Kidney Function and Damage

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I. INTRODUCTION
Clinical biochemistry in nephrology is mainly used to diagnose and monitor renal dysfunction or damage. This is of special importance in human medicine, because of the frequent observation of renal failure in elderly people, and in canine and feline medicine, especially for the early detection of chronic renal failure (CRF), which is frequent. In U.S. private practice, however, renal disease was not reported as one of the 29 most common disorders in dogs and was ranked 17th in cats (Lund et al., 1999). In U.S. veterinary university hospitals, renal disease was observed in 15% of dogs more than 10 years old, in 33% of cats more than 15 years old (Polzin et al., 1989), and in 30% of cats more than 15 years old (Krawiec and Gelberg, 1989).

Kidney diseases are uncommon in equids and cattle (Fetcher, 1985). The latter show better resistance to a loss of kidney function than monogastric animals because of the filtration function of the rumen epithelium as shown by bilateral nephrectomy (Fetcher, 1986).

II. KIDNEY MORPHOLOGY AND FUNCTION
A. General Structure of the Kidneys
The mammalian kidney consists of tens of thousands to millions of nephrons that function as parallel units. The larger the species, the greater the number of nephrons per kidney (Kunkel, 1930; Ryland, 1937–1938; Vimtrup, 1928). This ranges from about 10,000 in mice (Cullen-McEwen et al., 2003), 175,000 in cats (Brown et al., 1993), 300 to 700,000 in dogs (Finco and Duncan, 1972), and 7 million in elephants, as compared to about 1 million in humans. The number of nephrons progressively increases during fetal development and is complete at birth (e.g., in sheep or humans) or during the few days following birth (e.g., in rat) (Gimonet et al., 1998). The number of nephrons in the dog decreases slightly (5%) during the 2 first months of life, whereas the glomerular volume increases by 33% (Horster et al., 1971). However, great interindividual variability is observed within species. In dogs, the size or the weight has little influence on the number of glomeruli, but the size of these latter is larger in larger breeds (Finco and Duncan, 1972; Kunkel, 1930). In sheep, the number of nephrons in twins was about 30% lower than in single lambs (Mitchell et al., 2004) or showed no difference (Bains et al., 1992).
The general architecture of the nephrons is identical in all species (Fig. 16-1) (Kriz and Bankir, 1988), but their disposition within the kidney differs between species (Bankir and de Rouffignac, 1985).

Blood is supplied to the kidneys by the renal arteries. These divide into interlobar and arcuate arteries located at the corticomedullary junction. Branches of the latter supply blood to the afferent arterioles of the tuft of capillaries in the glomerulus, from which it is collected by the efferent arterioles. Depending on the location of the glomeruli, blood is then supplied to a network perfusing the cortical tubules or to the vasa recta vessels, which penetrate deep into the medulla in “hairpins” parallel to the loops of Henle. See the review in Pallone et al. (1998). The total blood supply to the kidneys (renal blood flow, RBF) is very high, about 20% of the cardiac output, and most of it goes to the cortex. Only a fraction of the plasma flow (renal plasma flow, RPF) is filtered resulting in the glomerular filtration rate (GFR). This is the filtration fraction (FF), which generally amounts to 20% to 30% of RPF: GFR = RPF × FF.

The RBF remains quite stable, because of autoregulation, even with variations in systemic blood pressure. The precise mechanism of autoregulation is unknown but results from vasoconstriction/dilation of the afferent and efferent glomerular arterioles, which maintains an almost constant hydrostatic pressure within the glomerulus. Autoregulation is efficient in healthy dogs between 70 to 180 mmHg and may be altered in disease, especially during CRF (Brown et al., 1995).

B. Glomerulus and Filtration

Each glomerulus consists of a tuft of anastomosed capillaries within the Bowman’s capsule, which collects the primitive urine formed by plasma filtration and opens into the tubule conducting urine to the renal pelvis. The filter between the plasma and urine consists of three layers (Fig. 16-2) (see reviews in Deen et al. [2001], and Rennke and Venkatachalam [1977]):

- The fenestrated endothelium of the capillaries, allowing direct contact between the plasma and the basal membrane via large pores and fenestrae (~50 to 100 nm); the luminal face is coated with sulfated glycosaminoglycans and glycoproteins.
- The basement membrane made of a gel containing approximately 90% water and negatively charged sulfated glycosaminoglycans.
- The filtration slits (~25 nm) between the footlike processes of the podocytes (i.e., the visceral epithelial cells covering the external surface of the capillaries) (Gubler, 2003). The slits are made porous by the slit diaphragm in which proteins, consisting mainly of the extracellular part of the nephrin molecule anchored in the membrane of the
podocytes, are arranged in zipper-like fashion (Wartiovaara et al., 2004). This slit diaphragm seems to be the basis of filter selectivity, although this has long been considered a function of the basement membrane; it may also act as a signaling system to modulate filtration (Benzing, 2004).

The driving force of filtration is hydrostatic pressure of cardiac origin (Fig. 16-3). This is opposed by plasma colloidal (oncotic) pressure produced by plasma proteins and urine hydrostatic pressure within the Bowman’s capsule. The colloidal pressure in the Bowman’s capsule is negligible because of the almost total absence of proteins. As a result, the effective filtration pressure is 10 to 15 mmHg (Navar et al., 1999). This slit diaphragm seems to be the basis of filter selectivity, although this has long been considered a function of the basement membrane; it may also act as a signaling system to modulate filtration (Benzing, 2004).

The drivers of filtration are the hydrostatic pressures within a glomerular capillary, which is opposed by hydrostatic pressure within Bowman’s space (P fb) and oncolytic pressure in the capillary (P o). Values are pressures in dogs and cats.

The limits of filtration (see the review in Tryggvason, 1999) are as follows:

- **Size and shape:** Neutral molecules with a diameter <2.5 nm diffuse freely. Then as diameter increases, filtration decreases to approximately 0 when the diameter is >3.5 nm (i.e., the approximate diameter of the albumin molecule).
- **Charge:** Because of the high concentration of sulfated glycosaminoglycans at the surface of endothelial cells and in the GBM, the filtration slit tends to repel negatively charged molecules (i.e., most plasma proteins at blood pH).

As a result of glomerular filtration, all small hydrosoluble plasma molecules, including water and ions, are freely filtered but high molecular weight proteins are not. Albumin, which has an MW = 67,000 and a pI = 4.9 (Portell et al., 1979), is very close to the limit of filtration so that only a minimal amount is filtered by “normal” kidneys. The albumin concentration in primitive urine is ~20 to 30 mg/l and many smaller proteins are also present, most of which are reabsorbed in the tubule.

**C. Tubule: Reabsorption and Secretion**

1. **Parts of the Tubule**

The tubule is divided into different segments (Fig. 16-2) (see standard nomenclature in Kriz and Bankir, 1988):

- The proximal tubule begins with a convoluted portion followed by a straight section dipping toward the medulla; the epithelium of the proximal tubule consists of thick cuboidal cells with a very dense brush border on the lumenal side, which provides an immense surface of exchange with the glomerular urine; this is the portion of the nephron where most solutes and water are reabsorbed.

- The loop of Henle produces a “hairpin” bend within the medulla, ending close to the glomerulus at the juxtaglomerular apparatus. Long and short loops descend only into the inner or outer medulla, respectively. The loop of Henle is essential to urine concentration mechanism and it is often stated that long loops are mostly observed in species living in desert areas, and thus related to higher concentrating ability. Almost all the nephrons in dogs and cats are long looped (Bulger et al., 1986), whereas those in humans and pigs are mostly short looped (Bankir and de Rouffignac, 1985), and the average urines are more concentrated in dogs and cats than in humans or swine. The two main points regarding function are that (1) the water and urea permeability of the descending thin limb is high, because of the presence of the aquaporin 1 water channel and (2) the ascending thin limb shows very low water and high sodium chloride permeability.

- The juxtaglomerular apparatus is a morphological entity at the confluence of the afferent and efferent arterioles of the glomerulus and a differentiated part of the loop called the macula densa (Spangler, 1979a, 1979b). The cells in the macula densa respond to decreases in blood pressure or hyponatremia by secreting renin stored in the granules, thus activating the angiotensin-aldosterone response.

- The distal convoluted tubule stretches from the macula densa to the confluence into a collecting tubule within the cortex. The reabsorption capacity is lower than in the proximal nephron (e.g., only ~5% to 10% of sodium and chloride), and secretion of potassium may occur. See the review in Reilly and Ellison (2000).

- The collecting tubules leading to the renal pelvis. The final regulation of urine volume and solute excretion occurs in the final segment of the distal tubule and the collecting tubule, and it is partly regulated by hormones.

2. **Functions of the Tubule**

The main tubule functions are the reabsorption of water, electrolytes, and small molecules and, to a lesser extent, the secretion of ions and small molecules. Reabsorption is dominant in healthy animals and mainly occurs in the proximal tubule by active and passive transport. Further adjustment of urine excretion occurs in the distal tubule and is controlled by hormones, so that the final urine is usually more concentrated than the ultrafiltrate. See the review in Reilly and Ellison (2000). Reabsorption and secretion continually adapt to maintain an almost constant plasma composition, whereas the intake and utilization of ions and small molecules vary with food and water supply.
proximity of meals, environment, physical effort, and so on. This is why the following occurs:

- Urine composition can show large variations in the same healthy or diseased subject.
- A notable overlap of urinary analyte concentrations is observed in healthy and diseased animals.
- The reference intervals for urine analytes are of little relevance when determining spot urine compositions.

a. Small Hydrophilic Molecules

Glucose, amino acids, and low-molecular-weight proteins are mostly reabsorbed in the proximal tubule. Almost 100% of the glucose, amino acids, and proteins are reabsorbed. The former return to the plasma, whereas small proteins are degraded in the tubular cells. Other small hydrophilic molecules are not or only poorly reabsorbed (e.g., creatinine) (see Section II.A.1.a).

Glucose reabsorption is permitted by the SGLT1 (sodium glucose transporter) in the apical membrane, which couples glucose reabsorption with sodium transport down a gradient produced by an Na/K-ATPase in the basolateral membrane, across which glucose diffuses in a concentration gradient by means of GLUT-2 transporters. This glucose reabsorption capacity is limited by the finite number of transporters: maximal tubular reabsorption (Tm) is attained when plasma glucose concentration (P-Glucose) is about 12 mmol/l in dogs, 15 to 18 mmol/l in cats, and 8 to 10 mmol/l in cows.

No urea reabsorption occurs before the medullary part of the collecting duct because of the presence of urea transporters activated by antidiuretic hormone (ADH). This is part of the mechanism creating a high inner medullary osmolality. A small amount of urea is even secreted into the medullary part of the ascending branch of the loop of Henle. Urea reabsorption is increased when urine flow is low (e.g., during dehydration or volume depletion). Urea is central to the concentrating mechanism in the kidney and is ensured by the presence of urea transporters at the thin extremity of the descending loop of Henle and corresponding segments of the vasa recta, which enable urea diffusing from the medullary part of the collecting tubule to enter the nephron and to a lesser degree the blood. See the review on urea transporters in Smith and Rousselet (2001).

b. Electrolytes

Electrolytes are mostly reabsorbed in the proximal tubule. The rate of reabsorption differs considerably according to the internal balance of each ion. Under “normal” conditions it is almost 100% for sodium, chloride, calcium, and phosphates, but much lower for potassium, especially in ruminants owing to their high dietary intake.

The extent of reabsorption can be estimated from the fractional excretion (FE) of solutes (i.e., the fraction of the filtered load of an electrolyte that is finally excreted in urine) (see Section II.C.3):

- Sodium concentration is kept low in tubule cells, as in other cells, by an Na/K-ATPase in the basolateral membrane. See the review in Feraill and Doucet (2001). This active transport of sodium ions from the urine accounts for most sodium reabsorption. It also creates a sodium concentration gradient that allows cotransport of amino acids, glucose and other ions, and so on. See the review in De Weer (1992). Further reabsorption of sodium and chloride occurs in the ascending branch of the loop of Henle via an Na-K-2Cl cotransporter in the luminal membrane. See the review in Russell (2000). Final adjustment in the distal part of the nephron is hormonally controlled by aldosterone and natriuretic peptides.

- Chloride is the most abundant anion in the extracellular compartment. The plasma concentration and elimination of chloride are usually concomitant with those of sodium, except in the case of acid-base disorders. In metabolic acidosis, bicarbonate ions secreted by the kidney cells are exchanged with chloride by an Na-independent Cl/HCO₃ exchanger, thus increasing urine chloride elimination and the plasma anion gap.

- Potassium is reabsorbed in the proximal tubule (~70%) and in the ascending part of the loop of Henle, the distal tubule, and the medullary collecting duct. Potassium is also secreted by the distal tubule and cortical collecting duct, mainly during hyperkalemia (Berlin and Kennedy, 1948). See the review in Hebert et al. (2005). Part of this process is based on aldosterone secretion, which induces the synthesis of Na/K-ATPases, thereby favoring potassium excretion and sodium reabsorption.

- The inorganic phosphates (P i ) in plasma comprise about 80% HPO₄²⁻ and 20% H₂PO₄⁻. They are reabsorbed in the proximal tubule by a sodium cocraduate, which is inhibited by PTH, thus increasing phosphodiuresis. Some newly identified peptides may also decrease tubular phosphate reabsorption without any alteration of glucose and amino acid reabsorption and thus lead to renal loss of phosphates. See the reviews in Laroche and Boyer (2005) and Ritz et al. (2003). The Tm for phosphate reabsorption is higher in ruminants than in other species (Summerill and Lee, 1985; Symonds and Manston, 1974).

- Calcium: Free and complexed calcium ions are freely filtered by the glomerulus. Calcium is mostly reabsorbed in the proximal tubule and in the ascending branch of the loop of Henle, except in horse and rabbit, which excrete significant amounts of this ion when supply is sufficient. Reabsorption occurs by paracellular route. Epithelial channels in the distal part of the nephron permit the transepithelial transport of about 15% of filtered calcium. See the
reviews in Hoenderop and Bindels (2005) and Hoenderop et al. (2005). This final reabsorption is mainly under the hormonal control of PTH.

