperthyroid cats and decreased significantly following treatment with I-131 at one month without further significant decrease by six months. BUN and serum creatinine were significantly increased at one month. Creatinine continued to increase at three and six months likely the result of increased muscle mass, but BUN did not increase further [209].

SDMA was increased more frequently than serum creatinine at baseline, one, three, and six months following treatment of hyperthyroidism in cats of one study [210]. Body weight, creatinine, and SDMA increased up to 30 days

following treatment for hyperthyroidism. Creatinine but not SDMA continued to increase during weight gain in these cats [200].

Though SDMA and serum creatinine were positively correlated in another study of hyperthyroid cats, SDMA was increased in 30% of samples at a time that serum creatinine was within the reference range. In 5% of samples, serum creatinine was increased when SDMA was normal. SDMA was relatively higher than creatinine in hyperthyroid compared to euthyroid or hypothyroid cats. The assignment of renal status was often discordant between SDMA and serum creatinine in this study [202].

Overt azotemia following treatment of hyperthyroidism is more likely to occur in cats during iatrogenic hypothyroidism. It can take up to six months for hypothyroidism to develop in some cats after radioiodine treatment [207]. Significantly more cats with hypothyroidism had azotemia (16 of 28) compared to euthyroid cats (14 of 47) following treatment in one study [207]. It has been estimated that about 10% of cats are expected to develop iatrogenic hypothyroidism following I-131 treatment for hyperthyroidism [206], but this was encountered in nearly half of cats following bilateral thyroidectomy [202]. Hypothyroidism was documented in 45–67% of cats with "unmasked" azotemic CKD following I-131 or bilateral thyroidectomy treatment [201, 206]. In hyperthyroid cats that were nonazotemic before treatment, a >2.1 mg/dL serum creatinine developed at a median of six months in 16% of these cats.

Overmedication with antithyroid medications in cats with hyperthyroidism can cause iatrogenic hypothyroidism (low T4, high TSH) and an increase in serum creatinine concentrations. Restoration of euthyroidism following dose reduction of antithyroid medications was associated with a significant reduction in plasma creatinine (median 2.61 versus 2.07 mg/dL) in one study [211]. Serum creatinine decreased in all hypothyroid cats following supplementation with thyroxine in another study, but most creatinine concentrations were still above the reference range [206].

An increased SDMA concentration above the reference interval prior to treatment had a high specificity but poor sensitivity for the prediction of post-treatment azotemia [210] similar to results from a previous study [201]. SDMA concentration showed few false positives for the prediction of azotemia, but failed to predict the emergence of azotemia in most cats [201].

INTERPRETATION OF RENAL FUNCTION TESTS IN NONRENAL DISEASES

Gastrointestinal Disorders

GI bleeding can increase BUN concentration more so than serum creatinine because digested blood provides an endogenous protein load that preferentially increases BUN over serum creatinine. The elevation in BUN is largely related to the amount of hemorrhage [90] but can also be influenced by the degree of ECFV contraction associated with the hemorrhage (prerenal azotemia) that can increase both BUN and serum creatinine as a form of prerenal azotemia. In a very early study of normovolemic dogs, the maximal increase in BUN was proportional to the amount of protein fed in the form of whole blood, plasma, packed red blood cells (RBCs), lean round steak, or casein. The globin component of hemoglobin from RBCs was demonstrated to be readily digested in this study [212]. An increase in BUN by two to threefold over baseline was commonly observed in dogs fed blood [87, 212]. Greater increases in BUN were seen in dogs fed blood when they were dehydrated or hypotensive [87]. Dogs with upper GI hemorrhage in one clinical study had significant increases in both serum creatinine and BUN. There was a larger increase in BUN that was obvious, while the increase in creatinine was still within the reference range, resulting in a nearly doubling of the BUN-to-serum-creatinine ratio [185]. In one study of dogs with protein-losing enteropathy, mean serum creatinine was 0.4–0.7 mg/dL and mean BUN was 12–16 mg/dL [213], suggesting loss of lean muscle mass affecting creatinine without an increase in BUN from bleeding. Anecdotally, chronic bleeding from bad teeth and periodontal disease can cause an increase in the BUN/creatinine ratio in addition to classic GI bleeding.

In one study of dogs with chronic enteropathy, serum creatinine was low in 75% and within the reference range in 25%, while the urea nitrogen was above the reference range in 14% and normal in 85%. Consequently, the urea-to-creatinine ratio will be increased most of the time since creatinine is low most of the time regardless of whether the urea is increased or not [214]. Occult fecal bleeding was common in one study of CKD, even in IRIS CKD Stage 2. The urea-nitrogen-to-creatinine ratio was increased in dogs of this study from stage 2 to stage 4 [215].