- Magnesium: Non-protein-bound magnesium is filtered by the glomerulus. Only about 25% are reabsorbed in the proximal tubule. Most reabsorption occurs in the ascending branch of the loop of Henle (50% to 60%) (Rosol and Capen, 1996).

c. Water

Most water (~75%) is reabsorbed passively in the proximal tubule along with ions and small hydrophilic molecules, so that the fluid entering the descending branch is isotonic. Water is reabsorbed in the descending branch of Henle’s loop as a result of the corticopapillary osmolar gradient, whereas the ascending branch is impermeable to water. Final reabsorption occurs in the collecting tubule, mainly under the hormonal control of the antidiuretic hormone (ADH). This hypothalamic nonapeptide is secreted by the posthypophysis when osmolality increases and during hypovolemia or decreased blood pressure. Receptors in the collecting tubule cells trigger the AMPc-dependent synthesis and shuttling of aquaporins, mainly the AQP2 water channel, into the apical and basolateral membrane, thereby permitting water reabsorption and the final adjustment of urine concentration. See the review in Nielsen et al. (2002). The final urine concentration is usually much greater than that of the glomerular filtrate (Fig. 16-4).

d. Acid-Base Regulation

The main organs involved in acid-base regulation are the kidneys and lungs. The lungs modulate the elimination of CO2 and the kidneys the elimination of protons and generation of bicarbonate ions.

The carbonic acid (H2CO3)-bicarbonate (HCO3- ) buffer system is the most efficient extracellular buffer system. It is based on the low dissociation of carbonic acid (pKa ~6.1) at the pH of the extracellular/intravascular compartment and the fact that it is an open system eliminating CO2 in the lungs. Its efficiency is enhanced by the action of carbonic anhydrase, which accelerates the hydration of carbonic anhydride (CO2) into carbonic acid. High concentrations of carbonic anhydrase occur in many tissues including the kidney tubule. The relationship between CO2, H2CO3, and HCO3 is expressed in the following equation:

\[ \text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \]

Bicarbonate ions filtered by the glomerulus are mainly reabsorbed in the proximal tubule (~80%) as CO2, which is lipophilic and able to diffuse across the membrane. Within the cell, CO2 is hydrated by carbonic anhydrase into carbonic acid, which dissociates into bicarbonate ions and protons. The secretion of protons into the tubule lumen ensures the conservation of bicarbonate and its transfer to the plasma. This occurs principally in the distal tubule, where protons are excreted and buffered in urine with filtered organic anions, phosphate, and ammonia generated from glutamine in the tubule cells by the action of glutaminase and glutamate dehydrogenase. This process is enhanced during acidosis, when distal tubule and collecting tubule cells excrete protons against a concentration gradient by the action of an H+-ATPase in the basolateral membrane.

e. Endocrine Functions

Two major hormones, erythropoietin (EPO) and 1α, 25-dihydroxycholecalciferol (calcitriol), are synthesized by the kidneys and released into the blood.

EPO is a cytokine that regulates erythrocyte production synthesized in the peritubular cells in response to hypoxia. Minor amounts are also produced in the liver, mainly in the newborn. EPO binds to receptors of bone marrow progenitor cells and acts synergistically with other growth factors to proliferate and mature the erythroid progenitor cells. In advanced chronic renal disease, the synthesis of EPO decreases and is insufficient to meet the demands for new red cell production, resulting in anemia. See the review in Fisher (2003).

Calcitriol is a secosteroid hormone derived from vitamin D3. It is produced in the proximal tubule cells by the
action of 1α-hydroxylase on 25-hydroxyvitamin D₃ produced by liver hydroxylation of vitamin D$_3$. Calcitriol is a major antihypocalcemic hormone acting at the transcriptional level to induce active intestinal absorption of calcium. It acts in synergy with PTH to activate calcium release from bone. PTH increases the expression and activity of 1α-hydroxylase activity during calcium and vitamin D$_3$ deficiency. The direct modulating effects of calcium and phosphates are weaker. 1α-Hydroxylase activity was recently shown to be down-regulated by phosphaturic peptides called phosphatins. See the reviews in Ebert et al. (2006), Jones et al. (1998), and Kumar (1984). Calcitriol synthesis is decreased in CRF, and its administration is recommended for the treatment of animals with CRF and concomitant hyperparathyroidism. See the review in Nagode et al. (1996).

III. TESTS OF KIDNEY FUNCTION

Kidney function can be evaluated from the concentrations of plasma or urine analytes, which are mainly dependent on their elimination (e.g., P-Creatinine). These indirect markers can be easily and rapidly measured, but their sensitivity is poor and generally remains unaltered until 75% of renal function has been lost and their concentrations may be modified by extrarenal factors. Direct tests of kidney function are based on the elimination kinetics of markers of glomerular filtration, blood flow, or tubule reabsorption/secretion and are based on the clearance concept. These tests are more difficult and take longer to perform but allow earlier detection of reduced function.

A. Indirect Tests of Glomerular Function

P-Creatinine is the test most often used to diagnose and monitor kidney disease in human and animal clinical pathology. P-Urea is also used frequently but is subject to more numerous extrarenal factors of variation. These molecules are almost totally eliminated by glomerular filtration, so that in the case of kidney failure their plasma concentrations increase. However, neither test is sensitive in the early diagnosis of kidney disease because of the large functional reserve of the kidneys. Moreover, variations of P-Urea and P-Creatinine are not proportional to the number of functional nephrons (e.g., a mean increase of 85% in P-Creatinine and of 140% in P-Urea was observed after a two-fold reduction of GFR, with values close to the upper limit of the reference interval) (Letebvre et al., 1999).

1. Creatinine
   a. Creatinine Metabolism

Creatinine is a small molecule (MW 113) produced by degradation of creatine and creatine-phosphate, an energy-storing molecule mainly present in skeletal muscles. See the reviews in Braun et al. (2003), Perrone et al. (1992), and Wyss and Kaddurah-Daouk (2000). Creatine is synthesized from the amino acids glycine, arginine, and methionine, the final step occurring in the liver (Fig. 16-5). It is then taken up by the muscles where it is reversibly phosphorylated to creatine phosphate by creatine kinase. Creatine is the product of the spontaneous, irreversible, non-enzymatic, internal dehydration of creatine, and dephosphorylation of creatine phosphate.

![FIGURE 16-5 Schematic representation of creatinine metabolism.](image)

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Creatinine mainly circulates in a free form in the plasma and is distributed into the whole body water compartment (Schloerb, 1960; Watson et al., 2002a). It was reported that, in dog plasma, 6% were bound to plasma proteins (Kennedy et al., 1952). Creatinine is freely filtered by the glomerulus and is not reabsorbed or secreted in cats (Finco and Barsanti, 1982) and ponies (Finco and Groves, 1985), but it may be strongly secreted in horses.
(Bickhardt et al., 1996). In dogs, either no secretion has been observed (Finco et al., 1993; Watson et al., 2002a) or very weak proximal tubule secretion has been reported in males but not in females (O’Connell et al., 1962; Swanson and Hakim, 1962). This secretion is slightly increased following reduction of the renal mass (Robinson et al., 1974). Secretion of creatinine by active transport in the proximal tubule has been reported in humans, in whom it is nonuniformly increased in renal failure (Walser et al., 1988), also in sheep (Bickhardt and Dungelhof, 1994), rabbit (Matos et al., 1998), pig (Wendt et al., 1990), and goat (Ladd et al., 1957). Creatinine is reabsorbed by the tubules in newborn rabbits and humans, probably by back-leak through the immature tubules (Matos et al., 1998).

As observed in humans and rats, extrarenal intestinal catabolism may be suspected in cases of renal failure. This may involve the bacterial flora, as demonstrated in the rat by accumulation creatine bacterial catabolites including methylguanidine (Jones and Burnett, 1972; Mitch et al., 1980). This hypothesis is supported by the observed increase in cats with ARF (Ohashi et al., 1995). Finally, creatinine is rapidly cleared from plasma (half-life of 3 h in dogs; Watson et al., 2002a) and eliminated in urine, where its total excretion depends on body weight (Gartner et al., 1987).

b. Preanalytical Factors of Variation

- Specimen: Serum and plasma creatinine concentrations are identical, although a slightly higher serum value has been reported in dogs (Thoresen et al., 1992).
- Differences between jugular and cephalic vein may be overlooked (5 µmol/l in dogs) (Jensen et al., 1994). Canine P-Creatinine is not changed by storage at −20°C or −70°C for up to 8 months (Thoresen et al., 1995) or by three freeze-thaw cycles (Reynolds et al., 2006).
- In human urines, the creatinine concentration decreased by about 20% to 30% in 1 week at any temperature between 4°C and −80°C, and was not altered by five freeze-thaw cycles (Schneider et al., 2002), whereas others found that it was stable up to 30 days when stored at 4°C and minimally decreased at 25°C (Spierto et al., 1997).
- Diet and meals: P-Creatinine in dogs and cats was increased by meals containing meat, especially when this was cooked (Epstein et al., 1984; Evans, 1987; Harris and Lowe, 1995; Lowe et al., 1998; Sagawa et al., 1995; Watson and Church, 1980; Watson et al., 1981) or after oral loading with creatine (Lowe et al., 1998), although large interindividual differences were observed. P-Creatinine was higher in dogs fed chicken-based diets than egg- or casein-based diets (Bartges et al., 1995a). No differences in P-Creatinine were observed between cats receiving high- or low-protein diets (Adams et al., 1993). P-Creatinine was moderately higher in young dogs on a low-salt diet (Bagby and Fuchs, 1989) and about 50% higher in goats fed on a low-protein diet (Vallonen et al., 1982).
- Hydration status: P-Creatinine was only moderately increased in dogs deprived of water for 4 days, even when the weight loss was >10% (English et al., 1980; Hardy and Osborne, 1979).
- Physical exercise: P-Creatinine was decreased (by about 40%) by physical training in sled dogs (Kronfeld et al., 1977; Querengaesser et al., 1994). It was not significantly changed after strenuous physical exercise in untrained dogs (Chanoint et al., 2002). It was increased by about 20 µmol/l after a 400min sprint in greyhounds (Rose and Bloomberg, 1989; Snow et al., 1988) and by approximately 50% after strenuous sprint efforts in sled dogs (Hammel et al., 1997; Querengaesser et al., 1994), but not after very long races (up to 415 miles) (Hinchcliff et al., 1993; Querengaesser et al., 1994).
- Housing: P-Creatinine was slightly higher (10 to 20 µmol/l) in dogs kept indoors than outdoors (Kuhn and Hardegg, 1988; Rautenbach, 1988), whereas others found no difference (Spangenberg et al., 2006).
- Drugs: P-Creatinine was decreased in dogs receiving glucocorticoids (Braun et al., 1981), whereas U-Creatinine was increased (Iversen et al., 1997). P-Creatinine was unchanged or little affected by a single dose of nonsteroidal anti-inflammatory drugs (NSAID) (Lobetti and Joubert, 2000; Mathews et al., 2001) or by halothane anesthesia (Lobetti and Lambrechts, 2000), and moderately increased by furosemide administration (Adin et al., 2003) and high-dose trimethoprim-sulfadiazine in dogs (Lording and Bellamy, 1978). P-Creatinine could increase (Kitagawa et al., 1997), remain unchanged (Atkins et al., 2002), or decrease (Pouchelon et al., 2004) in dogs treated with ACE inhibitors for heart failure.

c. Analytical Factors of Variation

HPLC is considered to be the reference method (Blijenberg et al., 1994; Hanser et al., 2001), but routine analyses are based on the nonspecific Jaffé reaction (alkaline picrate) and enzymatic procedures (Guder et al., 1986). See the reviews on creatinine measurement in Spencer (1986) and recommendations for improvement in Myers et al., (2006). The enzymatic methods give slightly lower results than HPLC and Jaffé reaction, −5 and 20 µmol/l, respectively, in dog plasma (Evans, 1987; Palm and Lundblad, 2005). The main interferents in the Jaffé reaction are glucose, ketones, and hemoglobin, but canine plasma was unaffected by hemolysis up to 25 g/l (Jacobs et al., 1991, 1992; O’Neill and Feldman, 1989). Interference in the kinetic Jaffé technique is limited in normal dog plasma as the other chromogens react more slowly than creatinine (Palm and Lundblad, 2005). There is less interference in urine because of the lower proportion of Jaffé chromogens, so that calculated creatinine clearances may differ greatly according to the technique used, as shown in rats (Jung et al., 1987).

The accuracy of plasma creatinine measurement is far from satisfactory in human medicine and cannot be
expected to be better in veterinary medicine (Miller et al., 2005; Myers et al., 2006). The measurement of urine creatinine has not been validated in animals, but a poor correlation between different techniques was shown in dogs, especially in concentrated urines (Trumel et al., 2004).

d. Reference Intervals and Physiological Factors of Variation

Reference intervals for P-Creatinine have been poorly defined in most species because of animal selection and the chosen analytical method. The reference intervals for dogs were shown to differ considerably from one textbook to another (Lefebvre et al., 1998b), so that any interchanging of reference limits or decision levels would be risky:

- Breed: P-Creatinine is higher in large breeds of dogs (Braun et al., 2002; Feeman et al., 2003; Hilippo, 1986; Medaille et al., 2004), even in puppies (Kühl et al., 2000).
- Gender: No differences related to gender were observed in dogs (Broulet et al., 1986; Passing, 1981), although moderately higher concentrations (~+10%) were observed in 1- to 3-year-old and 9- to 11-year-old male beagles (Fukuda et al., 1989).
- Age: A high P-Creatinine concentration was reported in newborn calves, puppies, and foals, probably because of the accumulation of creatinine ingested from the allantoic fluid (9 to 23 µmol/l in bovines) (Edwards et al., 1990; Kühl et al., 1985; Kühl et al., 2000; Lupke et al., 1967). This decreased during the following weeks and then showed a moderate increase again. In dogs and cats, P-Creatinine almost doubled from the first weeks to 1 year of age, then remained stable up to 10 years (Kraft et al., 1996; Passing and Brunk, 1981; Strasser et al., 1993, 1997; Swanson et al., 2004; Vajdovich et al., 1997; Wolford et al., 1988), although other authors reported a regular decrease with age in dogs (Fukuda et al., 1989; Lowseth et al., 1990). In piglets, P-Creatinine almost doubled between 2 and 6 months of age (Krog et al., 1979).
- Biological rhythms: Both a circadian and a seasonal rhythm were observed in dogs, but they were of limited significance (Singer and Kraft, 1988; Sothorn et al., 1993; Strasser et al., 2001), whereas no such rhythms were reported in bulls (Boehnke, 1980). In healthy sheep, creatinine was 25% higher in summer than in winter (Nawaz and Shah, 1984).

e. Variations of P-Creatinine in Disease

An inverse curvilinear relationship was observed between P-Creatinine and GFR in dogs (Fig. 16-6) (Finco et al., 1995, 1993; Miyamoto, 2001a; Westhoff et al., 1993). This was the same in both sexes (Finco et al., 1995). The assessment of GFR from creatinine is hazardous because of interindividual variability (e.g., P-Creatinine = 200 µmol/l could be observed in dogs with GFR ranging from 0.4 to 1.4 ml/min/kg) (Finco et al., 1995). In cats the relationship is almost linear (Hall et al., 2003).

The sensitivity and specificity of P-Creatinine for the diagnosis of CRF are not very high in dogs and neither are the predictive values (Braun and Lefebvre, 2005; Gleadhill, 1994). The critical difference for P-Creatinine in dogs is 35 µmol/l (Jensen and Aaes, 1993). These criteria have not been reported for other species.

It was suggested that the evolution of renal disease in dogs could be monitored by repeated creatinine measurement and that the time of death could be predicted from the 1/P-Creatinine versus the time curve (Allen et al., 1987); however, this approach gave false estimates in humans (Walser et al., 1988).

P-Creatinine is the most efficient indirect marker of GFR in mammals. It is increased in chronic and acute renal failure, and also in some conditions not directly involving the kidney. For example, see the review as it pertains to dogs in Table 16-1 and in Braun et al. (2003). Similar variations were also observed in cats, equines, and bovines.

2. Urea

a. Urea Metabolism

Urea is a small hydrophilic molecule (MW 60) synthesized in the liver from bicarbonate and ammonium in the Krebs-Henseleit cycle. Urea is the main form in which nitrogen is eliminated in mammals. After synthesis, it is distributed into the total body water compartment (Dunegan et al., 1978; Scholebr, 1960). It is freely filtered by the kidney glomeruli and reabsorbed from the collecting tubule. Its passive reabsorption is increased when urine flow in the tubule is reduced (Park and Rabinowitz, 1969).
TABLE 16-1 Main Causes of Variations of P-Creatinine in the Dog

<table>
<thead>
<tr>
<th>P-Creatinine</th>
<th>Primary renal disease</th>
<th>Intoxication by arsenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>amyloidosis</td>
<td>fluoride</td>
</tr>
<tr>
<td></td>
<td>glomerulosclerosis</td>
<td>citrinin</td>
</tr>
<tr>
<td></td>
<td>polycystic disease</td>
<td>ochratoxin</td>
</tr>
<tr>
<td></td>
<td>uremic crisis</td>
<td>vitamin D</td>
</tr>
<tr>
<td></td>
<td>kidney graft rejection</td>
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</tr>
<tr>
<td></td>
<td>congenital renal diseases</td>
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</tr>
<tr>
<td></td>
<td>Secondary renal disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>babesiosis</td>
<td>leishmaniasis</td>
</tr>
<tr>
<td></td>
<td>leptospirosis</td>
<td>borreliosis</td>
</tr>
<tr>
<td></td>
<td>trypanosomiasis</td>
<td>encephalitozoonosis</td>
</tr>
<tr>
<td></td>
<td>histiocytosis</td>
<td>heartworm disease</td>
</tr>
<tr>
<td></td>
<td>Extrarenal disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ureteral obstruction</td>
<td>uroperitoneum</td>
</tr>
<tr>
<td></td>
<td>Portosystemic shunts</td>
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</tr>
<tr>
<td></td>
<td>Early babesiosis</td>
<td>cachexia</td>
</tr>
<tr>
<td></td>
<td>Hyperthyroidism</td>
<td>kidney graft</td>
</tr>
</tbody>
</table>

For reference, see Braun et al. (2003).

which can lead to increased P-Urea in dehydrated patients or in patients with hemorrhage or to decreased P-Urea in overhydrated patients. Some urea also filters into the intestine, where it is degraded by bacteria into ammonium, which is absorbed and provides a notable proportion of the ammonium supply to the liver. Another important source of ammonium is the catabolism of amino acids. Proteins are thus a major source of ammonium for urea synthesis. Intense recycling of urea occurs in ruminants by transfer to the gastrointestinal tract and to saliva. Urea can also be added to ruminant food (Cirio et al., 2000; Marini and Van Amburgh, 2003), whence it is incorporated into bacterial proteins. The dietary supply of urea is low in other species.

b. Preanalytical Factors of Variation

- Specimen: No difference between canine serum and heparin plasma was observed and only minor changes were noted when specimens were stored frozen for up to 8 months (Thoresen et al., 1995). P-Urea is little affected by hemolysis (up to 25 g hemoglobin/l) and icterus in cattle, horses, cats, or dogs; it is decreased by lipemia in dogs (Jacobs et al., 1992; O’Neill and Feldman, 1989). P-Urea is stable in plasma and whole blood stored for up to 3 days at 20°C (Thoresen et al., 1992) and in serum or plasma stored frozen at −20°C and at −70°C up to 8 months (Thoresen et al., 1995).
- Diet and meals: P-Urea is increased in dogs after meals. Peak postprandial increase can be as high as 7 mmol/l about 6 h after the meal and last for more than 18 h; it is greater with high-protein diets or in animals fed large amounts (Anderson and Edney, 1969; Epstein et al., 1984; Evans, 1987; Vogin et al., 1967). In most species, basal P-Urea reflects the balance between nitrogen utilization and excretion and can be greatly influenced by nutrition (Kohn et al., 2005). The fasting concentration of P-Urea was lower in dogs on low-protein diets with normal or reduced renal function (Polzin et al., 1983, 1991; Reynolds et al., 1999), in horses (Doreau and Martin-Rosset, 1985), in sheep (Rabinowitz et al., 1973), in goats (Valtonen et al., 1982), and in cats (Hesta et al., 2005). P-Urea was also increased by prolonged fasting, because of catabolism of body proteins (Rabinowitz et al., 1973).
- Hydration status: Dehydration had little effect on P-Urea in dogs (≤12.5 mmol/l in 4 days, with weight loss up to 16%) (Hardy and Osborne, 1979), but it produced a two-fold increase in calves within 4 days (Bianca et al., 1965).
- Drugs: P-Urea was unchanged by halothane anesthesia in dogs (Lobetti and Lambrechts, 2000) and was increased at high doses of trimethoprim-sulfadiazine (Lording and Bellamy, 1978).
- Physical exercise: P-Urea in sled dogs was higher after 12 weeks of training, probably as a result of increased protein intake (Reynolds et al., 1999). In greyhounds, P-Urea was unchanged by a 235 m sprint and moderately increased 30 min after a 420 m run (Snow et al., 1988).

c. Analytical Factors of Variation

Most techniques are based on the specific action of a bacterial urease. The accuracy of P-Urea measurements is not usually reported.
d. Reference Intervals and Physiological Factors of Variation

1 mmol/l = 6 mg/dl. There is no good reason to continue using BUN, as the analytical procedure was abandoned long ago and is merely a source of confusion. If required, the factors are BUN (mg/dl) × 0.356 = Urea (mmol/l), and BUN (mg/dl) × 21.4 = Urea (mg/l).

- Gender: No significant effect of gender was observed in young beagles (Passing and Brunk, 1981) or in adult dogs (Broulet et al., 1986).
- Age: P-Urea decreases in dogs (by about 50%) between birth and 1 to 2 months (Kühl et al., 2000; Woldorf et al., 1988), after which irregular but moderate variations have been reported (Broulet et al., 1986; Cowgill and Spangler, 1981; Lowseth et al., 1990; Passing and Brunk, 1981; Strasser et al., 1993, 1997; Vajdovich et al., 1997). P-Urea increased from birth to 6 months in Great Danes, except those fed on low-protein diets (Nap et al., 1991). P-Urea was similar in adult horses and newborn foals, decreasing by about 50% in foals on the first day and remaining low for at least 2 months (Edwards et al., 1990). P-Urea decreased during the first week in calves (Hartmann et al., 1987), although others did not report any change during this time (Klee et al., 1985).
- Individuals: Intraindividual variations in horses were more important than the variations observed before and after foaling (Doreau and Martin-Rosset, 1985).
- Biological rhythms: P-Urea in sheep was 30% higher in summer than in winter (Nawaz and Shah, 1984), but other authors found no seasonal difference in dogs (Strasser et al., 2001).

e. Pathological Factors of Variation

The efficiency of P-Urea in renal failure diagnosis has not been reported. The critical difference in dogs was 2.4 mmol/l around a mean value of 5 mmol/l (Jensen and Aaes, 1993). A threshold of decision of 10 mmol/l has been suggested for cattle with suspected renal disease (Campbell and Watts, 1970).

The relationship between P-Urea and P-Creatinine in dogs was reported to be low (Gabrisch, 1973) or high and linear (Toutain et al., 2000), except for frequent increases in P-Urea concentration, with normal P-Creatinine likely the result of poor preanalytical conditions (Medaille et al., 2004). Because of great interindividual differences, the urea/creatinine ratio cannot be related to specific diseases (Finco and Duncan, 1976).

The variations in P-Urea with disease are similar to those of P-Creatinine, but numerous extrarenal factors may contribute to increased P-Urea, such as gastrointestinal hemorrhage, fasting, or sepsis, which increase protein catabolism; thyrotoxicosis, which lowers P-Urea by increasing GFR; and decreased renal perfusion, which increases renal reabsorption (DiBartola et al., 1996; Praise and Grauer, 1998). Other factors of decreased P-Urea include portosystemic shunts, malnutrition (Davenport et al., 1994), liver insufficiency, and Krebs-Henseleit cycle enzyme defects. See the reviews in Dial (1995) and Sutherland (1989). These extrarenal factors of variation explain why P-Urea is less specific than P-Creatinine for the diagnosis and management of CRF and should not be recommended as a test of renal function. However, as P-Urea greatly depends on protein supply, it is a useful tool for monitoring the effects of dietary protein restriction (Devaux et al., 1996).

P-Urea is less effective in cattle than P-Creatinine to evaluate decreased GFR in diarrheic calves (Brooks et al., 1997), and only 30% of cattle with P-Urea above the upper limit of the reference interval had renal disease (Campbell and Watts, 1970).

f. Effects of Prolonged Increases of P-Urea: Carbamylated Hemoglobin

The carbamylation of proteins is an irreversible nonenzymatic reaction occurring between amino groups of proteins and isocyanate, the active form resulting from cytanate isomerization. This latter is derived from the spontaneous dissociation of urea into ammonium and cyanate ions. Thus, the higher and longer the concentration of P-Urea, the higher the concentration of carbamylated proteins, for instance, of hemoglobin (a process analogous to protein glycation in diabetes mellitus). As proposed in human medicine (Stim et al., 1995; Wynckel et al., 2000), carbamylated hemoglobin may be useful for assessing canine CRF and distinguishing it from ARF (Heiene et al., 2001b), whereas others found a significant overlap with specificity and sensitivity equal to 96% and 84%, respectively, at a 100 µg/g Hb threshold (Vaden et al., 1997a).

3. Cystatin C

Cystatin C is a small constitutive protein (MW ~14,000) synthesized by all nucleated cells and only cleared by glomerular filtration. The plasma concentration is thus increased in cases of renal failure. Cystatin C is considered the most sensitive marker of renal failure in humans. See the reviews in Filler et al. (2005), Latezza et al. (2002), and Price (2000). It can be measured with reagents used in humans in plasma from dogs (Almy et al., 2002; Jensen et al., 2001; Martin et al., 2002) but not cats (Martin et al., 2002). A 50% decrease of P-Cystatin C was reported in dogs following a meal, so sampling should be done after a 12-hour fast (Braun et al., 2002). The upper limit of the reference interval in dogs is 1.3 mg/l (Braun et al., 2002) and similar to the human threshold. P-Cystatin C is well correlated with P-Creatinine and GFR in normal dogs and in dogs with reduced GFR (Almy et al., 2002; Braun et al., 2002). At present, no diagnostic advantage of P-Cystatin C over P-Creatinine has been demonstrated in dogs.
B. Direct Tests of Glomerular Function

The determination of glomerular filtration rate (GFR)—that is, the volume of ultrafiltrate produced per unit of time (e.g., ml/min) by glomerular filtration—is considered the best way to evaluate kidney function. This is based on the hypothesis of the “intact nephron”: as “the surviving nephrons of the diseased kidney largely retain their essential functional integrity” and “retain a remarkably uniform relationship between glomerular and tubular function” (Bricker et al., 1997).

GFR depends on the size of the animal, so different modes of expression have been proposed. One of the most frequently used is to relate GFR to body weight (ml/min/kg) or better still to lean body mass in humans (Swaminathan et al., 2000). Another approach is to use the body area (m²/min/m²), but as the equations used to calculate this from BW have not been validated in all species, this may introduce further inaccuracy (Price and Frazier, 1998). Another mode of expression consists of relating GFR to the volume of the extracellular compartment, as one kidney function is the regulation of body water content. This indexing is rarely adopted but has been used in humans (Peters et al., 2000) and dogs (Gleadhill, 1994; Gleadhill and Michell, 1996).

1. Determination of GFR

There is no easy method for determining GFR from a single blood or urine specimen. In human clinical pathology, there are equations to estimate GFR from P-Creatinine, gender, weight, and age. The most frequently used are the Cockcroft-Gault’s equations, but these are imprecise and the results depend on the techniques used for P-Creatinine (Grubb and Nordin, 2006; Wuys et al., 2003). No such equations are available for use in animals. To our knowledge, the only study relating GFR to P-Creatinine showed that the estimation of canine GFR from P-Creatinine was imprecise, and the authors did not recommend using the equation to estimate GFR (Finco et al., 1995).

The measurement of GFR is based on the clearance of markers freely filtered by the glomerulus and having no or minor secretion and reabsorption. See the review in Heiene and Moe (1998). The accepted reference for GFR determination is the urinary clearance of inulin, a low-molecular-weight polysaccharide (Shannon, 1935). The total amount of this marker eliminated in urine over a period of time is equal to the filtered load:

\[
\text{Total amount eliminated during time } t = (U-\text{Inulin}) \times (U-\text{Volume})
\]

\[
\text{GFR} = \frac{(U-\text{Volume}) \times (U-\text{Inulin})}{(P-\text{Inulin}) \times t}
\]

in which U-Inulin and P-Inulin are in the same units, volume is in ml, and time in min, thus GFR is in ml/min.

However, such determinations are labor intensive; they necessitate the maintenance of a constant plasma concentration by constant infusion of the marker, the accurate determination of urine volume, and precise and accurate measurement of the marker. Urine collection is often impractical as the total urine is required, which implies careful washing of metabolic cages (Watson et al., 2002a) or the use of indwelling catheters. See the review on urine collection and preservation in cats and dogs in Osborne (1995).