Cardiac Conditions

BUN may be increased in cardiac conditions. This can be attributed to prerenal factors that result in reduced delivery of blood to the kidneys and or to high-level activation of the renal tubular urea transporters during the body's perception of reduced cardiac output and increased ADH release [76, 216]. There is discordance between BUN and serum creatinine in this setting as BUN is increased more so than would be predicted by decreased GFR alone. The BUN-to-creatinine ratio is increased in part due to the effects of increased circulating ADH on renal tubular urea transporters that increase the reabsorption of urea into the blood stream. Additionally, the BUN could be preferentially increased due to catabolism or intake of higher protein intake diets, whereas the creatinine could be decreased due to loss of lean muscle mass due to cardiac cachexia [217].

It appears that BUN can be used as a biomarker for the prognosis of heart failure in humans by serving as a surrogate for the degree of neurohormonal activation [76, 216]. A high

BUN/creatinine ratio was an independent predictor of death in humans with acute heart failure [218]. Discordance of BUN and serum creatinine has been noted in some dogs or cats with congestive heart failure (CHF). Both BUN and serum concentrations were significantly increased in a review of 31 dogs with aortic thrombosis, but the BUN was above the reference range more frequently than creatinine [219]. In a study of 145 dogs and cats with acute CHF, 52% exhibited elevated BUN at the time of admission, but creatinine was increased in only 17% at the same time [220]. Cats with CHF and cachexia had higher BUN and BUN/serum creatinine ratios than those cats with CHF but without cachexia; serum creatinine concentrations were not different between these groups. Maximal values for BUN were 112 mg/dL, 4.6 mg/dL for serum creatinine, and BUN/creatinine ratio of 77 in cats of this study [217]. There was no difference in BUN or serum creatinine concentrations in dogs with or without cardiac cachexia, but dogs with cardiac cachexia had a significantly higher BUN-to-creatinine ratio. BUN was as high as 107 mg/dL and serum creatinine as high as 2.7 mg/dL in this study [221].

Portosystemic Shunt

In animals with portosystemic shunts (PSS), ammonia metabolism is disrupted, and hyperammonemia develops as a result of shunting of portal blood into the systemic circulation [79]. Both dogs and cats with PSS typically have low BUN concentrations, most likely due to decreased urea cycle function to synthesize urea with decreased hepatic perfusion [222–224]. Impaired creatine synthesis in the liver can contribute to lower serum creatinine concentrations [225, 226]. GFR is higher than normal in dogs with shunts, which can in part additionally account for the finding of low serum creatinine and BUN that occurs in this population. After correction of the shunt, BUN and serum creatinine raise to a normal level as GFR declines and as more normal liver metabolism returns [226].

Periodontal Disease

In a study of 38 healthy dogs with periodontal disease, BUN concentration significantly increased after treatment of the periodontal disease from a median of 13–17 mg/dL but remained within the reference range (5–30 mg/dL); the creatinine did not change before or after treatment [227]. The reasons for the discordance between the change in BUN and serum creatinine in this study were not apparent.

Primary Hyperparathyroidism

Sixty-three percent of dogs with primary hyperparathyroidism in one study had a BUN below the reference compared to only 4% of dogs with a low serum creatinine. The reason for this discordance was not identified but might be explained by polyuria preferentially affecting BUN [228].

Exercise

With vigorous exercise, muscle produces increasing amounts of ammonia, which potentially could increase BUN generation in the liver. An increased release of creatinine from muscle is also possible depending on the degree of muscle insult. The effect of vigorous exercise on BUN and creatinine in dogs is highly variable. No change in BUN or creatinine was seen following agility competition in one study [229]. A decrease in BUN was observed in Labrador Retrievers following field trial competition; creatinine was not measured [230]. An increase in BUN occurred after exercise in sled dogs without a change in creatinine in another study [231].

Creatinine variably increased, was not changed, or decreased in dogs undergoing heavy exercise in four other studies. Creatinine significantly increased by about 20 μmol/L (0.23 mg/dL) after sprinting in Greyhounds at a time of no change in BUN [232]. Creatinine increased by 50% over baseline following sled dog performance in one study; BUN was not measured [233]. Increases in serum creatinine could result from increased muscle release of creatinine or decreased creatinine clearance following decreased GFR associated with exercise. Creatinine decreased and BUN increased at some time points following sled dog racing of another study, but the magnitude of these changes was small [234]. Creatinine decreased by about 10% from baseline in untrained Beagles after running for reasons that were not apparent; BUN was not measured [235].

Immune-mediated Thrombocytopenia

In a study of 73 dogs with immune-mediated thrombocytopenia, a high BUN at the time of admission was significantly associated with failure to survive compared to dogs without increased BUN. BUN exceeded the reference range more often than serum creatinine (22% versus 5%); serum creatinine was not associated with survival in this study. The disparity between the number of dogs with BUN and serum creatinine increases is likely due to those that had melena, which was also associated with failure to survive [236].