Easier procedures have been proposed, based on the following:

- The use of other markers: Some markers; such as ferrocyanide (Ladd et al., 1956), thioulate (Bing and Effersoe, 1948; Dalton, 1968), and sulfanilate (Maddison et al., 1984; Ross and Finco, 1981) have been abandoned. The endogenous or exogenous clearance of creatinine is the most frequently used technique, whatever the species. Iodinated radiocontrast media (e.g., iohexol) provide a good alternative to inulin, but most laboratories are not equipped to measure iodine (Westhoff et al., 1993, 1994). The metabolism of these various markers has not been documented in domestic animals. In some cases extrarenal metabolism can be significant, as shown in cats for diethylenetriaminepentaacetic acid (DTPA), which first concentrates in the heart and lung, then accumulates moderately in the liver before being concentrated in the kidneys (Drost et al., 2000; Uribe et al., 1992). Radiolabeled markers (see the review in Daniel et al. [1999]) allow easier and more accurate measurement of concentrations, but their use is restricted to specially equipped centers: 113I-Inulin, 51Cr-EDTA (Biewenga and van den Brom, 1981; van den Brom and Biewenga, 1981), and 99mTc-DTPA (Gleadhill et al., 1999). They have also been used in large species such as horses (Matthews et al., 1992; Walsh and Royal, 1992).

- The technique of administration: Single injection instead of continuous infusion and determination of plasma clearance of the marker, with calculations based on the decreased plasma concentration of the marker (Pihl and Nosslin, 1974; Summerville and Treves, 1986).

- The use of nuclear imaging techniques and 99mTc-labeled tracers such as DTPA. These permit the functions of each kidney to be evaluated separately (Assaillly et al., 1977; Drost et al., 2000; Krawiec et al., 1988), as these may be similar in healthy but not always in diseased dogs (Cowgill and Hornhof, 1986; Lourens et al., 1982). Determination of GFR by imaging is not as precise as clearance methods (Barthez et al., 1998; Kampa et al., 2002).

2. Variations of Results of GFR Determination According to Procedure

a. Preanalytical Factors of Variation

- Anesthesia: In most cases, anesthesia/sedation was reported to have little or no effect on GFR determination in the dog (Bostrom et al., 2006; Gagnon et al., 1982; Lourens et al., 1992; ...
et al., 1982; Newell et al., 1997), but a decrease was reported after acepromazine alone (Lourens et al., 1982) and others found that GFR was slightly higher in anesthetized than in nonanesthetized dogs (Balint and Forgacs, 1967), maybe as a consequence of the intravenous fluids. Anesthesia could lead to reduction of GFR in dogs with reduced renal mass (Stone et al., 1981). Thiopental anesthesia had no effect on GFR in sheep (Cirio et al., 1990).

- Hydration status: GFR was higher in dogs that were hyperhydrated, so hydration status should be standardized (Kerr, 1958; Kunze et al., 2006; Tabaru et al., 1993). In cats, infusion of Ringer lactate at 1 to 2ml/min produced on average a 40% increase of GFR (Rasmussen et al., 1985). In sheep, GFR was lower in summer than in winter because of hemococoncentration (Nawaz and Shah, 1984).

- Diet: GFR was higher in dogs fed animal proteins rather than other proteins (Bartges et al., 1995a, 1995b, 1995d; Kerr, 1958). It was higher with high levels of proteins in the diet in normal dogs and cats and after renal mass reduction (Adams et al., 1994; Bartges et al., 1995c; Bovee, 1992; Bovee and Kronfeld, 1981; Robertson et al., 1986). In partially nephrectomized dogs, low- or high-sodium diets had little influence on GFR (Greco et al., 1994). In cats, GFR was moderately reduced by low-sodium diets (Buranakarl et al., 2004), unchanged in cats receiving potassium-depleted food, but lowered when the same food was acidified with ammonium chloride (Dow et al., 1990). In cows, GFR was unchanged by mineral supply (Hartmann et al., 2001). GFR was higher in sheep fed a normal or high rather than a low protein diet (Cirio and Boivin, 1990a, 1990b; Cirio et al., 1990; Eriksson and Valtonen, 1982; Rabinowitz et al., 1973; Valtonen et al., 1982).

- Meals: GFR increases ranging from 10% to 45% were observed after a meal of proteins in normal dogs and in dogs with renal mass reduction (Bourgoignie et al., 1987; Brown, 1992; Ewald, 1967; Jolliffe and Smith, 1932; Moustgaard, 1947; O'Connor and Summerill, 1976; Reinhardt et al., 1975). This postprandial increase of GFR was not observed in dogs with experimental Fanconi’s syndrome (Woods and Young, 1991). It was not reduced by fasting for more than 1 day (Moustgaard, 1947). No difference in GFR was observed when ponies were fed twice a day or received the same amount of food every 2h (Clarke et al., 1990).

- Exercise: GFR was unchanged by training in horses and dogs (McKeever et al., 1985, 2002) or by submaximal exercise for 20 or 60min despite hemococoncentration (Hinchcliff et al., 1990; McKeever et al., 1991). During effort under anaerobic conditions in horses, GFR decreased sharply then returned to prerun values within 15min (Schott et al., 1991).

- Drugs: In dogs and sheep, GFR was increased by glucocorticoids, probably by an increase of plasma flow rate as in rats (Baylis and Brenner, 1978; Gans, 1975). GFR was decreased by furosemide in dogs and cats (Boström et al., 2003; Hanna et al., 1988). Controversial effects of NSAIDs were reported in dogs: either no change of GFR with mecloxicam, carprofen, and ketoprofen (Crandell et al., 2004; Ko et al., 2000; Narita et al., 2005) or a moderate decrease with carprofen or ketoprofen (Forsyth et al., 2000). GFR was moderately decreased after repeated excretory urograms in dogs (Feehey et al., 1980a). In cats with experimental CRF, the ACE inhibitor benazepril increased GFR up to 30% (Brown et al., 2001), whereas ACE inhibitors could decrease GFR in sodium-restricted dogs (Hall et al., 1979).

- Biopsy: GFR was not affected after repeated renal biopsies in dogs (Drost et al., 2000; Grroman et al., 2004).

b. Techniques of GFR Measurement

The procedures used to calculate plasma clearances may also influence the results as they depend on the mathematical model chosen (Heiene and Moe, 1999; Powers et al., 1977; Watson et al., 2002a) and on the proposed limited sampling strategies (Barthez et al., 2001; Blavier et al., 2001; Finco, 2005; Watson et al., 2002a). Others have suggested measuring the concentration of an exogenous marker at a given time after injection (e.g., P-Creatinine after IV. load in dogs) (Labato and Ross, 1991; Watson et al., 2002a).

The measurement of creatinine concentrations by Jaffé technique (discussed earlier) led to erroneously high values for creatinine clearance in early studies. Because of the larger proportion of interfering substances in plasma than in urine with the Jaffé technique, P-Creatinine was overestimated in comparison to U-Creatinine (e.g., in dog urine) (Shannon et al., 1932). This discrepancy between creatinine and inulin clearances does not exist when the measurements are based on the more accurate enzymatic techniques (Finco et al., 1993).

The results of GFR determination may vary according to the technique so their transferability is limited (Driehuys et al., 1998; Finco, 2005; Gagnon et al., 1971; Izzat and Rosborough, 1989; Oester et al., 1968; Rogers et al., 1991), and caution is required when using decision limits from other laboratories or from the literature. Correcting factors have been proposed in some cases, for instance, for iohexol plasma clearance and exogenous creatinine urinary clearance in dogs (Finco et al., 2001; Gleedhill and Michell, 1996). However, such results remain controversial. The estimation of GFR based on iohexol clearance, for example, was considered reliable in dogs and cats (Brown et al., 1996), whereas others showed differences of up to 0.95ml/min/kg with creatinine clearance (Miyamoto, 2001b). The urine clearance of endogenous creatinine and the urine and plasma clearance of exogenous creatinine gave similar results (Finco et al., 1981, 1991; Lee et al., 1983; Watson et al., 2002a), but plasma DTPA clearance was 1.15 time higher than plasma iohexol clearance in dogs (Moe and Heiene, 1995).
Dog  Cat  Equine  Cattle  Sheep  Goat
d. Variations with Disease

Decreased GFR is the gold standard of renal failure, whatever its cause or subsequent evolution (discussed later). A critical approach should always be applied in the clinical evaluation of renal function (Finco and Barsanti, 1989), especially as the results obtained differ according to the method used (discussed earlier).

Alterations of GFR may be secondary to many extrarenal diseases. GFR is decreased in hypothyroidism (Adams et al., 1997) and hypoxia (Lobetti et al., 1996). It may be decreased or not in experimental or spontaneous diabetes mellitus (Kaneko et al., 1978, 1979). GFR evaluation is also useful for monitoring the toxicity of drugs eliminated by renal filtration (e.g., carboxplatin) (Bailey et al., 2004; Shapiro et al., 1988) and as an indicator of the effects of renal dysfunction on the pharmacokinetics of drugs (e.g., oxytetracyclin in dogs) (Duffee et al., 1990).

C. Tests of Tubule Function

1. Concentrating Ability

a. Urine Osmolality versus Urine-Specific Gravity

One of the most important functions of the kidney in mammals is to reabsorb more than 99% of the filtered water, so that the final urine is more concentrated than the glomerular ultrafiltrate.

Urine concentration is best evaluated by osmolality (Bovee, 1969)—that is, the concentration of particles in a solution, independently of their chemical characteristics (1 sodium ion has the same osmotic effect as 1 molecule of urea or as 1 molecule of albumin). The main determinant of specific gravity in dog urine is urea (Meyer et al., 1997). The laws of osmolality are only valid for dilute solutions in which the different particles are completely independent and this is not verified in urine or plasma. See the review in Sweeney and Beuchat (1993).

The urine concentration is determined in routine tests from the specific gravity (U-SG)—that is, the ratio of the weight of 11 of solution to 11 of water, which depends on the relative proportions and the molecular weight of all the compounds in solution (for instance, 300mOsm/kg solutions of NaCl, urea, and glucose would have SGs of approximately 1.009, 1.014, and 1.054, respectively; the latter would correspond to 1mOsm/kg solution of albumin).

Urine osmolality and specific gravity were highly correlated in dogs (Bovee, 1969; Dossin et al., 2003; Harvey, 1973; Hendriks et al., 1978; Meyer et al., 1997), sheep (English and Hogan, 1979), and cats (Lees and Osborne, 1979). The correlation was weaker in calves (Thornton and English, 1976). Maximum concentrations could be as high as 1400mOsm/l in rabbits, 2400mOsm/l in dogs, and 3200mOsm/l in cat and sheep (Anderson, 1982).

b. Preanalytical Factors of Variation

- Specimen: In cats, U-SG was not changed by freezing (Lees and Osborne, 1979). Spot urines could give very different U-SG results in the same animal depending on the time of sampling.
- Diet: U-SG was lower in cats supplied with moist food than with dry food (Palmore et al., 1978). In cows, U-osmolality was little changed when the mineral supply was reduced by 50% or increased to 200%, but the urine volume was greatly increased during the high mineral supply period (Hartmann et al., 2001).
- Physical exercise: In greyhounds, U-Osmolality was decreased (~8%) by training because of increased diuresis resulting from plasma volume expansion (McKeever et al., 1985). U-SG was transiently decreased for 1 to 2h after an effort under anaerobic conditions in horses (Schott et al., 1991). The 25% to 75% interval for U-SG in postrace Thoroughbred horses was 1.021 to 1.033 (Cohen et al., 2002).
- Environment: Water intake, urine volume, and osmolality differed significantly in sheep depending on whether the environment was cool or hot, dry or humid (Guerrini et al., 1980).
- Drugs: U-SG was increased in dogs after the administration of radiographic contrast media (Feeney et al., 1980b). In dogs, urine volume was increased and osmolality decreased after medetomidine (Burton et al., 1998) and after glucocorticoids administration, which interferes with the action of vasopressin on the kidney (Joles et al., 1980; Sirek and Best, 1952; Waters et al., 1997). In horses, U-Osmolality was increased after oral sodium bicarbonate loading (Rivas et al., 1997) and was low after treatment with furosemide (median 1.018) (Cohen et al., 2002).
- Anesthesia: U-SG was not changed by halothane anesthesia in dogs (Lobetti and Lambrechts, 2000) but was decreased by isoflurane anesthesia in horses because of increased diuresis, maybe from the fluid support (Watson et al., 2002b). In horses, U-Volume was increased, and U-SG and U-Osmolality were decreased by xylazine and detomidine (Gasthuys et al., 1986, 1987; Nunez et al., 2004; Steffey and Pascoe, 2002; Thurmon et al., 1984; Trim and Hanson, 1986).

c. Analytical Factors of Variation

Measuring osmolality requires expensive equipment, thus is not easy to do in most veterinary clinics. Freeze-point osmometers are usually unsuitable for the direct measurement of highly concentrated urines, especially in cats (Lees and Osborne, 1979).

U-SG must be measured by refractometry. See the review in George (2001). The reagent strips available for U-SG
d. Reference Values and Physiological Factors of Variation

The range of variations of U-SG or U-Osmolality in healthy animals is large in all species, so that reference intervals are devoid of any relevance for spot urines.

- Breed: U-SG was higher in miniature schnauzers than in labradors and might be a factor in oxalate stone formation (Stevenson and Markwell, 2001).
- Gender: Sex had no effect on canine U-SG (van Vonderen et al., 1997).
- Age: U-SG was lower in aged dogs than in young adults (van Vonderen et al., 1997). U-SG was low at birth in puppies, then increased over the first 2 months to values higher than those of mature dogs (Faulks and Lane, 2003; Laroute et al., 2005). U-SG/osmolality in newborn foals was similar to or moderately lower than that of adults, then it decreased to a state of hypostenuria during the first day of life and remained so for at least 2 months (Edwards et al., 1990).
- Inter- and intraindividual variability: U-SG high in canine urine, CVs were in the range of 30% to 40% (van Vonderen et al., 1997).
- Biological rhythms: U-SG in dogs was slightly lower in evening than in morning samples (van Vonderen et al., 1997).

e. Pathological Factors of Variation

As variations in healthy animals can be large, hypo- or isostenuria must either be confirmed on repeated samples or observed in moderately dehydrated or azotemic animals to be interpreted as an indicator of kidney dysfunction.

A concentrating ability below the commonly accepted limits of 1.030 and 1.035, in dogs and cats, respectively, is considered inadequate. See the review as this pertains to dogs in Watson (1998).

2. Tests of Water Deprivation

When plasma osmolality increases, osmoreceptors in the hypothalamus stimulate the release of ADH in blood, thus increasing water reabsorption and equilibrating plasma osmolality. Diabetes insipidus is an uncommon condition characterized by polyuria and polydipsia without glucosuria. It can be congenital or acquired (Harb et al., 1996) and results from decreased secretion of ADH by the hypothalamus (central diabetes insipidus) or from insensitivity of kidney cells to the effects of ADH. See the reviews in Cohen and Post (2002) and Neiger and Hagemoser (1985).

Tests of water deprivation are based on the fact that sudden or progressive withholding of water produces progressive dehydration. The resulting increase in extracellular fluid osmolality triggers the release of ADH, thus an increase of U-Osmolality and U-SG in healthy subjects. The concentrating ability after exogenous ADH administration can be measured to test whether the absence of urine concentration results from a central defect of ADH secretion or from peripheral resistance to ADH. See the review in Finco (1995a). Protocols for test combination have been proposed (Mulnix et al., 1976), as well as for ADH measurement, but they have not gained wide acceptance (Biewenga et al., 1987). In dogs as in humans, urinary excretion of aquaporin-2 occurs in parallel to ADH action and can be used as a marker of collecting duct responsiveness (van Vonderen et al., 2004).

Water deprivation tests may be hazardous, so should only be used to investigate polydipsia-polyuria once significant kidney damage has been ruled out (MacDougall, 1981). They are contraindicated in dehydrated, azotemic, or hypercalceemic dogs or cats (Barsanti et al., 2000).

Water restriction in 10-month-old beagles produced maximum U-SG = 1.070 after up to 23 h of water deprivation (Balazs et al., 1971). In adult dogs, maximum U-Osmolality was 2738mOsm/kg with corresponding U-SG of 1.076 after 72 h and a weight loss of 16% (Hardy and Osborne, 1979). In horses, water deprivation for 72 h led to an average loss of weight of 8%, and an increase in U-SG (mean ~1.050), the hematocrit, and P-Proteins (Genetzky et al., 1987; Rumbaugh et al., 1982).

3. Urine Excretion of Ions

a. Fractional Excretion

Many electrolytes are intensely reabsorbed after filtration, mainly in the proximal tubule; their excretion is thus increased when tubule dysfunction occurs. Urine electrolyte concentrations also depend on the alimentary supply as the homeostatic mechanisms aimed to stabilize plasma concentration modulate tubule reabsorption. They can thus greatly differ as a function of the diet in all species and on the proximity of meals in monogastric animals.

The most meaningful information would be obtained from daily urine excretion, which is often impossible to obtain because of the difficulties associated with urine collection. Expressing the urinary elimination of a solute (X) as the ratio of the filtered load that is found in urine has been proposed, whence the name fractional excretion (FE):

\[ \text{FE}_X = \frac{\text{amount in urine}}{\text{amount filtered}} = \frac{(U-X \times U-\text{Volume})}{(P-X \times \text{GFR})} \]

in which U-X and P-X are the urine and plasma concentration of X respectively.
If creatinine clearance is used as a measurement of GFR, it can be demonstrated that FE is equal to the ratio of the solute clearance to creatinine clearance, thus the synonym fractional clearance may be preferred (Constable, 1991). FEs can easily be determined in “spot” samples of plasma and urine according to the following equation:

\[ FE = \left( \frac{U-X}{P-X} \right) \times \left( \frac{P-Creatinine}{U-Creatinine} \right) \]

Such spot measurements are often well correlated with daily elimination. See the reviews in Coffman (1980) and King (1994). However, spot determinations in cats are highly variable compared with 72-h values, and should thus be interpreted with caution (Finco et al., 1997). FE\(_{\text{Na}}\) and FE\(_{\text{K}}\) are poor indicators of the daily excretion of these ions in animals with renal failure (Adams et al., 1991). FEs and standard clearances are highly correlated in horses for sodium, potassium, and phosphates (Traver et al., 1977) (e.g., spot measurements of FE\(_{\text{Pi}}\) are within 0.1% of the calculated 24-h value) (Lane and Merritt, 1983). In sheep FE\(_{\text{Na}}\), FE\(_{\text{K}}\), FE\(_{\text{Ca}}\), and FE\(_{\text{Pi}}\) are highly correlated with the respective daily urine excretion of these ions (Garry et al., 1990c).

b. Preanalytical Factors of Variation of Ion Excretion

- Diet: Daily elimination and FE\(_{\text{Na}}\), FE\(_{\text{K}}\), FE\(_{\text{Ca}}\), and FE\(_{\text{Pi}}\) are higher in fed than in nonfed healthy dogs, whereas the excretion of phosphate is unchanged (Lulich et al., 1991). Diuresis and urine mineral composition differ according to food composition and intake in dogs (Zentek et al., 1994) and cats (Sauer et al., 1985a, 1985b) (e.g., cats fed a low K diet had lower FE\(_{\text{K}}\) and higher FE\(_{\text{Ca}}\)) (Dow et al., 1990); cats supplemented with magnesium showed a four-fold increase of FE\(_{\text{Mg}}\) (Norris et al., 1999a), and FE\(_{\text{Ca}}\) was increased in phosphate-depleted dogs (Goldfarb et al., 1977). FE\(_{\text{K}}\) is lower in dogs fed low-phosphate diets (Polzin et al., 1991) and low-protein diets (Polzin and Osborne, 1988). In cattle (see a review in Lunn and McGuirk [1990]) the composition of the diet greatly influences electrolyte balance, especially for calcium and magnesium (Gray et al., 1988): FE\(_{\text{Mg}}\) was about three times higher in cows receiving oral magnesium hydroxide than in controls (Kasari et al., 1990) and is mainly used to test magnesium status (Sutherland et al., 1986). In cows receiving a high mineral diet, FE\(_{\text{Ca}}\), FE\(_{\text{Mg}}\), and FE\(_{\text{Pi}}\) were increased (Hartmann et al., 2001). In sheep artificially loaded with NaCl by oral or intraruminal route, GFR, FE\(_{\text{Na}}\), and FE\(_{\text{K}}\) increased, whereas P-Sodium remained stable (Meintjes and Engelbrecht, 1993). In cattle, sodium bicarbonate loading increased FE\(_{\text{Na}}\) and FE\(_{\text{Mg}}\), decreased FE\(_{\text{Mg}}\) and FE\(_{\text{Ca}}\), but had no effect on FE\(_{\text{K}}\), FE\(_{\text{Ca}}\), and FE\(_{\text{Pi}}\) (Roby et al., 1987). In mares, FE\(_{\text{Ca}}\) and FE\(_{\text{Pi}}\) are dependent on the diet (e.g., FE\(_{\text{Pi}}\) ranged from almost 0% to 20% when mares fed on pasture alone or with a high-P mineral supplement) (Caple et al., 1982a, 1982b). FE\(_{\text{Ca}}\), FE\(_{\text{Mg}}\), and FE\(_{\text{Pi}}\) were increased in cows receiving a high-mineral diet (Hartmann et al., 2001).

- Meals: In cats, urine excretion of phosphates and magnesium is moderately higher in the postprandial period (Finco et al., 1986). Postprandial variations of FE\(_{\text{Na}}\) and FE\(_{\text{K}}\) were observed in cats fed a high-phosphate high-sodium diet (Finco et al., 1989). FEs were altered after large meals in ponies (Clarke et al., 1990).

- Physical exercise: In horses, FE\(_{\text{K}}\), FE\(_{\text{Na}}\), and FE\(_{\text{Cl}}\) were decreased by training (McKeever et al., 2002). FE\(_{\text{Na}}\) was increased, whereas FE\(_{\text{Ca}}\) was decreased, and FE\(_{\text{K}}\) remained unchanged during submaximal exercise (McKeever et al., 1991) or transiently decreased immediately after effort under anaerobic conditions (Schott et al., 1991).

- Drugs and fluid therapy: In dogs, FE\(_{\text{K}}\) is increased in volume expansion by NaCl infusion (Massey et al., 1969). FE\(_{\text{K}}\) and FE\(_{\text{Cl}}\) but not FE\(_{\text{Na}}\) are increased after medetomidine administration in dogs (Burton et al., 1998). In horses, IV. infusions of glucose or saline solutions increased FE\(_{\text{Ca}}\), FE\(_{\text{Cl}}\), and FE\(_{\text{Pi}}\), and FE\(_{\text{K}}\) was also increased after glucose infusion (Roussel et al., 1993). In ponies, administration of xylazine increased FE\(_{\text{K}}\), FE\(_{\text{Na}}\), and FE\(_{\text{Cl}}\) (Trim and Hanson, 1986).

c. Analytical Factors of Variation

Ion concentrations in urine are usually measured with the same techniques as in plasma with ad hoc dilutions when necessary, but techniques have not been validated in animal species. An interfering substance in the urine of sheep, cattle, horses, and cats, but not dogs, causes falsely low results for potassium but not sodium with ion-selective electrodes (Brooks et al., 1988).

d. Reference Intervals and Physiological Factors of Variation

Mean values of FEs collected from the literature are summarized in Figure 16-8. It should be remembered, as emphasized in horses (Morris et al., 1984), that interindividual variations are large and the values observed in individuals may be outside the limits of each group.

- Age: In puppies, FE\(_{\text{Na}}\) increased during the first 6 months of life, whereas FE\(_{\text{K}}\) peaked at about 4 months, and FE\(_{\text{Ca}}\), FE\(_{\text{Cl}}\), and FE\(_{\text{Pi}}\) were little changed (Lane et al., 2000).

- Pregnancy-lactation: In cows, significant changes in FE\(_{\text{Na}}\), FE\(_{\text{K}}\), FE\(_{\text{Cl}}\), FE\(_{\text{Ca}}\), and FE\(_{\text{Pi}}\) were observed prepartum and during lactation (Ulutas et al., 2003). FE\(_{\text{Na}}\), FE\(_{\text{K}}\), and FE\(_{\text{Cl}}\) were unchanged with the stage of lactation, whereas FE\(_{\text{Pi}}\), FE\(_{\text{Ca}}\), and FE\(_{\text{Mg}}\) differed (Fleming et al., 1992).

- Biological rhythms: In fasted dogs, FE\(_{\text{Na}}\) and FE\(_{\text{Ca}}\) were higher in the morning and FE\(_{\text{Pi}}\) in early afternoon (Hartenbower et al., 1974). In cows, FE\(_{\text{Na}}\), FE\(_{\text{K}}\), FE\(_{\text{Cl}}\), FE\(_{\text{Ca}}\), FE\(_{\text{Pi}}\), and FE\(_{\text{Mg}}\) did not vary significantly over 24h
but most showed great interindividual variability (Fleming et al., 1991). Other authors have observed significant changes in Pi, Na, and K total excretion and FEs with maximal values at the middle of the day, which did not depend on age or production category (high or low) (Fleming et al., 1992).

e. Pathological Factors of Variation

The main difficulty when interpreting FEs in the diagnosis of renal disease is that they are greatly influenced by all extrarenal factors involved in the regulation of plasma electrolyte balance, mainly by dietary supply (Finco and Barsanti, 1989; Finco et al., 1992a). As a result, the interpretation of increased FEs in terms of tubular dysfunction is often hypothetical. Only repeated measurements under well-controlled conditions (e.g., experimental settings) may offer some relevance.

In cats with severe CRF, the FEs of ions were normal in most animals, and their measurement did not seem to improve diagnosis (Filippich, 1992). In dogs, it was shown that FE_{K} was a less accurate indicator of CRF than P-Creatinine (Gleadhill, 1994).

FEs are also changed in nonrenal diseases. In diabetic cats, FE_{Mg} was about 20 times higher than in controls and could be responsible for the frequent hypomagnesemia observed in diabetes mellitus (Norris et al., 1999b). In the cat, a case of increased FE_{P} with normal P-Phosphates is described, probably because of decreased reabsorption and resulting in rickets-like symptoms (Henik et al., 1999). In horses, FE_{K} is moderately but insignificantly lower during rhabdomyolysis (Beech et al., 1993).

IV. TESTS OF KIDNEY DAMAGE

A. Glomerular Damage

1. Proteinuria

Proteinuria is one of the most frequent abnormalities in routine urinalysis and was observed in about 43% and 50% of canine and feline samples submitted to a university hospital (Barlough et al., 1981). See the review on human proteinuria in Waller et al. (1989). Although glomerular damage is the cause of the most intense proteinurias, it is not the only one: these can also originate from the tubules and their cause may be pre- or postrenal (Table 16-2). See the consensus statement on canine proteinuria in Lees et al. (2005).

The following systematic approach is required in cases of confirmed proteinuria: (1) check that it is persistent, (2) evaluate the magnitude, and (3) localize the origin. See the reviews in Hurley and Vaden (1995), Kunze et al. (2006), and Lees et al. (2005).

a. Origin of Urinary Proteins

The characteristics of the glomerular filtration slit (see Section IB) are such that almost no or very little plasma protein is filtered. The MW of albumin is closest to the filtration threshold, so this is the first plasma protein to
ine, which is inversely related to urine dilution, is used as the need to collect urine for 24 h. The concentration of creatinine by measuring total daily excretion, but this is difficult due to better estimates of proteinuria would be obtained sp u urine protein concentration can differ considerably within a given animal, depending mainly on the urine concentration. Better estimates of proteinuria would be obtained by measuring total daily excretion, but this is difficult due to the need to collect urine for 24 h. The concentration of creatinine, which is inversely related to urine dilution, is used as a correction in spot samples as its excretion in a given animal is supposed to be fairly constant. Urine protein excretion is thus expressed as the U-(Protein/Creatinine) ratio (U-P/C), in which the concentrations of the two analytes are expressed in mg/l. This ratio has gained general acceptance in clinical pathology. See the reviews in Lulich and Osborne (1990) and Price et al. (2005).

The U-P/C ratio in spot urines is well correlated with 24-h urine excretion in healthy and CRF dogs and cats (Adams et al., 1992; Barsanti and Finco, 1979; Grauer et al., 1985; Monroe et al., 1989; White et al., 1984).

c. Preanalytical Factors of Variation

- Specimen: The method of urine collection (natural voiding, catheterization, cystocentesis) was not found to have a significant effect on U-Proteins in dogs (Barsanti and Finco, 1979). U-P/C could be increased in cases of cystitis and blood contamination of urine (Bagley et al., 1991).
- Diet and meals: U-Protein was higher in dogs with CRF, fed high-protein diets (Polzin et al., 1983, 1984). U-P/C is moderately decreased 4 to 8 h after a meal (Jergens et al., 1987).
- Housing: U-P/C was higher in hospitalized non-proteinuric dogs than in nonhospitalized ones and was generally lower than 0.5 (McCaw et al., 1985).
- Drugs: In dogs, long-term glucocorticoid therapy produced a regular increase of U-P/C > 0.5 at 2 weeks.