Infectious and Parasitic Diseases

Infectious and parasitic diseases can decrease renal function through mechanisms that promote an inflammatory process to the organism in the kidneys, creative oxidative stress and renal injury, or damage the kidneys through immune complex injury in response to the infecting organism [237–243]. Animals that are sick from a systemic infection can have reduced ECFV and reduced GFR to account for some of the increases in BUN and creatinine (prerenal azotemia). These processes are likely to affect BUN and serum creatinine to the same degree, unless there is GI bleeding that will preferentially increase BUN or if there is chronic catabolism with loss of muscle mass in which the creatinine will be preferentially decreased. There was discordance in the frequency of increased creatinine compared to increased BUN in one study

of dogs with babesiosis. BUN was increased about twice as often as was the finding of an increased serum creatinine (62.4% versus 30.7%) and the degree of azotemia was mild in most cases. A reason for this discordance was not apparent. A decreased BUN happened rarely that was attributed to hepatic dysfunction and decreased synthesis of urea [244]. In 51 dogs with vena caval syndrome due to heartworm infection, BUN and serum creatinine were elevated presurgically and decreased significantly after surgical removal of the heartworms in dogs that survived. Ten days post worm removal, the mean BUN/serum creatinine ratio was 18 in survivor dogs and 33 in nonsurvival dogs [245].

Uroperitoneum (Uroabdomen)

BUN and serum creatinine both progressively increase following the accumulation of urine in the peritoneal cavity as one form of postrenal azotemia. Ruptured bladder is the most common cause for uroperitoneum, but urine leakage can also occur following major trauma to the kidneys, ureter, and urethra. The concentrations of creatinine and urea in urine initially entering the peritoneal cavity are much higher than that in the circulation, which favors concentration-dependent movement of these molecules from the abdominal fluid into the circulation. Urine that enters the peritoneal cavity is modified by reabsorption of solutes from the abdominal fluid and entry of water into the abdominal fluid by osmotic and concentration-dependent dynamics. An increase in BUN is the first biochemical abnormality to be detected in uroabdomen. Urea is more readily absorbed across the peritoneal membranes than is creatinine due to its lower molecular weight resulting in an increase in BUN before serum creatinine in the early hours following urine accumulation in the abdominal cavity. The greatest discordance between BUN and serum creatinine is observed early when azotemia is minimal. In an experimental model of uroabdomen in the dog, an increased BUN above the reference range was the first significant biochemical change observed by 5 hours and an increased serum creatinine above the reference range was documented by 21 hours after urine entered the abdominal cavity. There was a concordant increase in both BUN and serum creatinine from 21 to 69 hours following the development of uroabdomen when moderate to severe azotemia had developed. In the clinical condition, the magnitude and rate of increase in BUN and serum creatinine concentrations may be magnified by concomitant prerenal factors that commonly accompany trauma such as shock and hypotension [246]. The higher molecular weight of creatinine in the abdominal fluid urine causes it to be reabsorbed across the peritoneal membranes more slowly than urea. This allows the ratio of abdominal fluid creatinine to serum creatinine to definitively identify the abdominal fluid as urine when a large gradient is detected, even before serum creatinine is increased. The concentration of urea nitrogen in the abdominal fluid is often nearly the same as in the blood after 24 hours or more making this ratio of little value later on. A large gradient of abdominal fluid potassium to serum potassium is also useful to confirm the diagnosis of uroabdomen [246–250]. The combination of metabolic acidosis, hyperkalemia, hyponatremia, and azotemia that often are discovered in patients with uroperitoneum can be confused with similar findings in hypoadrenocorticism, especially when there is no clear history of trauma.

In summary, the kidneys are integral in maintaining the constancy of the internal milieu, largely through the excretion of metabolic waste products, conservation or excretion of water and electrolytes, and acid–base balance. In addition, the kidneys are integral in the regulation of arterial blood pressure and the secretion, metabolism, and excretion of hormones. The kidneys are endocrine organs that secrete erythropoietin to stimulate RBC production from the bone marrow, as well in the synthesis and secretion of the most active metabolite of vitamin D ($1,25(OH)_2$-vitamin D or calcitriol) vital in the regulation of calcium metabolism and in general cellular health. Pathology in any part of the kidney can have vast consequences on a variety of physiological processes resulting in serious disease. Failure to conserve water, glucose, amino acids, and plasma proteins are processes that can be detected in urine as well as additions of cells (RBC, white blood cells, epithelial cells) from the kidneys or lower urinary tract that can be detected in urine sediment. Many severe or advancing kidney diseases are associated with the loss of ability to elaborate concentrated urine and renal origin proteinuria. The specifics of these alterations in urinalysis will be discussed in sections of this book that follow.

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