<table>
<thead>
<tr>
<th>TABLE 16-2</th>
<th>Categories of Causes of Proteinuria Based on the Site or Mechanism of the Underlying Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prerenal</td>
<td>(Definition: due to abnormal plasma content of proteins that traverse glomerular capillary walls having normal permselectivity properties.) Normal proteins that are not normally present free in the plasma (e.g., hemoglobin or myoglobin). Abnormal proteins (e.g., immunoglobulin light chains) (Bence-Jones proteins).</td>
</tr>
<tr>
<td>Renal</td>
<td>(Definition: due to abnormal renal handling of normal plasma proteins.) Functional (Definition: proteinuria that is due to altered renal physiology during or in response to certain transient phenomena (e.g., strenuous exercise, fever, and so on). The key distinction here is that the proteinuria is not attributable to presence of renal lesions. The hallmarks of this type of proteinuria are that it is mild and transient—that is, it promptly resolves when the condition that is generating it resolves. Pathological (Definition: proteinuria that is attributable to structural or functional lesions within the kidneys, regardless of their magnitude or duration.) Glomerular (Definition: due to lesions altering the permselectivity properties of the glomerular capillary wall.) Tubular (Definition: due to lesions that impair the tubular recovery of plasma proteins that ordinarily traverse glomerular capillary walls having normal permselectivity properties.) These plasma proteins traffic into the urine from glomerular capillaries. They consist mainly of low-molecular-weight proteins but may also include small amounts of moderate molecular weight proteins (e.g., albumin). Interstitial (Definition: due to inflammatory lesions or disease processes (i.e., acute interstitial nephritis) causing exudation of proteins into the urine from peritubular capillaries.</td>
</tr>
<tr>
<td>Postrenal</td>
<td>(Definition: due to entry of protein into the urine after it enters the renal pelvis.) Urinary (Definition: due to entry of proteins derived from hemorrhagic or exudative processes affecting the walls of the urine excretory pathway, renal pelvis, ureter, urinary bladder, and urethra (including into the urethra from the prostate gland in males.) Extraurinary (Definition: due to entry of proteins derived from secretions or from hemorrhagic and/or exudative processes affecting the genitai tract and/or external genitalia during voiding or in the process of collecting urine for analysis.)</td>
</tr>
</tbody>
</table>

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and peaked slightly above 1.0 after 4 weeks (Waters et al., 1997). Nephroangiography often produces transient proteinuria (Holtas et al., 1981).

d. Techniques
- Detection: The different screening techniques do not provide the same results. See the review in human medicine (Thysell, 1969). Proteinuria detection is most often based on the use of test strips, the detection limit being 0.25 to 0.30g/l for albumin but much higher for globulins (Behr et al., 2003). Moreover, as the reagent patch is based on a pH indicator, falsely positive results are often observed in alkaline urines. Hemoglobin interference remains negligible as long as the urine is not colored, even if the “blood” patch is strongly positive (Jansen and Lumsden, 1985; Vaden et al., 2004). False positives are not encountered with denaturation tests such as the sulfosalicylic acid and nitric acid ring tests, which give identical reactions with globulins and albumin. Caution should be taken in the interpretation of dipstick results for the effects of urine dilution/concentration, and readings must be interpreted with U-SG as a possible correction factor.

- Quantification: The quantification and fractionation of proteins should preferably be performed in nonconcentrated urines, although the U-Protein content is usually low, necessitating special procedures. Concentration techniques may alter the relative composition of urine proteins in human urines (Ala-Houahala et al., 1984). The biuret reaction cannot be used unmodified as its quantification limit is too high (a few g/l). Older methods based on heat denaturation, turbidimetry (Hendriks et al., 1976), and more recent ones using special stains such as Ponceau S and Coomassie Blue have mostly been abandoned. The main stain currently used is pyrogallol red, which gives slightly higher results with albumin than with globulins (Behr et al., 2003). Calibrations are performed with different specimens (albumin, dilution of serum, concentrated urines, etc.), thus urine protein measurements can greatly differ from one laboratory to another, as with older techniques (Barsanti and Finco, 1979). U-Protein and U-P/C can be measured in canine but not feline urine with special dipsticks used for human urine (Welles et al., 2006).

e. Reference Values and Physiological Factors of Variation
Proteinuria is not detected by routine techniques in urines of healthy animals, except for possible “traces” or “+” readings in highly concentrated urines. In most species, the upper limit of U-Proteins is about 0.3 to 0.4g/l as observed in piglets (Ruhmann et al., 1986) and horses (Edwards et al., 1989). In normal dogs, the urinary loss of proteins is very low and the median is 6mg/kg BW/day. The value in 80% of dogs was ≤10mg/kg/day (Biewenga et al., 1982), whereas others reported 14mg/kg/day (DiBartola et al., 1980a). The upper limits of U-Proteins in cats were <21/mg/kg/d and 27mg/kg/d in females and males, respectively (Monroe et al., 1989). The commonly accepted reference limits for U-P/C are 0.5 and 0.4 in dogs and cats, respectively (Lees et al., 2005; Elliot, 2007).

- Age: In puppies, total proteinuria and U-P/C decreased in the first 6 months of life (Lane et al., 2000). Total daily excretion of proteins was lower in 2-month-old puppies than in adults (Laroute et al., 2005), but others detected proteins in urines of almost all dogs aged less than 3 months (Faulks and Lane, 2003). In newborn piglets there was a transient increase of U-Proteins between 6 and 24h (Parker and Aherne, 1980) because of absorption of fragments of colostral IgGs during the first day of life, and after 3 days proteins become almost undetectable (Martinsson, 1972).

- Gender: U-Protein is higher in naturally voided urine of male than female dogs; the difference is not observed in urines obtained by cystocentesis (Barsanti and Finco, 1979).

- Intra- and interindividual variations: Interindividual variations in proteinuria are much greater in adult dogs than in 2-month-old puppies (Laroute et al., 2005) and are very large in cats (Russo et al., 1986). In sheep, total protein excreted per 4h and U-P/C were stable for 2 days (Garry et al., 1990c).

- Biological rhythms: In dogs, no differences in protein output and U-P/C were observed between night and day samples (McCaw et al., 1985).

f. Pathological Factors of Variation
In human medicine, U-P/C is considered a safe method to rule out the possibility of significant excessive 24-hour protein excretion (Price et al., 2005). One-quarter of a colony of beagles 4 to 6 years old with no clinical signs and normal U-SG showed transient or permanent proteinuria (Stuart et al., 1975).

Glomerulopathies are the cause of the most severe urine protein losses. See the review in Lulich et al. (1996). Dramatic increases in protein excretion are observed in canine and feline amyloidosis and membranous glomerulonephritis with values attaining 900mg/kg/day and U-P/C >10 (Biewenga and Grays, 1986; Center et al., 1985, 1987; DiBartola et al., 1980b; Minkus et al., 1994). Proteinuria is the earliest sign of glomerular disease in experimental glomerulonephritis of the cat (Bishop et al., 1991).

Proteinuria is also observed in extrarenal conditions: exercise in the dog (Epstein and Zambraki, 1979) as in humans, plasma protein overload in dogs when P-Proteins exceed 95 to 100g/l (Terry et al., 1948), and urinary infections in 90% to 95% of cases when leukocytes or bacteria are identified (Fettman, 1987, 1989). About half of the dogs...
with pituitary-dependent hyperadrenocorticism show moderate increases of U-P/C (Hurley and Vaden, 1998; Ortega et al., 1996).

2. Albuminuria-Microalbuminuria

More than 99% of filtered albumin is reabsorbed in the proximal tubule. See the review in Gekle (1998). Maximal reabsorption capacity is lower in rodents, so that a minimal increase in plasma concentration results in urine excretion, which is not observed in dogs (Gartner, 1981). The word microalbuminuria is used to qualify the urinary elimination of traces of albumin, below the detection limit of urine total proteins (i.e., below ~300mg/l) but above 20 to 30mg/l. These thresholds correspond to those used in human medicine for the early diagnosis of renal complications of diabetes mellitus.

Microalbuminuria cannot be detected in canine or feline urine with tests used for human microalbuminuria (Pressler et al., 2002). Special tests are commercialized for the semi-quantitative evaluation of microalbuminuria in canine urines (Pressler et al., 2002). Preliminary studies showed that microalbuminuria was observed in a large proportion of dogs without any clinical sign of renal disease, but the upper limit of “normal” urine elimination of albumin and the effects of possible factors of variation have not been determined in dogs. Moreover, the detection of microalbuminuria does not provide any information about the existence of a possible evolutive disease of the kidney. More basic studies will be required before any use of this new test.

U-albumin in human urine is stable for up to 5 months when stored at −20°C and correctly homogenized after thawing (Brinkman et al., 2005). An ELISA test with a limit of quantification of 10mg/l has been set up for use in dog urine (Vaden et al., 2004). Albuminuria is unchanged in dogs after exercise for 20 min at 8km/h (Gary et al., 2004).

3. Urine Protein Electrophoresis

The diagnostic use of electrophoresis of urinary proteins is based on the identification of selective proteinuria versus unselective proteinuria. The latter results from severe glomerular damage allowing massive transfer of all plasma proteins (unselective) including high MW immunoglobulins. The former is observed during the first stages of glomerular damage, when only low MW plasma proteins are eliminated or when tubular damage impairs the reabsorption of filtered low MW proteins, producing selective proteinuria. Protein identifications are most frequently performed in SDS-agarose gels where the proteins are separated according to their MW (Meyer-Lindeenberg et al., 1997; Muller-Peddinghaus and Trautwein, 1977; Schultze and Jensen, 1998). Protein electrophoresis permits the identification of severe glomerulo- and tubulointerstitial nephropathies but not their differentiation (Zini, 2004). The technique is 100% sensitive for glomerular damage but only 40% specific in dogs (Zini et al., 2004). A more selective identification of urine proteins can be obtained by mass spectrometry; for instance, retinol-binding protein is only present in the urine of dogs with kidney damage and Tamm-Horsfall protein excretion is reduced in kidney disease (Forterre et al., 2004).

In normal dogs and cats, electrophoresis shows only traces of albumin and rarely of proteins migrating in the globulin zone—that is, mainly transferrin and α1-microglobulin (Groulade et al., 1977, 1978; Harvey and Hoe, 1966; Meyer-Lindeenberg et al., 1997; Pages and Trouillet, 1990; Yalcin and Cetin, 2004) and sometimes low-molecular-weight proteins (Muller-Peddinghaus and Trautwein, 1977; Zaragoza et al., 2003).

Monoclonal or polyclonal immunoglobulin light chains (Bence-Jones proteins) are identified in ~40% of cases of monoclonal gammapathy (Leifer and Matus, 1986; Matus et al., 1986), in spontaneous ehrlichiosis (Varela et al., 1997), and in plasma cell malignancy (Hurvit et al., 1971).

B. Tubule Damage

1. Urine Enzyme Activities
a. Pathopathology

The enzymes found in urine have two origins. See the reviews in Dubach and Schmidt (1979) and Jung et al. (1992). Low MW plasma enzymes are filtered by the glomerulus (e.g., lysozyme or amylase) and are almost totally or partially reabsorbed by the tubule. High MW plasma enzymes cannot be filtered by the glomerulus, but they can be released by the tubule cell. The release of enzymes by other parts of the urinary tract seems negligible.

Most of the enzyme markers used in routine analysis occur mainly in the proximal tubule cell. Renal damage causes their excretion into urine to increase, but there are no increases in plasma enzyme activity, except in severe cases (Shaw, 1976). The kidney specificity of enzymes used as markers of tubule damage is not an issue as long as the MW of the enzymes is large enough to preclude glomerular filtration of the corresponding plasma circulating enzyme.

The localization of enzymes along the nephron is not homogeneous. In rats, proximal and distal tubule damage can be distinguished by measuring the respective glutathione-S-transferase and lactate dehydrogenase (Bombard et al., 1990) but this has not been used in domestic species. The intracellular localization of enzymes differs considerably, for example, alkaline phosphatase (ALP), alanine aminopeptidase (AAP), GPDAP (glycyl-prolyl-dipeptidyl aminopeptidase), and gamma-glutamyltransferase (GGT) occur in the brush borders, β-glucuronidase, N-acetyl-β-glucosaminidase (NAG) in the lysosomes, lactate dehydrogenase (LDH) and glutathione-S-transferase (GST) in the cytoplasm, and glutamate dehydrogenase (GLD) in the mitochondria. This has been used in experimental toxicology to study the progression of cell damage (Bret et al., 1993), but it has not been used in clinical cases.
Enzymes cannot accumulate in urine, as they do in plasma because of their elimination with each urination. Thus, the amount of enzyme eliminated in a urine sample reflects the amount of kidney damage that has occurred since the preceding urination. However, the urine concentration of enzymes not only depends on the release of these latter from the kidneys but also on urine concentration/dilution, hence the frequent use of the U-(Enzyme/Creatinine) ratio as a correction factor in the determination in spot urines (Gossett et al., 1987).

b. Preanalytical Factors of Variation

- Specimen: Many enzymes are unstable in refrigerated canine urine and are almost totally inactivated by freezing (e.g., GGT, LDH, and amylase) (Keller and Freudiger, 1984), whereas NAG and AAP are stable for one month at 4°C and −18°C after gel filtration (Reusch et al., 1991). GGT is stable for 3 days at +4°C (Adams et al., 1985; Gossett et al., 1987).
- Anesthesia: U-GGT and U-(GGT/Cr) were increased after prolonged sevoflurane anesthesia in horses (Driessen et al., 2002).

c. Analytical Factors of Variation

No technique has been validated for the measurement of urine enzyme activities, so results may differ greatly from one laboratory to another.

Better measurements of AAP and NAG enzyme activities in dog urine are obtained after the elimination of small-molecular-weight inhibitors by dialysis or gel filtration (Reusch et al., 1991). Others have reported that GGT and NAG in urine from dogs (Sato et al., 2002a), cattle (Sato et al., 1997), horses (Adams et al., 1985; Gossett et al., 1987), and cats (Sato et al., 2002b) can be measured without prior preparation.

d. Reference Intervals and Physiological Factors of Variation

As with plasma enzyme activity measurements, no validly transferrable reference intervals are available for urine enzymes because of the lack of standard techniques and primary control material to ensure interlaboratory controls of accuracy. It is thus unwise to take such information from the literature without checking values of the laboratory.

- Gender: U-NAG was approximately two times higher in male dogs than in females (Nakamura et al., 1983; Reusch et al., 1991; Sato et al., 1997), and was reduced by castration and by vasectomy (Higashiyama et al., 1983). U-NAG was also higher in steers than in cows, but the U-(NAG/Creatinine) ratio was not influenced by sex in cattle (Sato et al., 1997) and cats (Sato et al., 2002b). U-GPDAP activity was higher in males than in females (Uechi et al., 1997).
- Age: Neither age nor gender affected U-GGT and U-ALP activities in horses (Brobst et al., 1986).
- Individuals: Inter- and intra-individual variations were large in dogs and cats (Ogura, 1986; Reusch et al., 1991).

- Biological rhythms: No circadian rhythm of U-GGT or U-NAG was observed in cats (Uechi et al., 1998), but the excretion of both enzymes in dogs was higher between 12:00 and 16:00 (Uechi et al., 1994b) and that of GPDAP was higher between 8:00 and 12:00 (Uechi et al., 1997). U-GGT and U-ALP were moderately higher in morning urines in horses (Brobst et al., 1986).

e. Pathological Factors of Variation

Increased urine enzyme excretion is a sign of acute kidney damage, whatever its cause, but does not imply organ dysfunction (Ellis et al., 1973b, 1973c). In many cases, significant increases of urine enzyme excretion are observed without or earlier than any alteration of the function markers (Rivers et al., 1996). Urine enzyme excretion shows little or no modification in chronic renal diseases (e.g., canine CRF) (Heiene et al., 1991).

Although NAG is largely used in toxicology, GGT is often preferred in routine clinical practice because of its ease measurement without urine predialysis and use of readily available reagents. Only urine NAG was increased initially when primary damage was located in the papillary zone of dogs (e.g., ethyleneimine) (Ellis et al., 1973a).

Enzymuria is probably the most sensitive test for monitoring kidney damage induced by treatments with potentially nephrotoxic drugs such as gentamicin in dogs (Adelman et al., 1979; Davies et al., 1998; Grauer et al., 1994, 1995; Greco et al., 1985; Lora-Michiels et al., 2001; Martinez et al., 1996; Spangler et al., 1980), sheep (Brown and Garry, 1988; Garry et al., 1990a), and horses (Rossier et al., 1995). Similar changes were observed in horses treated with neomycin: enzyme excretion was increased whereas creatinine clearance and plasma creatinine concentration were unaltered (Edwards et al., 1989).

Urine enzyme excretion was also increased in renal damage secondary to extrarenal disease, such as canine leishmaniasis (Palacio et al., 1997), approximately 40% of cases of pyometra (de Schepper et al., 1989a, 1989b; Heiene et al., 2001a), and in heartworm disease (Uechi et al., 1994a). The U-NAG and U-(NAG/Creatinine) ratios were increased in cows with interstitial nephritis and amyloidosis (Sato et al., 1999).

2. Blood

Hematuria can originate from any part of the kidney or the urinary tract. See the review in Forrester (2004). It is detected by the color of the urine, ranging from light pink to red in macrohematuria, or more frequently by routine urinalysis for invisible microhematuria. This latter is detected by the peroxidase activity of hemoproteins and can therefore give false positives with other proteins such as myoglobin or catalase. The limit of detection of hemoproteins is low, so that occult blood can be detected in the absence of a positive reaction for proteins. See the review on techniques of blood detection in Syed et al. (2002).
Idiopathic kidney bleeding has been reported in Weimaraners (Hitt et al., 1985). See the review in Hitt (1986). Occult blood could be detected in approximately 25% of cases in 2-month-old dogs (Faulks and Lane, 2003). Blood in urine can result from sample collection, especially catheterization, as in large animals when indwelling systems are used (Godeau et al., 1990). Macroscopic and microscopic hematuria could result from kidney biopsy in cats (Nash et al., 1983) but only rarely (Osborne, 1971).

V. BIOCHEMICAL CHANGES IN KIDNEY DISEASE

Renal or kidney disease is a pathological process affecting any part of the kidney and may or may not be associated with alterations in kidney function. Kidney or renal failure (insufficiency) is characterized by a decrease in one or several kidney functions, first the urine concentrating ability, then the elimination of small-molecular-weight molecules from the plasma, characterizing azotemia (i.e., increases of P-Urea and/or P-Creatinine). Uremia is the syndrome resulting from renal failure. Cases of azotemia are not always primary renal azotemia caused by parenchymal damage but may be prerenal or postrenal azotemia resulting from reduced kidney perfusion and interferences with urine excretion, respectively. See the reviews in DiBartola (2005b) and Osborne and Polzin (1983).

Although the etiology of renal diseases and associated lesions may differ markedly, the differential diagnosis, prognosis, and monitoring of disease evolution or therapy is mainly based on clinical biochemistry. However, clinical biochemistry cannot identify the cause of the renal dysfunction/lesion, which requires other tools, such as renal biopsy, imaging, genetic testing, urine culture, cytology, and so on. Most of the literature available is concerned with dogs and cats and, to a much lesser degree, horses. The major renal syndromes of interest to the clinical pathologist are chronic renal failure, acute renal failure, nephrotic syndrome, and Fanconi-like syndromes because of their specific pattern of alterations in biochemical variables and the time course of these changes.

A. Chronic Renal Failure

Chronic renal failure is a slow irreversible deterioration of kidney function, which, on account of the very large functional reserve of the kidneys, occurs without clinical or biological signs over a long period. Chronic renal failure in dogs and cats is highly prevalent in old animals and is a frequent cause of death: it usually evolves more or less rapidly from early renal disease by a largely unknown process. See the reviews in Brown et al. (1997) and Finco et al. (1999). As renal disease does not necessarily imply CRF, the main challenge is to detect any kidney disease as early as possible to limit its progression by appropriate dietary and therapeutic renoprotective maneuvers. See the reviews in Braun and Lefebvre (2005), Grauer (1985, 2005), Greco (2005), and Lees et al. (1998).

1. Development and Progression of Renal Failure

Experimental models of chronic renal failure have helped to extend our knowledge of CRF progression and of the concomitant changes in biochemical variables. However, some results obtained in rodents seem not to be applicable to cats and dogs. The most commonly used model is the remnant kidney model, based on nephrectomy of the right kidney and reduction of the left kidney mass by selective ligatures of the renal arteries or by electrocoagulation of the left renal cortex. The intensity of left kidney mass reduction determines the postoperative decrease in renal function quantified by the decrease of GFR between the control and postoperative periods.

In dogs, a 5/6 renal mass reduction led to a 65% decrease in GFR and only moderate increases of P-Creatinine and P-Urea, which remained within the reference limits (Brown et al., 2000). One month after the renal mass had been reduced to 3/4 in dogs, GFR was approximately 0.75ml/min/kg and P-Creatinine approximately 300µmol/l (Finco et al., 1992b). In cats, 3/4 and 5/6 reductions of renal mass led to reductions in renal function of one-half and two-thirds, respectively (Adams et al., 1994; Miyamoto, 1998).

The only compensatory mechanism observed was hypertrophy of the remaining tissue except after subtotal kidney mass reduction, when hyperplasia occurred (Filippich et al., 1985). For example, the removal of one kidney in dogs was followed by a 40% increase in weight of the remaining kidney (Carriere, 1978). Compensation was progressive, being rapid for the first 2 to 12 weeks, then slower (Churchill et al., 1999). This explains why the immediate postoperative GFR value is not indicative of the GFR measured several weeks later (Lefebvre et al., 1998a). The GFR decrease was generally less than expected from the reduction of renal mass, and hypermetabolism was observed in the remaining kidney tissue (Fine, 1991). It is thus recommended to wait at least 6 to 8 weeks after surgery to obtain stationary conditions before testing renal function.

Compensation is dependent on many factors, especially nutrition. It was less effective in dogs fed a low-protein (White et al., 1991) or a high-phosphorus diet (Finco et al., 1992b) and in the case of hypertension (Finco, 2004), whereas dietary sodium supply had no effect, except when very low (Greco et al., 1994). Cats with experimental CRF fed a low-sodium diet had lower GFR values than cats fed a normal diet (Buranakart et al., 2004).

The progression of renal disease is poorly understood (Terzi et al., 1998) and leads to clinical disturbances of increasing severity. This has led experts to propose dividing canine and feline CRF into four stages based on P-Creatinine...
at presentation (Table 16-3) and substages based on proteinuria and blood pressure (Elliott, 2007). Experimental CRF does not necessarily lead to self-perpetuating renal disease (i.e., a spontaneous decline of GFR over time). Kidney function in dogs with a 75% reduction of renal mass was stable up to 4 years, except in some animals fed low-protein diets (Bovee et al., 1979), but not in others (Robertson et al., 1986). Following uninephrectomy in 7- to 8-year-old dogs, 2/3 animals survived 4 years, and the GFR remained stable at ~3.5 ml/min/kg, irrespective of dietary protein content (18% and 24%) (Finco et al., 1994). GFR was stable for 1 year, and U-P/C was moderately increased but remained below 0.3 after uninephrectomy in young cats (Finco et al., 1998). After 15/16 nephrectomy, renal function decreased rapidly in dogs fed a high-Ca, high-P diet (Brown et al., 1991).

Spontaneous CRF results mainly from tubular-interstitial disease, amyloidosis, and glomerulonephritis, which progress to the uremic syndrome (multiple organ dysfunctions) when end-stage renal failure is reached (Polzin et al., 2000). These dysfunctions probably result from the accumulation of solutes normally excreted by the kidney, the so-called uremic toxins. Ninety molecules, most of them nonidentified, are considered to be potential uremic toxins. See the reviews in Boure and Vanholder (2004), Vanholder and De Smet (1999), Vanholder and Glorieux (2003), and Yavuz et al. (2005), but urea and creatinine have little or no toxicity.

Familial renal diseases in dogs and cats provide unique models for human medicine, as most of them lead to the development of CRF. See the reviews in DiBartola (2005a) and Lees (1996). The biochemical findings in such patients when clinical signs are present are the same as in dogs with CRF (see the review in DiBartola, 2000). Proteinuria and hypercholesterolemia are present in primary glomerular disease, as observed in Bernese mountain dogs. Glucosuria may be observed in primary renal tubular defects in Norwegian elkhounds and basenjis (see later Fanconi-like syndromes). The major laboratory finding in Welsh corgis with renal telangiectasia is marked hematuria.

### 2. Biochemical Changes in Animals with Spontaneous CRF

The most frequent biochemical findings in CRF are isosthenuria, proteinuria, azotemia, hyperphosphatemia, and metabolic hyperchloremic acidosis. When the CRF diagnosis cannot be confirmed, GFR should be measured. Although it is relatively rare in horses, CRF produces biochemical alterations similar to those observed in dogs and cats. See the review in Schott (2004).

- Azotemia is a common finding in dogs and cats with CRF and is sometimes the only biochemical criterion used to diagnose CRF. However, it was absent or mild at presentation in about 50% of dogs with glomerulonephritis or renal amyloidosis (Cook and Cowgill, 1996; DiBartola et al., 1989). It was commonly observed in cats and sharpeis dogs with amyloidosis (Grauer and DiBartola, 2000). Survival was inversely correlated to P-Creatinine in cats and dogs with CRF (Allen et al., 1987; Elliott et al., 2000).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Creatinine (µmol/L)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt;125 dogs</td>
<td>Notazotemic</td>
</tr>
<tr>
<td></td>
<td>&lt;140 cats</td>
<td>Some other renal abnormality present (e.g., inadequate urinary concentrating ability without identifiable nonrenal cause), abnormal renal palpation or abnormal renal imaging findings, proteinuria of renal origin, abnormal renal biopsy results; increasing plasma creatinine concentration noted when serial samples have been collected</td>
</tr>
<tr>
<td>II</td>
<td>125–179 dogs</td>
<td>Mild renal azotemia (lower end of the range lies within the reference range for many laboratories, but the insensitivity of creatinine as a screening test means that animals with creatinine values close to the upper reference limit often have excretory failure)</td>
</tr>
<tr>
<td></td>
<td>140–249 cats</td>
<td>Clinical signs usually mild or absent</td>
</tr>
<tr>
<td>III</td>
<td>180–440 dogs</td>
<td>Moderate renal azotemia</td>
</tr>
<tr>
<td></td>
<td>250–440 cats</td>
<td>Many extrarenal clinical signs may be present</td>
</tr>
<tr>
<td>IV</td>
<td>&gt;440 dogs and cats</td>
<td>Severe renal azotemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Many extrarenal clinical signs are usually present</td>
</tr>
</tbody>
</table>

Reproduced from Elliott (2007).
indicator of the inability of the kidney to concentrate urine. U-SG and P-Creatinine are inversely related in cats (Elliott et al., 2000), but cats with CRF retained concentrating ability longer than dogs (Ross and Finco, 1981), although decreased U-SG was frequently observed in cases of severe CRF (Deguchi and Akuzawa, 1997; Elliott and Barber, 1998). As U-SG is higher in puppies than in adult dogs (noted earlier), the cutoff value for abnormally low U-SG should therefore be higher.

- Proteinuria may or may not be present in CRF. The identification of proteinuria does not mean that its cause is renal and this needs to be ascertained by rational diagnosis. When proteinuria is observed in dogs and cats with CRF, it is generally mild to moderate. However, there is some evidence that the presence of proteinuria in CRF dogs or cats aggravates prognosis (Brown et al., 1998a, Grauer, 2005). The persistence of proteinuria should be confirmed by repeated measurement over time (Lees et al., 2005).

- Hypoalbuminemia occurs in many dogs and cats with glomerulonephritis, and higher in the latter than in dogs with interstitial nephritis, proteinuria cannot be relied on for differential diagnosis of the underlying renal disease, which requires a renal biopsy (Grauer and DiBartola, 2000). When renal proteinuria is present in dogs or cats, the current recommendation is to treat it to avoid complications, when the U-P/C in nonazotemic animals is higher than 1, and in azotemic patients, higher than 2. See reviews in Lees et al. (2005) and Lulich et al. (1996). Angiotensin-converting enzyme inhibitors are the currently used antiproteinuric agents. The administration of such agents was shown to decrease U-P/C in dogs (Grauer et al., 2000) and cats (Brown et al., 2001). The magnitude of proteinuria may decrease in end-stage renal disease, when GFR decrease is severe (Jaenke and Allen, 1986).

- Hyperphosphatemia is frequently associated with moderate hypocalemia, which may be masked when hypercalcemia is the cause of renal disease. See the reviews in Kruger and Osborne (1994a, 1994b) and Kruger et al. (1996). In horses, CRF usually produced hypercalcemia and hypophosphatemia (Brobst et al., 1977, 1978a; Roberts and Setler, 1979) but hyperphosphatemia was also observed in a few cases (Brobst et al., 1977). This hypercalcemia may result from the fact that calcium is more strongly excreted by the horse kidney than in other species (see excretion of crystals of calcium carbonates) (Kruger et al., 1996). Hypocalcemia and hyperphosphatemia were observed in 70% of azotemic cattle but did not permit differentiation of prerenal from renal or postrenal causes (Brobst et al., 1978b).

- Hyperphosphatemia is a frequent finding in clinical CRF, but it is uncommon in patients with subclinical renal disease. It is currently considered that hyperphosphatemia per se does not directly contribute to clinical signs but is the main cause of renal secondary hyperparathyroidism, which is observed in models of CRF (Grunbaum et al., 1984) and in spontaneous cases (e.g., in about 84% of cats with CRF) (Barber and Elliott, 1998). The precise pathophysiology of secondary hyperparathyroidism in dogs remains unclear. See reviews in Nagode et al. (1996) and Yaphé and Forrestor (1994). Phosphate retention is increased as renal failure progresses. As a result of phosphate binding and decreased formation of calcitriol in the kidney, the ionized calcium concentration progressively decreases, thus inducing a progressive increase of PTH secretion, which enhances calcium mobilization from bone and absorption from the intestine and phosphate elimination by the kidney. Finally at the ESRF stage, PTH secretion becomes resistant to calcium suppression, leading to tertiary hyperparathyroidism. In rare cases, hyperphosphatemia may lead to renal osteodystrophy (Nagode and Chew, 1992), but PTH probably contributes to the pathophysiology of the uremic syndrome and may be a uremic toxin. The parathyroid concentration therefore needs to be measured in uremic animals for hyperparathyroidism diagnosis (Polzin et al., 2000), and results should be interpreted with regard to serum calcium concentration, especially that of ionized calcium. In small animal nephrology, minimizing hyperphosphatemia and hyperparathyroidism by dietary phosphorus restriction, the use of intestinal phosphate binding agents, and the possible administration of calcitriol is a therapeutic goal (Nagode et al., 1996). Careful monitoring of phosphatemia is therefore recommended in dogs and cats with CRF.

- Electrolyte and water disturbances are frequent, especially in advanced stages of CRF. Dehydration is highly prevalent in cats (~70%) (Lulich et al., 1992) and should be regularly determined during renal disease from repeated measurements of PCV, total proteins, and body weight. Metabolic hyperchloremic acidosis is due to the decreased excretion of hydrogen ions, bicarbonate wasting, and chloride retention. The estimated overall prevalence in cats was 63% to 80% (DiBartola et al., 1978b; Lulich et al., 1992), or 0% in nonuremic cats to about 50% in cats with end-stage CRF (Elliott and Barber, 1998).

Thus, the acid-base status should be investigated in all uremic patients. Acidosis may have several adverse effects on cardiovascular physiology, promote protein malnutrition, and induce bone demineralization. Hypokalemia has been reported in cats with CRF (Lulich et al., 1992) but is apparently uncommon in dogs. The muscle potassium content decreased in normokalemic cats with CRF (Theisen et al., 1997). The cause-effect relationship between kale- mia and CRF remains unclear, but hypokalemia may be
V. Biochemical Changes in Kidney Disease

B. Acute Renal Failure

1. Definition, Etiology, and Pathophysiology

Acute renal failure (ARF) is “a clinical syndrome characterized by the sudden onset of hemodynamic, filtration and excretory failure of the kidneys with subsequent accumulation of metabolic (uremic) toxins and dysregulation of fluid, electrolyte and acid-balance” (Cowgill and Elliott, 2000). ARF is generally reversible (unlike CRF) if diagnosed early and given adequate therapy. Whereas CRF results from a progressive decline of GFR, the cause of which most often remains unknown, ARF is associated with an acute decline in GFR frequently caused by an ischemic or toxic insult. Probably, one of the most difficult challenges in nephrology is to differentiate ARF from end-stage renal function or renal impairment at the onset of treatment, whereas P-Phosphates and P-Potassium were little changed. Many other models based on nephrotoxic agents such as mercuric chloride, uranyl acetate, and so on, surgery, radiation have been used, but the resulting alterations in urine/plasma biochemistry tend to differ as the various agents do not produce the same effects. Experimental models of ARF in various domestic animal species were first based on binephrectomy in ponies (Tennant et al., 1981), sheep (Simesen et al., 1979), and bulls (Watts and Campbell, 1970, 1971). Ruminants survived longer than monogastric animals (about 7 days) and showed almost linear increases of P-Urea and P-Creatinine, whereas P-Phosphates and P-Potassium were little changed and P-Calcium was decreased. Many other models based on nephrotoxic agents such as mercuric chloride, uranyl acetate, and so on, surgery, radiation have been used, but the resulting alterations in urine/plasma biochemistry tend to differ as the various agents do not produce the same initial events and subsequent cascade (Stein et al., 1975). Moreover, the biochemical alterations may differ considerably, according to the renal status of the animal. For example, in gentamicin-induced nephrotoxicity, polyuric hypokalemic ARF is observed in dogs with normal renal function or renal impairment at the onset of treatment, and this can lead respectively to reversible polyuric hypokalemic ARF or to fatal oligoanuric hyperkalemic ARF (Frazier et al., 1986).

2. Urine and Plasma Biochemical Findings in ARF

Urine and plasma biochemical findings are essential to distinguish between types of ARF. Generally, in prerenal ARF, hypersthenuric urine is produced as the ability of the kidney to concentrate urine. Renal parenchymal ARF is produced by intrinsic damage to the kidney caused by toxic insults, CRF, systemic diseases affecting renal function, or by pre-renal ARF. Postrenal ARF is the consequence of obstruction or diversion of urine outflow, and accumulation of excretory products in the body. See the review in Cowgill and Elliott (2000). Three consecutive phases of ARF have been described: the initiation phase (subclinical lasting from hours to days) in which the kidneys are subjected to the renal insult; the maintenance phase (lasting from a few days to 2 to 3 weeks), which develops when lesions of the renal tubules have been established; and the recovery phase associated with an improvement of renal function. The initiation phase is difficult to identify from a pathological point of view unless the onset of ARF is relatively slow. In such conditions, GFR decreases progressively, P-Creatinine and P-Urea increase but may remain within the reference intervals, urine-concentrating ability may change, and urine abnormalities (proteinuria, cylindruria, enzymuria) may be present. The maintenance phase is characterized by the most severe clinical signs and by plasma and urine biochemical alterations. The clinical signs and biochemical findings are generally resolved during the recovery phase unless the lesions are irreversible. Polyuria is usually observed. See the reviews in (Grauer and Lane, 1995; Lane et al., 1994a, 1994b; Lulich et al., 1992).

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responsible for general weakness, anorexia, and decreased renal function.

- Hyperlipidemia and hypercholesterolemia have been reported in dogs with glomerular diseases and nephrotic syndrome (Cook and Cowgill, 1996; DiBartola et al., 1989). For example, the estimated prevalence of hypercholesterolemia in dogs with glomerular amyloidosis was 86% (DiBartola et al., 1989). These changes probably result from a combination of increased hepatic synthesis and decreased catabolism of proteins and lipoproteins. Hypercholesterolemia and hyperlipidemia may contribute to further renal damage.

- Hematological alterations: CRF is associated with the development of progressive nonregenerating anemia. The main cause in dogs and cats (Oishi et al., 1993) is the decreased synthesis of erythropoietin (EPO). See the review in Cowgill (1992). However, an overlap of EPO concentrations was observed in anemic dogs or cats with and without CRF (Oishi et al., 1995; Pechereau et al., 1997). A loss of antithrombin III by increased filtration and platelet hypersensitivity resulting from hypoalbuminemia was reported in canine nephrotic syndrome and other forms of glomerulonephritis (Greco and Green, 1987; Green et al., 1985), resulting in the thrombus formation reported in cases of amyloidosis (Slauson and Gribble, 1971).
to concentrate urine is not impaired. If azotemia is present and the U-SG decreased, then renal parenchymal ARF should be considered. Distinction from CRF may be difficult. Nonregenerative anemia, normo- or hypokalemia, is more common in CRF (Grauer and Lane, 1995). Metabolic acidosis is generally more severe in ARF. For a similar degree of azotemia, animals with ARF exhibited more severe electrolyte disturbances than animals with prerenal azotemia or CRF (Cowgill and Elliott, 2000). The commonest life-threatening electrolyte disturbance in ARF is hyperkalemia, which may cause weakness, cardiac arrhythmia, and possible death of the animal. However, hypokalemia may also develop during the diuretic stage of ARF. Moreover, abnormally low plasma concentrations of sodium, calcium, magnesium, and potassium may exacerbate the development of ARF, especially in case of nephrotoxicity (e.g., caused by gentamicin) (Grauer, 1996). GFR measurements are not indicated in ARF as hydration status and renal function may vary considerably from one hour to another. Once the animal has recovered, GFR determination may permit quantification of the residual renal function, as ARF events may predispose to CRF development. In spontaneous ARF of dogs, P-Urea, P-Creatinine, or P-Phosphates were ineffective predictors of outcome, whereas oliguria was the best indicator of poor prognosis (Behrend et al., 1996).

Diagnosis of postrenal ARF is based on history, clinical signs, and imaging. Hematuria and peritoneal fluid with a higher creatinine concentration than that of plasma and similar to the urine creatinine concentrations are observed when rupture of the urinary tract occurs. Casts were detected in about 30% of dogs with ARF (Vaden et al., 1997b).

Urine enzyme activities are useful early markers of cell damage in ARF principally in nephrotoxicity studies, as they are more sensitive than other markers. In dogs treated with gentamicin, urine excretion of GGT increased by day 2 of administration, P-Creatinine remained below 180 μmol/L until day 9, and endogenous creatinine clearance remained within normal values until day 8 (Greco et al., 1985). The intensity of total enzyme excretion may help to quantify the extent of renal damage. The use of enzymuria in routine nephrology remains questionable except in toxicological settings and to monitor the effects of potentially nephrotoxic drugs.

Proteinuria is often detected in uremic animals because of frequent evidence of inflammation and hemorrhage in the urine sediment. However, its relevance in the differential diagnosis, prognosis, and follow-up of ARF patients, in contrast to CRF, remains unknown.

Unusual causes of ARF in dogs and cats have been reviewed recently (Stokes and Forrester, 2004). Many cases of spontaneous acute renal failure result from intoxications. A common example is the ingestion of ethylene-glycol (antifreeze) by dogs and cats, which is oxidized by alcohol dehydrogenase in the liver to glycolaldehyde and acids, with oxalic acid the terminal metabolite. These metabolites are responsible for severe metabolic acidosis and ARF with dramatic increases of P-Urea and P-Creatinine, proteinuria, and calcium oxalate crystalluria. In the initial phase, affected animals are polyuric with decreased U-SG and U-pH (Connally et al., 1996; Fox et al., 1987; Grauer et al., 1984; Hamlín, 1986; Thrall et al., 1984). In ruminants, equids, and swine, ARF is more frequently caused by nephrotoxic plants or mycotoxins than by infections; for example, ochratoxin A and citrinin produced increases in urinary enzyme excretion, proteinuria, and decreased U-SG in pigs (Szczech et al., 1973, 1974). Diagnosis is based on increases of P-Urea and P-Creatinine and a decrease of U-SG (Divers et al., 1982; Gouda et al., 1986).

Treatments with nephrotoxic drugs can also cause ARF. Cisplatin toxicosis is mainly due to damage of the lower segment of the proximal tubule. This produces a decrease of GFR in dogs and no change or increases of P-Urea or P-Creatinine and increases of FE_{Na} and FE_{Pr}, and of U-GGT and U-GGT/Creatinine (Forrester et al., 1993; Hardie et al., 1991). A decrease of GFR, U-SG, and P-Potassium was observed with amphotericin B, as was an increase of P-Urea and P-Creatinine (Randall et al., 1996).

Gentamicin nephrotoxicity determines progressive renal failure characterized by increases of urinary enzyme excretion, proteinuria, hematuria and cylindruria, azotemia, hyperphosphatemia, hypoalbuminemia, hyper- or hypokalemia, reduction of GFR, and an increase of FE_{Na} and FE_{K} in dogs (Daugaard et al., 1987; Riviere et al., 1984), cats (Hardy et al., 1985; Mealey and Boothe, 1994), and sheep (Garry et al., 1990b).

C. Nephrotic Syndrome

Nephrotic syndrome is characterized by the presence of proteinuria, hypoalbuminemia, hypercholesterolemia, and edema or ascites. It is mainly a consequence of diabetic nephropathy in humans. See the review in Ortih and Ritz (1998). In dogs and cats, it is caused by glomerular renal disease, mainly by immune-mediated glomerulonephritis and amyloidosis. See the reviews in Osborne and Jeraj (1980) and Relford and Lees (1996). A model has been obtained in dogs by repeated administration of cationized bovine albumin after endothox sensitization (Choi and Lee, 2004). The hallmark of nephrotic syndrome is severe proteinuria in the absence of active sediment. The prevalence of hypoalbuminemia and hypercholesterolemia in dogs ranged from 61% (Center et al., 1987) to 100% (Jeraj et al., 1984; Kurtz et al., 1972). Edema was usually less prevalent (from 0% to 15%) (Center et al., 1987; Kurtz et al., 1972). When the GFR decrease is substantial, animals with nephrotic syndrome become azotemic and present the clinical signs and biological findings characteristic of CRF and ARF. The prevalence of azotemia in dogs with glomerular disease varied from 20% (Wright et al., 1981) to 100% (Jeraj et al., 1984; Kurtz et al., 1972). In cats, azotemia, hypercholesteremia, hypoalbuminemia, anemia, and edema/ascites
were reported to occur in 67%, 77%, 96%, 63%, and 75% of cases, respectively (Arthur et al., 1986).

D. Fanconi-Like Syndromes

Fanconi-like syndromes are observed in some breeds of dogs, mainly basenjis (Noonan and Kay, 1990). They are characterized by multiple defects in the reabsorption of glucose, sodium, potassium, calcium, phosphate, amino acids, and water by the tubule (Bovee et al., 1979; Settles and Schmidt, 1994) producing decreased U-SG, proteinuria, and glucosuria (Darrigrand-Haag et al., 1996; Easley and Breitschwerdt, 1976). They may also be acquired as a result of gentamicin toxicity (Brown et al., 1976). They may also be acquired by maleic acid (Al-Bander et al., 1985) or 4-pentaenoate administration (Gougoux et al., 1989), or they may be transient and of unknown etiology (Hostutler et al., 2004; Jamieson and Chandler, 2001).

GFR in dogs with Fanconi-like syndrome may be decreased or remained unchanged (Bovee et al., 1979; Breitschwerdt et al., 1983). Glucosuria, which is one of the major findings in Fanconi-like syndromes, was not correlated with a reduction in GFR or with defective reabsorption of phosphate and sodium (Bovee et al., 1978a,b). The cause of death is apparently not progressive nonspecific renal failure, as the final events are papillary necrosis and pyelonephritis (Bovee et al., 1978b, 1979).

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